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**RENAL SODIUM-DOPAMINE RELATIONSHIP AND
THE EFFECTS OF SODIUM LOADING ON NATRIURETIC
AND HORMONAL RESPONSES IN CHINESE**

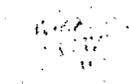
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Summary of Thesis

'Renal sodium-dopamine relationship and the effects of sodium loading on natriuretic and hormonal responses in Chinese'

Thomas Yan Keung Chan

Dopamine (DA) is an important intrarenal natriuretic, vasodilatory substance in the body. In my work, my main objective was to establish whether Chinese have an efficient renal DA system that will enable them to handle a salt load effectively without showing an increase in blood pressure (BP). A reduction in sympathetic nervous system activity, which is reflected by a fall in urinary free noradrenaline (NA) output, and enhanced renal kallikrein production, as indicated by increased urinary kallikrein output, may also contribute to the natriuretic response to salt loading. The relative importance of these two mechanisms in sodium homeostasis in Chinese were also studied. In my studies, urinary free DA and NA were measured by HPLC with electrochemical detection.

If DA is an important intrarenal natriuretic substance in Chinese and dietary sodium is the major determinant of its intrarenal synthesis, there should be a positive correlation between 24-hour urinary sodium and free DA outputs even under basal conditions. There should be a marked and sustained increase in urinary free DA output during the natriuretic response to salt loading. Their renal DA system should also be sensitive to small and gradual increases in oral salt intake.

In my first cross-sectional study, 89 normotensive subjects (BP <140 mmHg) with (5 M, 25 F, aged 48.3 ± 1.3 years) or without (21 M, 38 F, aged 43.4 ± 2.0 years) a family history of hypertension and 24 hypertensive patients (8 M, 16 F, aged 45.5 ± 1.7 years, systolic BP >160 and/or diastolic BP >95 mmHg) were recruited in

1989-1991. There was a positive correlation between 24-hour urinary sodium and free DA outputs in normotensives without a family history of hypertension ($r=0.42$, $p<0.001$). However, such a relationship was not seen in normotensives with a family history of hypertension ($r=0.22$, $p<0.5$) or hypertensives ($r=-0.02$, $p>0.5$). In my second cross-sectional study, 85 normotensive subjects (BP $<120/80$ mmHg) with (14 M, 13 F, aged 39.1 ± 1.4 years) or without (23 M, 35 F, aged 42.1 ± 1.2 years) a family history of hypertension and 47 hypertensive patients (25 M, 22 F, BP $>140/90$ mmHg) were recruited in 1996-1998. A positive correlation between 24-hour urinary sodium and free DA outputs was seen in both normotensives ($r=0.57$, $p=0.004$ and $r=0.36$, $p=0.006$) and hypertensives ($r=0.44$, $p=0.002$). Variations in findings between these two studies could be in part due to differences in the inclusion criteria (BP and its measurement), recruitment method and subject characteristics (age, body weight, BP levels and salt intake). Hypertensives in the second study were slightly younger and had less severe hypertension than hypertensives in the first study. Perhaps, a positive urinary sodium-free DA relationship is seen only in the earlier stages or when the hypertension is less severe. In the second study, hypertensives had a higher 24-hour urinary free DA output than normotensives. This hyperdopaminergic response may represent an early antihypertensive compensatory mechanism.

Forty-three sibling-pairs discordant for hypertension were also studied under basal conditions. The normotensives (14 M, 29 F, aged 39.0 ± 1.0 years, BP $<120/80$ mmHg) and hypertensives (21 M, 22 F, aged 40.0 ± 0.9 years, BP $>140/90$ mmHg) were significantly different with regarding to their body weight (62.7 ± 1.6 vs 69.1 ± 1.7 kg), BP ($120.0 \pm 1.3 / 71.8 \pm 1.2$ vs $147.0 \pm 2.5 / 91.4 \pm 1.5$ mmHg) and urinary free DA output ($1,457 \pm 107$ vs $1,765 \pm 108$ nmol/day). A positive

correlation between 24-hour urinary sodium and free DA outputs was seen in both groups ($r=0.54$, $p=0.0000$ and $r=0.37$, $p=0.015$).

I also studied the effects of large acute changes in dietary salt intake in eight healthy Chinese males (aged 22-24 years, body weight 58.3 ± 1.9 kg) without a family history of hypertension. They were given a constant diet containing 20 mmol sodium/day for 10 days (days -4 to 0 and days 1 to 5). On days 1 to 5, they also received 'Slow sodium' tablets equivalent to 200 mmol sodium/day. Urine was collected for 24 hours before and throughout the high salt intake period. After salt loading, their mean arterial blood pressure (MAP) remained unchanged (76.8 ± 1.8 and 75.0 ± 2.8 mmHg, $p>0.05$) and there was an 8- to 9-fold increase in sodium excretion ($p<0.01$). There was an 8-17% increase in urinary free DA output during the first three days of salt loading ($p<0.05$). There was a 22% reduction in urinary free noradrenaline (NA) output on the last day of salt loading ($p<0.05$). A similar renal DA response was seen in seven healthy Chinese males (aged 21-31 years, 75.5 ± 3.4 kg) with a family history of hypertension. On changing from a 20 to 220 mmol/day sodium diet, there was a 7- to 10-fold increase in sodium excretion ($p<0.02$). They also showed a 23% increase in urinary free DA output on day 1 ($p<0.05$). From day 2 to day 5 of the high salt intake period, the increase in urinary free DA output also became attenuated (12-15% higher than the baseline) but still reached statistical significance ($p<0.05$) on days 2 and 5. Unlike those without a family history of hypertension, urinary free NA remained unchanged and there was a 3.7% increase in MAP (80.1 ± 2.5 and 83.1 ± 2.1 mmHg, $p<0.05$). In contrast, when changing from a low (90-40 mmol/day) to high (220-340 mmol/day) sodium intake, Caucasians in previous studies showed a much greater increase in urinary free DA output (26-59%) and the urinary free DA output remained elevated throughout the

high salt intake period. These findings suggest that renal DA contributes only partly to the early natriuretic response to dietary salt loading in Chinese. A reduction in SNS activity towards the end of the high salt intake period might help offset any tendency to hypervolaemia-related increases in BP in normotensives without a family history of hypertension.

Seven healthy Chinese males (aged 23-25 years, 70.2 ± 3.1 kg) without a family history of hypertension received a constant diet containing 20 mmol/day of sodium for 12 days (days -3 to 0 and days 1 to 8). From day 1 to day 8, they also received 'Slow sodium' tablets equivalent to 50 mmol sodium on day 1, 100 mmol on day 2, 150 mmol on day 3, 200 mmol on day 4, 250 mmol on day 5 and 300 mmol on days 6 to 8. Urine was collected for 24 hours before and throughout the high salt intake period. Following salt loading, their MAP was unchanged (79.4 ± 0.5 on day 0 and 81.6 ± 2.1 mmHg, $p > 0.05$). By day 8, there was a 13-fold increase in sodium excretion. However, urinary free DA output remained unchanged. Despite a sodium intake of 320 mmol/day on the last three days of the high salt intake period, there were no significant changes in urinary free DA output ($1,907 \pm 250$ on day 0 vs $1,967 \pm 261$ on day 6, $2,020 \pm 282$ on day 7 and $2,122 \pm 278$ nmol on day 8, $p > 0.05$). In contrast, there was a 19.9-26.5% reduction in free NA output (226.5 ± 17.0 on day 0 vs 180.5 ± 22.6 on day 4 and 165.5 ± 12.3 nmol on day 6, $p < 0.05$). These findings suggest that the renal DA system in Chinese is insensitive to gradual and small increases in oral salt intake. The late reduction in SNS activity may help offset any tendency to hypervolaemia-related increases in blood pressure.

When seven healthy Chinese males (aged 23-25 years) were given an intravenous infusion of 0.9% saline (1,000 ml over two hours), hourly urinary free DA outputs during (71.0 ± 5.7 and 74.4 ± 3.5 nmol) and after (69.8 ± 7.0 and $73.5 \pm$

6.8 nmol) the saline infusion were not different ($p>0.05$) from the baseline (67.2 ± 4.9 nmol). Hourly urinary free NA outputs also remained unchanged. In contrast, hourly urinary kallikrein (measured by an amidolytic assay) showed an increase of 103.0-140.4% during the saline infusion (0.27 ± 0.05 at baseline vs 0.55 ± 0.13 in hour 1 and 0.65 ± 0.11 KU, $p<0.05$). Urinary kallikrein was still 74% higher than the basal level one hour after completion of the infusion (0.47 ± 0.08 KU, $p<0.05$).

In summary, my findings suggest that Chinese do not have an efficient renal DA system. Their renal DA response is insensitive to gradual, small increases in oral salt intake and a modest intravenous salt load. Even sudden, large increases in oral salt intake is associated with a relatively small and unsubstantiated renal DA response. The absence of early changes in urinary free NA output following salt loading may suggest that the natriuresis is not mediated by a reduction in SNS activity. However, a reduction SNS activity towards the end of high salt intake period may help offset any tendency to hypervolaemia-related increases in blood pressure. In contrast, the increase in urinary kallikrein during the natriuretic response to a modest intravenous salt load may suggest that the renal kallikrein-kinin system is the more important natriuretic system in Chinese.

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List of Abbreviations

3-MT	3-methoxytyramine
5-HIAA	5-hydroxyindoleacetic acid
5-HT	5-hydroxytryptamine
5-HTP	5-hydroxytryptophan
ACE	Angiotensin converting enzyme
ADM	Adrenomedullin
ADMA	Asymmetrical dimethyl-L-arginine
ADPKD	Autosomal dominant polycystic kidney disease
AI	Angiotensin I
AII	Angiotensin II
ANOVA	Analysis of variance
ANP	Atrial natriuretic peptide
bNOS	Brain nitric oxide synthase
BMI	Body mass index
BNP	Brain natriuretic peptide
BP	Blood pressure
BW	Body weight
CNP	C-type natriuretic peptide
cAMP	Cyclic adenosine monophosphate
cGMP	Cyclic guanosine monophosphate
CGRP	Calcitonin-gene related peptide
COMT	Catechol-O-methyl-transferase
DA	Dopamine
DAG	Diacylglycerol
DBP	Diastolic blood pressure
DOPAC	Dihydroxyphenylacetic acid
ECF	Extracellular fluid
eNOS	Vascular endothelial nitric oxide synthase
ET	Endothelin
GFR	Glomerular filtration rate
HCl	Hydrochloric acid
HPLC	High performance liquid chromatography
HT	Hypertensive
HVA	Homovanillic acid
IDDM	Insulin-dependent diabetes mellitus
iNOS	Macrophage inducible nitric oxide synthase
IP3	Inositol triphosphate
KCl	Potassium chloride
L-AAAD	L-aromatic amino acid decarboxylase
L-NAME	Nitro L-arginine methylester
L-NMMA	<i>N</i> ^G -monomethyl-L-arginine
MAO	Monoamine oxidase
MAP	Mean arterial pressure
mRNA	Messenger RNA
NA	Noradrenaline
Na	Sodium
NaCl	Sodium chloride

NaSO ₄	Sodium sulphate
NaHCO ₃	Sodium bicarbonate
NH ₄ Cl	Ammonium chloride
NIDDM	Non-insulin-dependent diabetes mellitus
NO	Nitric oxide
NOS	Nitric oxide synthetase
NPR	Natriuretic peptide receptor
NPY	Neuropeptide Y
NT	Normotensive
NYHA	New York Heart Association
PAMP	Proadrenomedullin N-terminal 20-peptide
PGD ₂	Prostaglandin D ₂
PGE ₂	Prostaglandin E ₂
PGF _{2α}	Prostaglandin F _{2α}
PGI ₂	Prostaglandin I ₂
Pi	Phosphorylation
PLA ₂	Phospholipase A ₂
PRA	Plasma renin activity
PRC	Plasma renin concentration
RAS	Renin-angiotensin system
RIF	Renal interstitial fluid
RPP	Renal perfusion pressure
SBP	Systolic blood pressure
SHR	Spontaneously hypertensive rats
SNS	Sympathetic nervous system
SP	Substance P
SR	Salt-resistant
SS	Salt-sensitive
STI	Sodium transport inhibitor
VIP	Vasoactive intestinal peptide

Publications from this Thesis

Full papers in peer-reviewed journals

1. Chan TYK, Critchley JAJH, Ho CS, Wong WKK, Tomlinson B, Swaminathan R. Effects of oral sodium loading on the urinary excretion of catecholamines in normotensive Chinese. *Pharmacol Commun* 1992; 2: 189-94.
2. Chan TYK, Critchley JAJH, Ho CS, Chan JCN, Wong WKK, Swaminathan R, Tomlinson B. Urinary dopamine and noradrenaline outputs during large acute changes in oral salt intake in healthy Chinese subjects. *J Auton Pharmacol* 1994; 14: 317-23.
3. Chan TYK, Critchley JAJH, Ho CS, Chan JCN, Tomlinson B. Urinary dopamine and noradrenaline outputs during oral salt loading in healthy Chinese subjects with a family history of hypertension. *J Auton Pharmacol* 1996; 16: 1-6.
4. Chan TYK, Critchley JAJH, Ho CS, Chan JCN, Tomlinson B. Urinary dopamine outputs do not rise in healthy Chinese subjects during gradually increasing oral sodium intake over eight days. *J Auton Pharmacol* 1996; 16: 155-9.
5. Chan TYK, Critchley JAJH, Ho CS, Tomlinson B, Chan JCN, Poon EWM, Lee ZSK, Critchley LAH, Swaminathan R. Renal kallikrein-kinin system, but not renal dopamine system, mediates the natriuretic response to intravenous saline infusion in healthy Chinese subjects. *J Auton Pharmacol* 2000; 20: 37-45.

Abstract in peer-reviewed journal

6. Chan TYK, Critchley JAJH, Wong WKK, Ho CS, Swaminathan R. Urinary dopamine, noradrenaline, adrenaline and sodium relationships in hypertensive and normotensive Chinese with or without a family history of hypertension (abstract). *Br J Clin Pharmacol* 1991; 31: 218P.

Chapter 1 Salt balance, renal dopamine and hypertension

1.1 Salt intake and hypertension

Both genetics and environmental factors as well as their interactions are thought to be important in the development of hypertension (1). In westernised societies, the most important modifiable environmental risk factors are high salt intake, alcohol intake, obesity and low physical activity (2). Recent data have also shed light on how stress can affect blood pressure (BP) (3).

Meta-analyses of observational data have confirmed and quantified an association between salt intake and high BP both between populations (4) and within populations (5). A difference in sodium intake of 100 mmol/day is associated with an average difference in systolic blood pressure (SBP) that ranges from 5 mmHg at age 15-19 years to 10 mmHg at age 60-69 years. The differences in diastolic blood pressure (DBP) are about half as great. Meta-analyses of randomised controlled trials have confirmed the favourable effect of a reduction in salt intake on BP (6,7). Thus, in people at age 50-59 years, a reduction in daily sodium intake of 50 mmol will, after a few weeks, lower SBP by an average of 5 mmHg, and by 7 mmHg in hypertensive subjects with a SBP of 170 mmHg (6). DBP will be lowered by about half as much.

High salt intake may theoretically induce hypertension through increases in cardiac output and effects on vascular reactivity and contractility (see Section 2.5). However, not all individuals respond to the same extent to alterations of sodium and extracellular fluid (ECF) volume status with corresponding increases or decreases in BP (8). In normotensive as well as hypertensive subjects, BP can be judged to be 'salt-sensitive' when observed to vary directly and substantially with the net intake of

sodium chloride (see Section 2.6.1). Subjects not showing such a phenomenon can be regarded as 'salt-resistant'.

Certain individuals are known to be more salt-sensitive (e.g. patients with hypertension and their first-degree relatives, blacks, older subjects, obese subjects, diabetic patients and patients with renal failure) (9). These individuals generally have a relative inability to handle a salt load efficiently, due to dysregulation of the natriuretic systems and/or antinatriuretic systems in the body (see Chapter 2).

1.2 The discovery of renal DA as an endogenous natriuretic factor

Dopamine (DA) was once regarded simply a metabolic precursor of noradrenaline (NA) and adrenaline. The role of DA as an intrarenal natriuretic, vasodilatory substance was not explored earlier because of the uncertainty of the origin of urinary DA and difficulties in measuring plasma DA (10).

Overall the past 30 years, the pioneer work of several investigators strongly suggests that renal DA is an important natriuretic factor in the body. [A detailed account is given in Chapter 3.] McDonald et al. (11) have shown that intravenous infusion of DA in pharmacological doses evokes a marked natriuresis and diuresis in humans. Alexander et al. (12) have shown that oral salt loading or intravenous infusion of 0.9% saline increases the urinary excretion of sodium and free DA in healthy subjects. Oates et al. (13) have shown that urinary free DA output increases after oral salt loading while plasma DA is unchanged. The calculated clearance values for DA far exceeds renal plasma flow. Both observations indicate that DA is formed inside the kidney. Using micropuncture techniques, Baines & Chan (14) have shown in rat kidney that L-dopa, the precursor of DA, is converted actively into

urinary free DA. Ball & Lee (15) have shown in healthy subjects receiving a high salt diet that inhibition of renal DA synthesis using carbidopa results in decreased urinary free DA excretion as well as attenuation of the natriuresis. The kidney has abundant DA receptors (16). Krishna et al. (17) have shown in healthy subjects that blockade of these receptors attenuates the natriuretic responses to ECF volume expansion by saline infusion. Aperia et al. (18) have shown that proximal tubular Na⁺-K⁺-ATPase, the enzyme responsible for sodium transport, is reversibly inhibited by DA and L-dopa.

1.3 Defects in renal DA production can cause hypertension

Lee (19) proposed in 1981 that the kidney 'fault' in essential hypertension may be a failure to mobilise adequately renal DA when dietary sodium intake is increased. His hypothesis was based on several observations.

Firstly, the Dahl salt-sensitive rat kidney does not seem to possess the same capacity to vasodilatation as Dahl salt-resistant rat kidney when the animal is given an oral salt load (20). Lee suggested that Dahl salt-sensitive rat might have a limited capacity to produce intrarenal vasodilatory and natriuretic substances such as DA.

Secondly, Luft et al. (21) studied the responses of normotensive blacks and whites to sodium intakes ranging from 10 to 1,500 mmol/day. With higher salt intakes, BP increases in both blacks and whites, but the increase is greater in blacks. Luft et al. (22) have also shown that blacks do not excrete an intravenous salt load as well as whites do, and their baseline plasma renin activity (PRA) is more suppressed. The concept of a genetic predisposition of salt sensitivity is supported by the observation that American blacks have a higher incidence of hypertension than

American whites, although both groups consume similar quantities of dietary salt (23).

Thirdly, many primitive communities have a daily sodium intake of less than 20 mmol. Therefore, when the ambient temperature is high, they are faced with the risk of salt depletion through sweating. It would be advantageous to be able to retain some of the sodium chloride. The evolutionary benefit of diminished salt excretion (24) from inability to mobilise renal DA is obvious (19). Two hundred years ago, the situation changed dramatically. Salt became readily available. The genes that previously protected against salt depletion are at a disadvantage with salt intake in excess of, say, 150 mmol/day. [Subsequent work by Lee, Critchley and co-workers, as detailed in Chapter 3, confirmed that sub-Sahara African populations do not show a positive correlation between the 24-hour urinary sodium and free DA output and their urinary free DA output does not rise in response to oral salt loading.]

Fourthly, the work by Lee and by others indicates that renal DA is an important natriuretic factor in the body (see Chapter 3).

Lee (19) has concluded that some individuals may be less efficient in excreting a salt load as a result of defective renal DA production. Such individuals may develop essential hypertension if presented with high salt loads by mouth during the critical inductive phase of childhood and adolescence. The kidney fault may be marked by a relatively low PRA and a relatively high plasma aldosterone level.

1.4 Other defects in the renal DA system can also cause hypertension

Apart from a reduction in renal DA production, defects in the transduction of renal tubular DA signal or renal vascular DA signal may lead to hypertension (25)

(see Section 4.1.3). This is not surprising since DA, via different DA receptor subtypes, regulates cardiovascular functions by its actions on the central and peripheral nervous systems, vascular smooth muscle, the heart and the kidney (see Section 3.1.2).

Jose et al. (26) described several human and animal models of hypertension in which there is a reduction in renal DA production and/or defects in coupling between DA₁ receptors and associated first messengers (see Section 4.1). They pointed out that this defect is: (a) genetic, since it precedes the onset of hypertension and co-segregates with the hypertensive genotype; (b) receptor-specific, since it is not shared by other humoral agents; (c) organ and nephron segment selective, since it occurs in proximal tubules but not in cortical collecting ducts or the brain.

1.5 Dietary changes and hypertensive diseases in Hong Kong Chinese

In Hong Kong, the local Chinese diet used to be low in fat and cholesterol and relatively high in carbohydrates and fibres (27). However, household surveys in the 1990s documented dietary shifts in the population from diet predominately of rice and small portions of meat, vegetables and fish to that with larger portions of all foods but rice and eggs (28). It is worth noting that the dietary sodium intake in the local population (29,30) is now approaching that of many western societies (31). For example, in my study of the relationship between sodium and potassium intakes and BP among healthy subjects mainly from the Shatin district in the late 1980s (29), the average sodium and potassium intakes were 145 and 40 mmol/day in men and 135 and 41 mmol/day in women. As part of her study of the metabolic syndrome in Chinese subjects, Lee (30) recruited healthy controls mainly from the Shatin district

in the late 1990s. The average sodium and potassium intakes then were 173 and 43 mmol/day in men and 162 and 42 mmol/day in women.

During 1988-1989, Woo et al. (31) studied the prevalence of hypertension among 1,513 Chinese employees of a public utility company and non-medical personnel of the Prince of Wales Hospital, the general teaching hospital for Shatin and the neighbouring districts. The prevalence of hypertension, defined as SBP \geq 140 or DBP \geq 90 mmHg, or a past history of hypertension, was 17% in men and 5% in women.

During 1995-1996, Janus et al. (32) studied the prevalence of cardiovascular risk factors and dietary intakes among 7,730 subjects aged 25-74 years randomly recruited from the whole of Hong Kong. Some of these subjects were selected for blood sampling and anthropometric measurements. About one in 10 men and one in nine women had 'definite hypertension' (SBP \geq 160 and/or DBP \geq 95 mmHg or on drug treatment for hypertension). About 34% of men and 29% of women with hypertension were untreated. About one in 12 men and 1 in 16 women were considered having 'borderline hypertension' (SBP 140-159 and/or DBP \geq 90-94 mmHg). About one in 100 men and one in 67 women had untreated isolated systolic hypertension (SBP \geq 160 and DBP $<$ 90 mmHg).

The work of my colleagues at the Chinese University of Hong Kong provides further evidence that hypertension is very common among Hong Kong Chinese. Kay et al. (33) reported that intracerebral haemorrhage accounts for 27% of all strokes in Shatin and the neighbouring districts. Intracerebral haemorrhage is seen between two to three times more frequently in Hong Kong Chinese than in westerners. Sanderson et al. (34) reported that hypertension is the most important identifiable risk factor (37%) in patients with congestive heart failure requiring admissions to the Prince of

Wales Hospital. Yip et al. (35) reported that diastolic heart failure is more common than systolic heart failure in Chinese patients with congestive heart failure requiring hospitalisation. They attributed their findings to older age at presentation and the high prevalence of hypertension in this community.

1.6 Do Chinese have an efficient renal DA system?

With the westernisation of their diets, Chinese in Hong Kong have a higher salt intake than before. Because of this and other undetermined factors, hypertension and hypertensive diseases are (increasingly) common (Section 1.5).

Therefore, several questions need to be addressed. Are Chinese particularly salt-sensitive with a significant rise in BP after an increase in salt intake? Do Chinese have a renal DA mechanism that will enable them to handle a salt load efficiently? If not, do they have other mechanisms? The first-degree relatives of hypertensive patients are known to be more prone to hypertension. Will they respond to a salt load differently compared to the offspring of normotensive subjects? It is with these questions in mind that my M.D. studies have been planned and performed (see Chapters 5 to 10).

1.7 The layout of this thesis

In Chapter 2, I have described the natriuretic and antinatriuretic systems in the body and the pathophysiological basis for salt-induced hypertension. This will allow a better appreciation of the relative importance of the renal DA system in ECF volume, sodium and BP homeostasis. In Chapter 3, I have summarised the evidence

supporting the role of DA as an intrarenal natriuretic substance. In Chapter 4, I have discussed how the dysregulation of the renal DA system may contribute to sodium and fluid retention and salt sensitivity in some common conditions such as hypertension and diabetes mellitus. With such background information, it would then be possible to compare and contrast my findings in Hong Kong Chinese with those from previous studies.

From Chapter 5 to Chapter 10, I have described the background (see Section 1.6) and objectives of my studies and discussed the main findings. I have focused on the renal DA system. With further developments of our assays that would allow the simultaneous measurements of urinary free NA and kallikrein, the role of these neurohormones in sodium and BP homeostasis have also been examined.

In Chapter 11, I have summarised my findings in Chinese and discussed the significance of these findings.

All the data in this thesis are presented as mean \pm SEM unless stated otherwise.

Chapter 2 Natriuretic and antinatriuretic systems and pathophysiological basis for salt-induced hypertension

Sodium chloride is the major ionic component that determines plasma and extracellular osmolality, and as such it exerts direct control on the ECF volume and plasma volume. The ECF represents about 20% of the body weight (36). Its two major compartments are the plasma (5% of body weight) and the interstitial fluids (15%).

2.1 Integrated responses of the kidney to changes in ECF volume

A comprehensive account of this subject has been described by Gonzalez-Campoy & Knox (37). In brief, sodium loading or depletion produces corresponding changes in the ECF volume and plasma volume. Changes in the ECF volume, plasma volume and parallel, concomitant changes in circulatory filling pressures are sensed by mechanoreceptors and baroreceptors distributed throughout the thoracic and abdominal visceral organs. The body mounts an integrated response aiming at counteracting any such changes, thus maintaining its internal environment constant. The kidneys are the ultimate effectors of the body's response to changes in ECF volume (Figure 2a). Thus, the ECF volume is tightly maintained by the balance of several haemodynamic (Section 2.2), natriuretic (Section 2.3) and antinatriuretic (Section 2.4) mechanisms not only within the kidney but in the body as a whole.

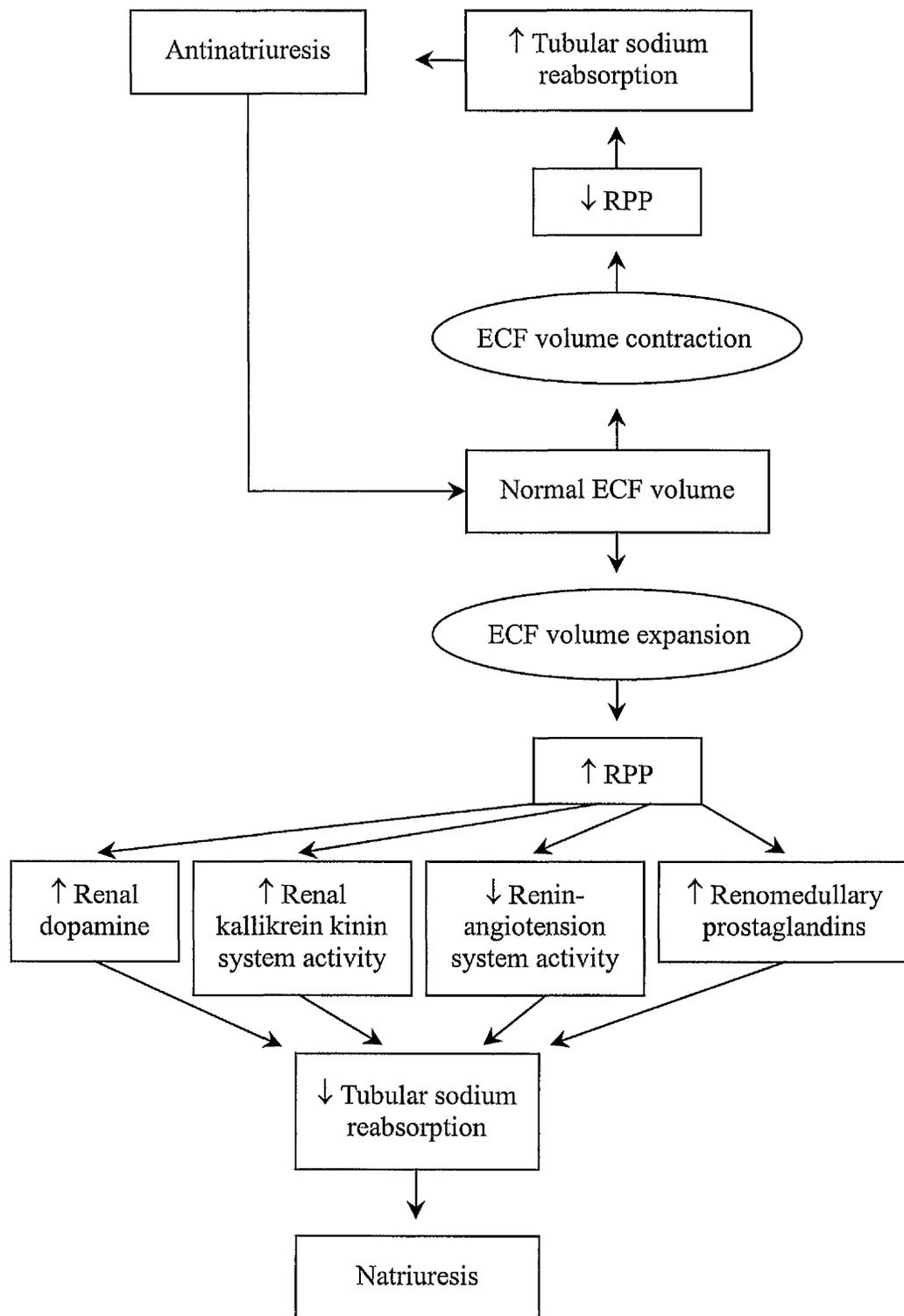


Figure 2a. Mechanisms whereby renal perfusion pressure (RPP) and intrarenal hormone systems regulate the ECF volume.
Adapted from Gonzalez-Campoy & Knox (37).

2.2 Arterial pressure-natriuresis

A central component of the feedback system for control of ECF volume and arterial pressure is the pressure-natriuresis mechanism, whereby acute changes in renal arterial pressure induce alterations in sodium excretion and urine flow (38). These changes occur in the absence of concomitant changes in whole-kidney blood flow or glomerular filtration rate (GFR). An alteration in renal medullary haemodynamics and/or changes in renal interstitial hydrostatic pressure during changes in renal arterial pressure may be responsible for this phenomenon (39).

Recent studies indicate that endothelium-derived nitric oxide (NO) (see Section 2.3.5) is by far the most important intrarenal mediator of pressure-natriuresis (40,41). Increases in renal arterial pressure increase NO formation, which inhibits sodium reabsorption via direct effects on the tubules as well as haemodynamically mediated effects (see Section 2.3.5). The pressure-natriuresis mechanism may also interact with a variety of endocrine, paracrine and neural factors, not only within the kidney but also in the body as a whole.

2.3 The natriuretic systems

By modulating the renal and systemic vascular resistance and acting on the renal tubules, circulating or locally produced vasoactive neurohormonal factors may regulate the renal excretion of sodium and water, the ECF volume and BP. Those with natriuretic properties are discussed in this section.

2.3.1 *The natriuretic peptides*

The natriuretic peptide family consists of structurally homologous peptides with natriuretic-diuretic and vasorelaxant properties: atrial natriuretic peptide (ANP), brain natriuretic peptide (BNP), C-type natriuretic peptide (CNP) and urodilatin (ularitide) (42,43). Three natriuretic peptide receptors (NPR) have been cloned (44). ANP has the highest affinity for NPR-A, followed by BNP and CNP. The order is reversed for NPR-B (CNP, BNP, ANP). NPR-C is the most prevalent of the three receptors and is regarded by some (45) as a storage-clearance binding site, regulating plasma natriuretic peptide concentrations. BNP has a much lower affinity than ANP for NPR-C, explaining the longer half-life of BNP. All three peptides exert actions via cyclic guanosine monophosphate (cGMP).

ANP, BNP and CNP are also degraded by neural endopeptidase, which has a wide tissue distribution, including the lung and the kidney. It has a greater affinity for CNP than for ANP and the affinity for BNP is much lower than those for the other two peptides (46).

Atrial natriuretic peptide

The cardiac atria are the major source of ANP. Atrial stretch is the primary stimulus for ANP secretion. ANP affects the kidney through NPR-A receptors. Increased GFR together with direct and indirect effects on the renal tubules are responsible for natriuresis and diuresis. The effect of ANP on GFR is accompanied by an increase in glomerular capillary hydraulic pressure, which is associated with a decrease in afferent arteriolar resistance and an increase in efferent arteriolar resistance. ANP also indirectly influences the kidney function and renal tubular

sodium and water handling by decreasing BP and suppressing the renin-angiotensin system (RAS), sympathetic nervous system (SNS) activity and vasopressin release.

Contraction of the plasma volume following salt restriction (47) or blood donation (48) decreases intra-atrial pressure and atrial stretch, reducing cardiac secretion and plasma ANP levels. Conversely, expansion of the ECF volume following oral salt loading (49,50) or intravenous saline infusion (48,51) increases atrial stretch and a rise in plasma ANP levels, producing marked natriuresis and diuresis. Infusion of ANP to healthy subjects in a dose to achieve physiological plasma levels results in natriuresis, decreased PRA and plasma aldosterone concentrations and a fall in BP (52).

Brain natriuretic peptide

BNP is synthesised predominantly in the ventricle. The release of BNP may involve a stretch-pressure mechanism and may be co-secreted with ANP. Like ANP, BNP is natriuretic in humans at physiological levels (53). Plasma BNP and ANP concentrations increase with oral sodium loading in healthy subjects (50,54). Plasma BNP levels fall during fluid removal by haemodialysis or ultrafiltration in patients with chronic renal failure and fluid overload (55,56). Acute intravenous saline infusion causes an increase in plasma ANP concentrations with no detectable increase in plasma BNP (50,54), suggesting a different regulation of BNP and ANP in normal subjects. The inhibitory effect of BNP on the RAS also contributes to its natriuretic effect.

Plasma ANP and BNP levels are markedly elevated in conditions associated with: (a) intravascular volume overload; (b) increased central venous pressure; (c) tachycardia; (d) reduced renal function (57). The levels are higher in hypertensive

patients, particularly in those with left ventricular hypertrophy (58). Plasma ANP and BNP increase with the severity of congestive heart failure (59). Plasma BNP measurement is better than ANP for the diagnosis of left ventricular systolic (60) or diastolic (61) dysfunction.

C-type natriuretic peptide

CNP is regarded as the endothelial component of the natriuretic peptide system (62). It is produced by vascular endothelium and may regulate vascular tone and growth in an autocrine/paracrine manner. It is also found in human kidney, mainly in the proximal tubules. Together with ANP and BNP, CNP may form a renal natriuretic peptide system that participates in the local regulation of sodium and water transport and the renal circulation. CNP is found in plasma of normal subjects at much lower concentrations than ANP and BNP.

Plasma CNP levels are increased in patients with chronic renal failure (63) and cor pulmonale (64), raising the possibility that CNP may be a circulating counter-regulatory hormone in conditions with overactive RAS. CNP has systemic cardiovascular actions, which include reductions in cardiac filling pressures and output, secondary to vasorelaxation and decreases in venous return, but has minimal renal actions (65). It is also a potent coronary vasodilator.

Urodilatin (ularitide)

Urodilatin is synthesised in the kidney and is detected only in the urine (66). Urodilatin exerts its renal effects in a paracrine manner. After its secretion from the distal tubular cells, it acts on the lumenally located NPR-A in the collecting duct. cGMP generation is followed by an interaction with apical amiloride-sensitive

sodium channels (which mediate sodium entry across the apical membrane), resulting in natriuresis and diuresis. Urodilatin is more potent than ANP as a natriuretic and diuretic agent when given intravenously.

In healthy subjects given a high salt meal, urinary urodilatin excretion, but not plasma ANP, parallels the postprandial natriuresis (67). The natriuretic and diuretic responses to urodilatin infusions are enhanced during high salt intake (68). Other actions which may contribute to urodilatin's diuretic and natriuretic properties include suppression of the RAS (69).

Urinary urodilatin excretion increases with the severity of congestive heart failure, suggesting that renal urodilatin synthesis may be stimulated to counteract sodium and water retention (70).

Urodilatin has been used prophylactically against acute renal failure related to major surgery with some encouraging results (71). Urodilatin improves sodium and urine output in cirrhotic patients with or without ascites by enhancing fluid delivery from the proximal tubules in addition to inhibiting fractional sodium reabsorption in the distal nephron (72). It may also be effective in patients with the hepatorenal syndrome (71). However, urodilatin fails to improve renal function in patients with established acute renal failure (73).

2.3.2 *Endogenous inhibitors of Na⁺,K⁺-ATPase*

De Wardener et al. (74) first suggested in 1961 that sodium homeostasis is, in part, attributable to a hormone, which can increase natriuresis by reducing sodium reabsorption in the nephron. There followed an extensive literature demonstrating that ECF volume expansion is associated with a substance in the plasma or urine which decreases the activity of the sodium transport enzyme Na⁺,K⁺-ATPase, and the

natriuresis of ECF volume expansion is the result of tubular rejection of sodium through inhibition of this sodium pump (75).

Endogenous inhibitors of Na^+, K^+ -ATPase that can be found in the circulation include: certain fatty acids such as linoleic acid and oleic acid, ouabain, digoxin and bufodienolides (76). There is evidence that such inhibitors may be present in the brain and may have a local action.

The hypothalamus may be the source of a sodium pump inhibitor (76). It is released in response to ECF volume expansion during salt loading. Once in the circulation, it inhibits the Na^+, K^+ -ATPase in the renal tubules and increases the renal excretion of sodium. Salt restriction and contraction of plasma volume appear to diminish the circulating levels of this hormone, leading to renal retention of sodium.

In healthy subjects given a single-dose intravenous ouabain, no changes in renal blood flow, GFR, urinary excretion of sodium, water and potassium are seen (77). If ouabain has an important influence on electrolyte and BP homeostasis, its action are likely to have a slow time course (77).

McDougall & Yates (78) have recently reviewed the natriuretic actions of ouabain. The natriuretic effect of renal arterial infusion of ouabain is relatively slow in onset and is sustained. The natriuretic effect of ouabain is markedly enhanced by acute ECF volume expansion or chronic mineralocorticoid treatment, both of which result in hypervolaemia and hypertension. The natriuretic response to small increments in BP is markedly enhanced by treatment with ouabain.

Increased concentrations of ouabain-like factor are observed in patients with asymptomatic left ventricular dysfunction due to dilated cardiomyopathy, suggesting that it may be an early marker of the disease (79).

2.3.3 *Renal kallikrein-kinin system*

The main components of the renal kallikrein-kinin system are the enzyme kallikrein, the substrate kininogen, various effector hormones or kinins, metabolising enzymes (several kininases, the most relevant being kinases I and II and neutral endopeptidase) and an unknown number of activators and inhibitors of kallikrein and kinases (80). In the kidney, tissue kallikrein has been localised to the tubular cells of the distal nephron. Kininogen has also been localised to the distal nephron in man, suggesting an intrinsic renal kallikrein-kinin system, which may inhibit sodium and water reabsorption and promote renal vasodilatation (81). The renal kallikrein-kinin system may also interact with other natriuretic (e.g. renal DA and ANP) and antinatriuretic systems (e.g. RAS).

In healthy subjects, the fractional excretion of sodium and inorganic phosphorus, which reflects the total and proximal sodium reabsorption, shows significantly positive correlations for both urinary kallikrein and kinin excretion (82). In normal subjects but not in patients with hypertension, the activity of the renal kallikrein-kinin system is augmented following the intravenous infusion of 0.9% saline, as evidenced by the increases in the plasma concentrations of prekallikrein as well as urinary kallikrein and kinin excretion and a decrease in urinary kinase excretion (82,83).

Epidemiological studies have shown an inverse relationship between BP and urinary kallikrein excretion (84). A large family pedigree study has shown that a dominant allele expressed as high urinary kallikrein excretion may be associated with a decreased risk of essential hypertension (85).

Song et al. (86) investigated the determinants of urinary kallikrein excretion in 204 normotensive subjects (119 Caucasians, 33 African-Americans, 52 Asians;

109 men, 95 women) living in the U.S. Urinary kallikrein excretion in African-Americans was approximately 50% lower than Caucasians or Asians. In all ethnic groups, women excreted 50% more kallikrein than men. Those with a family history of hypertension were over-represented in the lower stratum of a bimodal distribution of kallikrein excretion. Potassium excretion was diminished in Africans, and in a multivariate analysis, potassium excretion was the strongest correlate of kallikrein excretion.

Urinary excretion of kallikrein is significantly lower in salt-sensitive than in salt-resistant hypertensive patients (87). Oral administration of glandular kallikrein (derived from porcine pancreas) reduces BP in salt-sensitive hypertensive patients and increases urinary sodium excretion in both salt-sensitive and salt-resistant hypertensive patients (88).

Urinary kallikrein excretion is lower in women with preeclampsia and mild to moderate hypertensive pregnant women compared with normotensive pregnant women (89).

Insulin-dependent diabetes mellitus without microalbuminuria is associated with decreased basal and frusemide-stimulated kallikrein excretion, which is directly related to blood glucose level (90). The decreased activity of renal kallikrein-kinin system might be involved in the increased tendency to sodium retention in diabetic patients.

Reduced urinary or renal kallikrein levels have been observed in genetically hypertensive rats (91). In a rat strain inbred for low urinary kallikrein excretion, BP is higher than controls (92). BP is increased after dietary salt loading in the low-kallikrein group but remains unchanged in controls. In rat, monoclonal antibodies to rat urinary kallikrein effectively blocks the generation of kinins in the nephron

lumen, decreasing urinary kallikrein and kinin excretion by 74-85% and 76-79%, and inducing a 30% decrease in urine volume and a 20-40% decrease in urinary sodium excretion (93). Somatic gene delivery of human tissue kallikrein lowers the BP in spontaneously hypertensive rats (SHR) (94).

2.3.4 *Prostaglandins*

All the primary prostaglandins (PG) can be synthesised in the renal cortex (95). They act predominantly at their site of production.

Prostaglandins exert direct or indirect effects on the nephron throughout its entire length. Two major roles of prostaglandins in blood volume homeostasis have been extensively studied. The first is the protective effects of prostaglandins as vasodilators in the glomerular microcirculation during states of sodium depletion (96). The second role involves their effects on urinary sodium and water excretion, mediated either indirectly through vasodilatation or directly through tubular epithelial transport actions (97).

Studies on isolated renal microvessels have shown that both PGE₂ and PGI₂ attenuate angiotensin II (AII)-induced arteriolar vasoconstriction and PGI₂ antagonises AII-induced efferent arteriolar vasoconstriction (98). PGE₂ has been shown to counteract AII-induced contraction of isolated glomeruli and glomerular mesangial cells in culture (96). Conversely, cyclo-oxygenase inhibition augments these contractile responses. A similar counter-regulatory role of prostaglandins with respect to renal nerve stimulation has been shown in micropuncture studies (99) and an interaction between vasopressin and prostaglandins was shown in studies using the isolated blood-perfused kidney (100).

Intrarenal infusions of PGE₂, prostacyclin, PGD₂, and, less potently, PGF_{2α} increase the rate of sodium and water excretion (96,97).

To examine the mechanism of prostaglandin-induced natriuresis, two other experimental approaches have been tried. The effect of prostaglandin synthesis inhibition using indomethacin was studied in rats receiving a normal or high salt diet (101). Indomethacin administration increased fractional sodium reabsorption and the activity of the renal medullary Na⁺,K⁺-ATPase. Addition of exogenous PGE₂ to microsomal fractions from kidneys of normal rats was associated with suppression of the medullary Na⁺,K⁺-ATPase (101). These results suggest that the natriuresis associated with prostaglandin administration results from a direct inhibition of renal Na⁺,K⁺-ATPase.

In healthy subjects, the natriuretic response to intravenous saline infusion is accompanied by increased urinary PGE excretion (102). However, indomethacin administration does not alter the urinary natriuretic response to intravenous saline infusion (103).

It has been pointed out that only in situations in which endogenous prostaglandin production is enhanced does prostaglandin activity become relevant to total sodium balance. Under these circumstances, inhibition of cyclo-oxygenase will result in retention of sodium and water.

Hence, the role of prostaglandins may be to maintain the internal milieu of the renal tissues during extreme changes in the environment. They can dampen the effects of other neurohormones (e.g. AII) to protect the specific nephron segment, which otherwise suffer injury (104). This may be the reason why the effects of the inhibition of prostaglandin synthesis vary considerably depending on the overall physiological state of the subject (105).

2.3.5 Nitric oxide

Nitric oxide (NO) is synthesised from L-arginine by the enzymatic action of nitric oxide synthase (NOS). Three isoforms of NOS have been identified: brain bNOS, vascular endothelial eNOS and macrophage inducible iNOS. The vascular tone is partly controlled by NO generated from eNOS, activated by physical sheer stress (106). bNOS is more abundant and widely distributed than eNOS (107). The production of NO is determined by the type and quantity of NOS present and by availability of co-factors and substrates (106).

In the kidney, bNOS is found in the glomeruli and vasculature as well as the macula densa, collecting duct and inner medullary thin limb (108). eNOS is found in the arcuate and interlobular arteries, afferent arterioles and the glomerulus (109). Presumably, eNOS is present throughout the vascular endothelium of the renal circulation.

NO produced within the kidney plays a crucial role in sodium homeostasis and BP regulation by controlling the tone of glomerular arterioles and mesangium, pressure-natriuresis (Section 2.2), tubuloglomerular feedback, medullary circulation, renin release, and tubular function (110). In addition, NO may interact with other vasoactive and natriuretic/antinatriuretic systems.

In experimental animals, infusion of L-arginine increases renal blood flow and urinary excretions of sodium and water (111). Inhibition of NOS reduces renal blood flow by 25% and markedly reduces sodium excretion (112). The inhibition of NO synthesis markedly suppresses the slope of the arterial pressure-mediated response in sodium excretion. In support of the hypothesis that NO is involved in the mediation of the arterial pressure-natriuresis (Section 2.2), a direct relationship

between changes in arterial pressure and the rates of urinary excretion of sodium as well as nitrate and nitrite (a marker for endogenous NO activity) is seen.

In humans, infusion of L-arginine has been shown to increase the excretion of NO and its second messenger, cGMP, but the effect of this on sodium excretion seems to depend upon the salt intake status (113). Thus, L-arginine enhances sodium reabsorption when subjects have a low salt intake, but inhibits it when salt intake is high.

NO-induced natriuresis occurs independently of changes in renal perfusion pressure, indicating a tubular effect of NO (114). In support of this hypothesis, *in-vitro* studies revealed that NO inhibits both $\text{Na}^+\text{-K}^+\text{-ATPase}$ and $\text{Na}^+\text{-H}^+\text{-exchange}$ activity in the proximal tubule. In the collecting duct, NO has been shown to decrease sodium flux with no effect on $\text{Na}^+\text{-K}^+\text{-ATPase}$ activity.

On the other hand, inhibition of systemic NO synthesis by nitro L-arginine methylester (L-NAME) results in antinatriuresis and antidiuresis (115). These renal effects can be attributed to increases in sodium reabsorption both at proximal and distal nephron sites and decreases in renal diluting capacity. The direct association between the individual sodium-sensitivity and the pressor response to a NO synthase inhibitor, N^G -monomethyl-L-arginine (L-NMMA) suggests that there is increased dependence of vascular tone on NO in normotensive subjects whose BP is more salt-sensitive (116).

In healthy subjects, hyperinsulinaemia is associated with a decrease in renal generation of NO (117). In contrast, mild ECF volume expansion increases urinary excretion of NO metabolites. This insulin-induced attenuation of renal NO synthesis may contribute to anti-natriuretic actions of insulin.

In rats, it is possible to produce systemic hypertension by chronic blockade of NO synthesis using L-NAME. The hypertension is associated with marked renal vasoconstriction involving both preglomerular and efferent resistance vessels, as well as a fall in the glomerular capillary ultrafiltration coefficient (118). Glomerular BP appears to be elevated with moderate proteinuria.

Some animal models of genetic hypertension and certain forms of human hypertension are associated with a decrease in NO synthesis (119). Prolonged reduction of BP is seen in SHR after human eNOS gene therapy (120).

2.3.6 *Adrenomedullin and related peptides*

Adrenomedullin (ADM) shows structural homology with calcitonin-gene-related peptide (CGRP) and amylin (121). The ADM gene encodes ADM and the related proadrenomedullin N-terminal 20-peptide (PAMP) and the two may act synergistically in ECF volume and BP homeostasis.

Adrenomedullin

Immunoreactive ADM has been found in multiple human tissues, including the adrenal medulla, heart, aorta, brain, lung, kidney, and gastrointestinal organs (122). Both vascular endothelial cells and smooth muscle cells synthesise ADM (123,124). ADM is detected in human urine, and urinary ADM levels are much higher than plasma levels, suggesting that the kidney may be one of the major tissues for ADM production (125). ADM acts on target cells through its unique receptors and CGRP₁ receptors, with cyclic adenosine monophosphate (cAMP) being the main second messenger (126).

ADM is a potent and long-lasting vasodilator of the resistance and capacitance vessels in human forearm (127). Its vasodilator effects are mediated through a direct action on ADM receptors in vascular smooth muscle cells and an action on the endothelial cells to stimulate NO release (128). In experimental animals, ADM has renal vasodilator, natriuretic and diuretic effects (129).

Samson (130) has recently reviewed the role of ADM in fluid and electrolyte homeostasis. ADM stimulates NO production by endothelial cells, whereas PAMP acts presynaptically to inhibit adrenergic nerves that innervate blood vessels. Both peptides inhibit adrenocorticotropin release from the anterior pituitary gland and AII-stimulated aldosterone secretion. In the brain, ADM inhibits water intake and salt appetite. The natriuretic and diuretic effects of ADM reflect its unique actions on renal blood flow and tubular function.

Compared with normal subjects, plasma ADM levels are 26% higher in patients with hypertension and 45% higher in hypertensives with renal failure (131). In the latter patients, plasma ADM is directly proportional to the creatinine levels, suggesting that the kidney has a major role in the clearance of ADM. In patients with renal failure, the fall in plasma ADM after haemodialysis indicates that its plasma concentration is influenced by the ECF volume status (132). In patients with hypertension, left ventricular hypertrophy is associated with higher plasma ADM (133). In congestive heart failure, plasma ADM increases progressively with its severity (134). Plasma ADM is increased in the early phase of acute myocardial infarction and is even higher in those complicated by congestive heart failure (135). Elevated plasma levels of adrenomedullin are an independent predictor of prognosis in predominantly mild to moderate heart failure treated by conventional therapy

(136). Plasma ADM is also raised in patients with liver cirrhosis and chronic obstructive pulmonary disease (137).

In Dahl salt-sensitive rats, human adrenomedullin gene delivery lowers the BP and protects against salt-induced hypertension, cardiac hypertrophy, fibrosis and renal damage (138).

Calcitonin gene-related peptide

CGRP is synthesised and released from small, capsaicin-sensitive sensory nerves (139). This extensive network of sensory nerves is found in virtually all organs. CGRP has been shown to elicit potent vasodilation in the cerebral, coronary and peripheral vasculature.

In rat, intravenous infusion of CGRP results in a fall in BP with a marked increase in renal plasma flow and GFR (140). In healthy subjects, intravenous administration of CGRP lowers BP and increases the renal excretions of sodium and water (141,142).

In patients with severe hypertension, plasma CGRP-like immunoreactivity is lower than that of healthy subjects, even after treatment (143). In patients with moderate to severe pregnancy-induced hypertension, plasma CGRP levels are lower than those in healthy pregnant women (144). These findings suggest an involvement of the vascular afferent nerves in the aetiology of hypertension.

2.3.7 Vasoactive intestinal peptide

Vasoactive intestinal peptide (VIP) is found in the central and peripheral nervous systems and in some endocrine tissues, where it has both neurotransmitter and hormonal roles (145).

In healthy subjects, intravenous infusion of VIP causes a fall in pulmonary, systemic and coronary vascular resistance and BP, a reflex sympatho-adrenal stimulation with increased heart rate and myocardial contractility and a direct positive inotropic effect (146,147).

In-vitro studies using human saphenous vein indicated that VIP can produce endothelium or NO-dependent dilatation of the capacitance vessels (148).

The renal effects of VIP depend on the doses used. In rats given lower doses, VIP only induces a small increase in fractional and absolute excretion of sodium (149). With higher doses, significant falls occur in mean arterial pressure (MAP) and GFR and absolute sodium excretion tends to fall.

2.3.8 *Substance P*

Substance P (SP), which is a member of the tachykinin family of peptides, is found mainly in the central nervous system and peripheral afferent nerve fibres. It acts through stimulation of the neurokinin receptors, having a particularly high affinity for the neurokinin-1 receptors (150).

In dogs, intravenous SP infusion produces a fall MAP and systemic vascular resistance (140). In regions with high SP concentrations such as the muscular layers of the fundus and ileum, regional blood flow increases. SP and CGRP coexist in central and peripheral nerve endings of sensory neurons. In the pithed rats, CGRP is 10 times more potent than SP in producing vasodilatation, but CGRP has less than a third of the potency of SP in producing plasma extravasation (152).

In healthy subjects, SP infusion with plasma levels above the normal range produces lacrimation and sustained flushing, without significant changes in the pulse rate and BP (152). Brachial artery infusion of SP induces a dose-dependent

vasodilatation in the forearm (154). Skin oedema in the infused forearm and systemic vasodilatation occur at higher doses. Vasodilatation due to CGRP is prolonged, with a half-life of biological effects of 18 minutes (155). Vasodilatation due to SP has a half-life of 15 seconds only, due to the rapid development of tachyphylaxis. Simultaneous arterial infusion of both peptides produces additive, and possibly synergistic, effects on forearm blood flow. The vasodilator actions of SP are mediated in part via NO (156).

In rats, infusion of SP into the abdominal aorta above the renal arteries produces a natriuresis and diuresis (157). Intravenous SP infusion increases the urinary flow rate, clearance of water and urinary excretion of sodium, potassium and phosphate (158). In dogs, renal arterial infusion of SP increases renal blood flow and urinary excretion of water, sodium and potassium, with a fall in MAP (159). However, with higher (hypotensive) doses, progressive less diuresis, natriuresis and kaliuresis are seen. Since the fractional excretion of sodium correlates with urinary free DA excretion, the renal effects of SP may be mediated by increases in renal DA activities (160).

In rabbit proximal renal tubules perfused *in-vitro*, SP produces a 50% reduction in the rate of fluid absorption (161). The sensitivity of proximal tubules to SP and the existence of SP-containing afferent fibres within the renal cortex suggest a role for SP in the control of proximal tubule reabsorption.

2.4 Antinatriuretic systems

Circulating or locally produced neurohormonal factors with antinatriuretic, vasoconstrictive actions are discussed in the section.

2.4.1 Renin-angiotensin system

The RAS is a circulating neuroendocrine system (Figure 2b) in which the components produce the peptide hormone AII in the circulation, which then acts on such target organs as blood vessels, adrenal glands, and kidneys to maintain BP, ECF volume and sodium homeostasis (162,163).

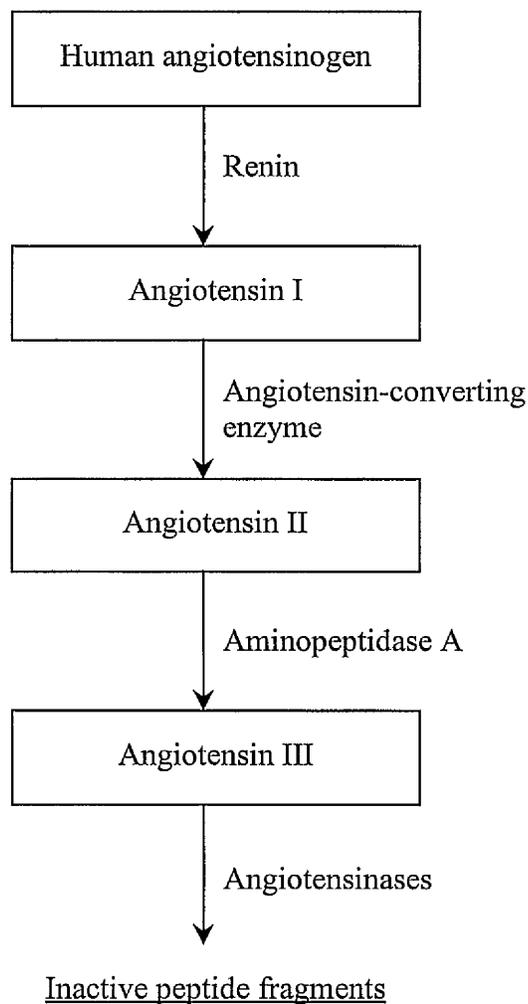


Figure 2b. The renin-angiotensin system.
Adapted from Gunning et al. (163).

There are also complete RAS within a variety of tissues and organs serving paracrine, autocrine, and intracrine functions. Tissue RAS exerts long-term effects on the function and structure of the cardiovascular, renal and other systems.

The liver is the primary site of angiotensinogen protein and messenger RNA (mRNA) synthesis. Angiotensinogen mRNA is widely distributed. Circulating renin seems to be derived from the juxtaglomerular apparatus in the kidney (Table 2a).

Table 2a. Regulators of renin release from juxtaglomerular cells.

Mechanism	Comments
<i>Macula densa mechanism</i>	
Macula densa cells in the distal tubule which sense urinary NaCl concentrations	<p>↓ salt intake → ↓ distal tubule delivery of sodium → ↑ renin release</p> <p>High/medium sodium concentration at macula densa → ↓ renin release</p> <p>Low sodium concentration at macula densa → ↑ renin release</p>
<i>Baroreceptor mechanism</i>	
Juxtaglomerular cells act as their own baroreceptor	<p>↑ renal perfusion → ↑ stretch of juxtaglomerular cells → ↑ renin release</p>
<i>Neural control</i>	
Sympathetic nerves predominantly innervate juxtaglomerular cells	<p>Renal nerve stimulation → ↑ renin release via β-adrenoreceptor</p> <p>α-adrenergic receptor stimulation → ↓ renin release</p>
<i>Endocrine/paracrine</i>	
↑ renin release	PGE ₂ , PGI ₂ , DA
↓ renin release	Angiotensin II (as negative feedback), endothelin, vasopressin (V ₁ receptor), adenosine (A ₁ receptor), ANP, NO

Adapted from Gunning et al. (163).

Renin cleaves off angiotensin I (AI) from angiotensinogen. An angiotensin-converting enzyme (ACE) splits AI into two fragments of which the larger represents the final hormone AII.

ACE (kinase II) exists as two isozymes. Somatic (endothelial) ACE is found in vascular endothelial cells, as well as in the renal proximal tubules. It plays a role in both the RAS (by converting AI to AII) and kallikrein-kinin system (by inactivating bradykinin). Circulating ACE is of endothelial cell origin. Pulmonary endothelium and peripheral tissues (especially the luminal surface of blood vessel walls) are the principal site of AI conversion to AII.

AII receptors are broadly divided into AT_1 and AT_2 (164). All the well known physiological and pathophysiological effects of AII have been attributed to AT_1 . In the kidney, AT_1 receptors are located on endothelial, epithelial and vascular smooth muscle cells. AT_2 has recently been shown to mediate antiproliferation and differentiation at least in some tissues and cells.

Through its direct action on smooth muscle cells, AII increases arteriolar resistance in renal, mesenteric, dermal, coronary, and cerebral vessels (165). AII exerts indirect pressor effects via the central and peripheral nervous systems. Its effects on the central nervous system include increased sympathetic discharge and decreased vagal tone (166). AII increases the vasoconstrictive response to renal nerve stimulation in dogs (167) and its inhibition attenuates the pressor response to NA in humans (168). The tissue RAS in the vasculature also contributes to the regulation of vascular tone.

AII stimulates aldosterone synthesis and secretion by zona glomerulosa cells of the adrenal cortex (169). In the cortical and medullary collecting duct and distal

tubule, aldosterone increases net sodium reabsorption and diminishes sodium leak-back.

In conditions of decreased renal blood flow, GFR is preserved over a wide range of perfusion pressures. Such autoregulation of GFR is dependent on AII, which preferentially vasoconstricts efferent arterioles while leaving afferent arteriolar resistance unaltered (170).

AII stimulates proximal tubule sodium chloride and sodium bicarbonate absorption (171). AII enhances proximal tubule sodium chloride transport indirectly by increasing presynaptic catecholamine release (172). Proximal sodium chloride and sodium bicarbonate absorption is in part mediated by AII-induced decrease in peritubular capillary hydrostatic pressure and an increase in peritubular oncotic pressure. The effects of AII on distal tubule transport of sodium and potassium are mediated through aldosterone release (169).

In healthy subjects, the natriuretic response to oral or intravenous salt loading is mediated partly by the suppression of the RAS, as reflected by a fall in PRA and plasma aldosterone concentrations (50). Thus, the natriuretic response to a saline infusion is greatly attenuated by the simultaneous infusion of aldosterone or AII to maintain plasma aldosterone or AII levels around basal values (173). Conversely, activation of the RAS contributes to the initial decline in renal sodium excretion seen after a sudden reduction in dietary sodium intake (174).

In experimental animals, intrarenal arterial infusion of ACE inhibitors or AII antagonists increases renal blood flow and natriuresis (175). In both healthy subjects and patients with borderline hypertension, low doses ACE inhibitors enhance the natriuretic response to intravenous saline infusion (176,177).

2.4.2 *Sympathetic nervous system*

By controlling cardiac output and the peripheral resistance, the SNS plays a dominant role in regulating the moment to moment cardiovascular homeostasis (178). Baroreceptors are located in the walls of the major arteries, including the carotid arteries and the aorta. When these stress receptors are stretched by high pressure, signals are transmitted to the brain stem, where they inhibit the sympathetic impulses to the heart and the vessels. This will allow the arterial pressure to fall towards normal. Conversely, when BP falls, decreased afferent impulses will diminish central inhibition, resulting in increased sympathetic outflow.

SNS-renal interactions

Stella & Zanchetti (179) and DiBona (180) have reviewed the ways in which the SNS can interact with the kidney. Like the heart, arteries and veins, the kidney is an important target organ for SNS activity. The kidney can influence not only the peripheral resistance by releasing vasoactive substances but also the body sodium and fluid composition by modulating sodium and water excretion. The main link between the SNS and the kidney is represented by the renal nerves, since changes in efferent renal nerve activity can elicit changes in renal functions, while afferent renal nerve activity driven by renal sensory receptors can interfere with the central and peripheral nervous system. Additional links are represented by circulating catecholamines acting on kidney function and by the actions of AII on the SNS.

The hallmark of kidney circulation is autoregulation, by which blood flow is maintained constant despite changes in arterial perfusion pressure. The main extrinsic factor is the SNS, which can also influence the intrarenal distribution of blood flow (179).

Renal SNS activity may alter urinary sodium and water excretions by at least two specific mechanisms: neural control of renin release from the juxtaglomerular cells (Table 2a) and renal excretory functions. Renal nerves control renin release by a direct action on the juxtaglomerular cells; this action is mediated via β_1 -adrenergic receptors (181). Circulating AII and aldosterone are then increased and renal sodium excretion is reduced. α -adrenergic receptors located on the juxtaglomerular cells exert an inhibitory action on renin secretion (182). The stimulation of selected areas of the hypothalamus can produce either a decrease or increase in renin release (183), suggesting that renin secretion can be influenced by emotional stimuli and the central nervous system can influence renin release by modulating the efferent sympathetic discharge to the kidney.

Using a micropuncture technique, Bencsath et al. (184) first established that stimulation of renal nerves favours tubular reabsorption of sodium and water. This was later confirmed by Moss (185), who used the renal denervation technique. The antinatriuretic and antidiuretic effects of sympathetic nerve stimulation are caused by increased sodium and water reabsorption across the proximal tubule and seem to be unrelated to the systemic or intrarenal haemodynamic changes (186). These effects also extend to the thick ascending limb of the loop of Henle. The increase in sodium transport associated with renal nerve stimulation in the proximal tubule and in the loop of Henle is entirely mediated by α_1 -adrenergic receptors and by activation of renal tubular Na^+, K^+ -ATPase (Section 3.3.1) and $\text{Na}^+ - \text{H}^+$ exchange (187).

Upright posture is known to activate efferent renal nerve fibers as a part of widespread activation of the SNS. During the first few minutes of the manoeuvre, renin release increases and sodium and water excretion decreases (188). When the cardiopulmonary afferent fibers are activated by manoeuvres affecting the central

venous volume and atrial pressure in an opposite way to upright posture (e.g. head-out water immersion and lower body negative pressure), an increase in natriuresis and diuresis is seen (189,190).

Increased SNS activity during behavioural stress induces sodium and water retention. These excretory responses are mediated primarily via increased tubular reabsorption rather than decreased GFR (191). In young men with a family history of hypertension, psychological stress has also been shown to reduce urinary sodium and water excretion (192).

Plasma and urinary free NA as indices of SNS activity

Techniques to measure human SNS activity have recently been reviewed by Grassi & Esler (193). In the past, measurements of plasma free NA and 24-hour urinary free NA have been the most frequent method employed because they are the simplest and safest. Almost all the circulating NA is released from the sympathetic nerve endings, but a relatively small amount is produced by the adrenal medulla (194). Only free catecholamines are considered to be active, since sulphoconjugation can rapidly inactivate excessive circulating free catecholamines to mitigate their actions and the conjugated fraction may act as a storage pool for free catecholamines (195). The percentage of the free fraction with respect to the total amount of circulating NA is about 32% for NA (196). In the interpretation of results, it should be remembered that plasma NA levels may vary widely with several physiological and environmental factors (e.g. psychological stress, exercise and orthostasis).

Studies in human subjects after tilting, exercise and stress suggested that urinary NA excretion reflects the overall SNS activity (197). NA excretion does not vary with urine volume but reflects the rate of infusion of NA into the plasma. Since

urinary NA excretion falls distinctly at the stage of advanced renal failure and severe reduction in excretory kidney function (198), it cannot be reliably employed in this situation.

The effects of salt loading on SNS activity

Contraction and expansion of the ECF volume in healthy subjects produce opposite changes in renal sodium excretion (see Section 7.4.4, Section 8.4.4, Section 9.4.3 and Section 10.4.3). These changes are mediated at least in part by increases and decreases in SNS activity, as reflected by the corresponding changes in plasma concentrations and urinary excretion of free NA.

Miller et al. (199) have shown in healthy subjects that plasma NA levels and urinary free NA excretion during sodium depleting with a low sodium diet and frusemide ingestion are greater than those obtained during ECF volume expansion using intravenous saline infusions. Others have shown that a fall in plasma NA concentration and urinary free NA excretion accompanies the natriuretic response to oral salt loading and intravenous saline infusion (200-204). Conversely, an increase in SNS activity will help decrease renal sodium excretion during sodium restriction (205,206). Adrenergic blockade (by reducing tubular sodium reabsorption) impairs sodium conservation during sodium deprivation in man (207).

2.4.3 *Neuropeptide Y*

Neuropeptide Y (NPY) is a vasoconstrictor peptide and a co-transmitter with NA in sympathetic nerve endings throughout the central and peripheral nervous systems including the renal nerves. Plasma NPY concentrations are considered less sensitive than those of plasma NA as an index of SNS activity (208). At high

concentrations, it has direct vasoconstrictor effect (209). In addition, it enhances the vascular effect of other vasoconstrictors, including NA and AII.

Bischoff & Michel (210) have recently reviewed the renal effects of NPY. The kidney expresses NPY receptors, among which Y_1 , Y_2 and Y_5 are involved in the regulation of renal function. Intrarenal or systemic administration of NPY causes potent renal vasoconstriction via Y_1 receptors. GFR is altered only little if at all by NPY, indicating a greater effect on the vas efferens than the vas afferens. NPY can inhibit renin release via Y_1 -like receptors. NPY can stimulate the proximal tubular Na^+,K^+ -ATPase via Y_2 receptors to produce antinatriuresis. NPY can antagonise the effects of vasopressin on isolated collecting ducts. It can act prejunctionally to inhibit NA release via Y_2 receptors. NPY antagonists enhance basal renal blood flow but do not alter basal natriuresis or diuresis, indicating that renovascular, but not tubular, NPY receptors may be tonically activated by endogenous NPY.

In renal proximal tubular cells, Holtback et al. (211) have shown that NPY determines the net effect of NA by its ability to attenuate the β - (inhibition of Na^+,K^+ -ATPase) and enhance the α - (stimulation of Na^+,K^+ -ATPase) adrenergic effects.

Increased circulating NPY levels are noted in patients with heart failure and acute myocardial infarction, reflecting changes in SNS activity and haemodynamics (212). In haemodialysis patients with fluid overload, the plasma concentrations of NPY correlate with the degree of fluid overload and arterial BP (213).

2.4.4 *Vasopressin (antidiuretic hormone)*

The primary physiological role of vasopressin is to maintain a normal plasma osmolality through its water-concentrating effects in the kidney. It also affects the

ECF volume and BP by having vasoconstrictor and antinatriuretic effects in the kidney (214), by regulating the cardiovascular function (215) and by modulating central nervous system activity involved with ECF volume and BP homeostasis. In the presence of water deprivation, plasma hyperosmolality, hypotension or hypovolaemia, osmoreceptors in the hypothalamus, baroreceptors in the carotid arteries and stretch receptors in the left atrium are activated, stimulating the release of vasopressin from the posterior hypothalamus and enhancing thirst. On the other hand, hypo-osmolality inhibits both vasopressin release and thirst.

Vasopressin exerts its biologic actions by binding to specific receptors. V_1 receptors are distributed throughout the kidney, in the glomerulus, the mesangial cells, the renal interstitial cells, and the myocytes of the renal vasculature (216). In the kidney, vasopressin acts on the V_2 receptors to enhance the permeability of the collecting duct to water, resulting in antidiuresis (217). At higher concentrations, vasopressin stimulates both the collecting duct and the thick ascending limb of the loop of Henle, resulting in increased electrolyte transport (218). The increased electrolyte transport by the thick ascending limb of Henle's loop contributes to the formation of a maximally concentrated urine and the maintenance of ECF volume in conditions of large, acute ECF volume contraction. Part of the antinatriuretic effect of vasopressin is due to potentiation of the effect of aldosterone at the cortical collecting duct (218). Vasopressin also modulates sodium reabsorption through its vasoconstrictive effects (219). Vasopressin in physiological concentrations decreases vasa recta flow by stimulating, directly, V_1 receptors, and, indirectly, via V_2 receptors. The decrease in vasa recta flow is due to efferent arteriolar vasoconstriction.

2.4.5 *Endothelins*

Three isoforms of human endothelin (ET) have been identified: ET-1, ET-2 and ET-3 (220). ET-1 is the most potent vasoconstrictor and pressor peptide known. It is synthesised in the endothelium and vascular smooth muscle cells. The ET_A receptor is highly selective for ET-1; the ET_B receptor is equally sensitive for all three isoforms of ET. The ET_A receptor is present on vascular smooth muscle cells and mediates predominantly contraction by ET-1. The ET_B receptor is present on the endothelium, where it mediates relaxation, and vascular smooth muscle cells, where it mediates vasoconstriction. The role of ET in cardiovascular and renal pathophysiology and the possible applications of ET antagonists have recently been reviewed (221,222).

The kidney is an important organ for ET synthesis and ET-1 is the most important. In the human kidney, ET_B receptor predominates over the ET_A receptor. ET_A receptor is localised in the vasculature and ET_B receptor in the renal tubules and medulla (223). ET-1 forms very strong ligand-receptor complexes that are rapidly internalised and inactivated, resulting in a very short half-life of circulating ET-1 (224). ET-1 exerts its actions in the kidney mainly as an autocrine/paracrine factor, which is also suggested by the fact that sites of ET synthesis and ET receptors are closely linked. Despite its short circulating half-life, the biological effects of ET-1 are relatively prolonged. ET-1 at high concentrations has potent antinatriuretic and antidiuretic actions by inhibiting sodium and water reabsorption in the renal medulla (225). Its other actions in the kidney include stimulation of renal cell growth (226), cell differentiation during organogenesis (227) and vasoconstriction (228). Big ET-1, its precursor, has surprisingly potent natriuretic and diuretic activities mediated mainly by stimulation of NO production coupled to ET_B receptor activation (229).

Abnormalities of the ET system occur in a range of diseases associated with vasoconstriction, vasospasm and vascular hypertrophy and ET-1 may be causal, or at least contributory, in some of these pathophysiological processes (221,222). For example, hypertensive patients with renal disease have higher plasma ET levels than those without renal damage (230). Plasma ET levels are higher in patients with salt-sensitive essential hypertension (231) or obese patients with hypertension (232) than in normotensive controls. In heart failure, activation of the endothelin system and elevated circulating levels of both ET-1 and big ET-1 are observed (233). Studies in both humans and animal models of cardiovascular disease show that inhibition of the endothelin function is associated with improvement of haemodynamics (233).

Hypertension could be mediated by high ET levels in the circulation or the vessel wall or by alterations in response to ET at the receptor level (221,222). A decrease in the response of the ET_B receptor (e.g. in the presence of dysfunctional endothelium) may attenuate the vasodilator response. Structural alterations of the vessel wall (e.g. vascular hypertrophy due to the mitogenic effects of ET) can play a role. ET may elevate BP by causing renal sodium and water retention. The actions of ET on the central and peripheral nervous systems can result in vasoconstriction through the release of other substances.

2.4.6 *Renal serotonin system*

Serotonin, or 5-hydroxytryptamine (5-HT), is a naturally occurring amine that can affect renal haemodynamics and the excretion of sodium and water. The kidney is rich in the enzymes required for the biosynthesis of serotonin as well as those necessary for its degradation (234). The essential amino acid L-tryptophan is first hydroxylated to 5-hydroxytryptophan (5-HTP) by the enzyme tryptophan-5-

hydroxylase. 5-HTP is then decarboxylated by L-aromatic amino acid decarboxylase (also known as dopa decarboxylase) (L-AAAD) to serotonin. The latter is degraded primarily by type A monoamine oxidase (MAO-A) to 5-hydroxyindoleacetic acid (5-HIAA).

In man, parenteral administration in man of serotonin decreases the renal blood flow, GFR, urine output and sodium excretion (234). Infusion of serotonin renal pro-drugs reduces urinary sodium excretion and urine flow rate without significant alterations in renal plasma flow and GFR, which are suggestive of a tubular effect (235). Pretreatment with carbidopa, which blocks the formation of serotonin by inhibiting L-AAAD, substantially attenuates the increase in serotonin excretion and abolishes the antinatriuretic effect (236).

In healthy subjects, dietary salt restriction stimulates the renal serotonergic system as evidenced by a significant rise in the urinary excretion of serotonin and 5-HIAA (206). Sodium loading is expected to have the opposite effects.

Studies performed in isolated proximal convoluted tubule preparations have shown that 5-HTP and L-dopa use the same transporter in order to be taken up into the tubular cells and both 5-HTP and L-dopa exert a competitive type of inhibition upon their cellular uptake (237). The decreased formation of serotonin in the isolated proximal convoluted tubule induced by L-dopa reflects a reduction in the intracellular availability of 5-HTP. In rats, the infusion of L-dopa significantly decreases the antinatriuretic and antidiuretic effects of serotonin (238).

Serotonin and DA also share a common metabolic pathway involving the MAO-A. Inhibition of this enzyme increases renal interstitial fluid (RIF) serotonin, concomitant with decreases in arterial BP and GFR (239). However, DA can also be metabolised by type B monoamine oxidase (MAO-B) and catechol-O-

methyltransferase (COMT) (see Section 3.2.4). Hence, available data suggest that serotonin may act as a counterregulatory paracrine substance to DA in the local regulation of sodium excretion (240).

2.5 The pathophysiology of salt sensitivity

There may be several mechanisms by which excessive dietary salt intake can elevate BP in susceptible subjects.

2.5.1 Increased blood volume and cardiac output

The principal mechanism for salt-induced hypertension is thought to be an expansion of the ECF volume and increased cardiac output (241). Such a tendency will normally be counteracted by a compensatory increase in natriuresis (Section 2.2) and changes in the activities of the natriuretic (Section 2.3) and antinatriuretic (Section 2.4) systems. However, in some individuals with defects in renal sodium excretion (e.g. due to dysregulation of the natriuretic or antinatriuretic systems) together with large sodium intakes exceeding excretory capacity, sodium and water retention and ECF volume expansion occur. Augmented venous return increases cardiac preload and consequently the cardiac output. The tendency for arterial BP to rise in response to the increase in cardiac output is offset because of activation of carotid and aortic baroreceptors and other receptors. The result, within a few seconds, is a strong decrease in sympathetic nerve signals to the entire circulatory system. However, this nervous feedback fades almost to insignificance (adapts) within days. The ECF volume and cardiac output begin to fall because of the

adaptation of the nervous control mechanisms. In order to maintain arterial pressure in balance with physiological needs, peripheral vascular resistance increases.

Thus, although the hypertension begins as a result of volume loading and is maintained as long as the volume loading continues, the evidence of excess volume and excess cardiac output disappears in the later stages of the hypertension. Substituted in their place is a greatly increased peripheral vascular resistance. This conversion is due to whole-body regulation, which means simply that the body regulates its tissue blood flow (partly through changes in vascular resistance) to match the tissue needs.

2.5.2 The role of blood vessel wall responsiveness

Vascular tone is the major determinant of arterial BP. Venous smooth muscle tone and venous blood volume determine the filling pressure of the heart and thereby influence cardiac output. Cardiac output and systemic vascular resistance determine arterial BP. Abnormalities of blood volume control have been implicated in hypertensive patients with increased blood volume and sodium sensitivity (242). The peripheral vessels themselves may also be important in determining the response to blood volume changes. Acute changes in blood volume are associated with a reduction in vascular resistance that offsets any increase in BP, whereas chronic changes in blood volume are associated with normalisation of cardiac output, but vascular tone is increased and this leads to elevated BP (243).

MacAllister & Vallance (243) have reviewed the contribution of the blood vessel wall during the systemic vascular adaptation to increases in blood volume. Mechanisms within the blood vessel wall enable the vessels to respond to local changes in BP and blood flow; these local mechanisms can influence the whole-body

response to increases in blood volume. Blood vessels demonstrate both flow-dependent and pressure-dependent (myogenic) autoregulation. It is believed that NO mediated, flow-dependent dilatation predominates in acute volume loading, but that with chronic volume loading under certain conditions, this system may become down-regulated or ineffective and therefore the myogenic response predominates and BP rises. This will tend to make the cardiovascular system volume-dependent. Hence, systemic vessels, by sensing pressure and shear stress, are important in determining the overall BP responses to volume changes.

2.5.3 *The natriuretic hormone hypothesis*

Excess dietary salt intake may elevate BP through hormonal mediators. The most commonly postulated mediator is an inhibitor of Na^+, K^+ -ATPase. This natriuretic hormone is thought to originate from the hypothalamus (Section 2.3.2). Chronic natriuretic hormone release may elevate BP through widespread inhibition of Na^+, K^+ -ATPase in vascular smooth muscle, depolarising the cell membrane, and eventually increasing calcium influx (244). The resulting reduction in the sodium gradient across the cell membrane diminishes calcium efflux via the $\text{Na}^+, \text{Ca}^{2+}$ exchange pump. Reduced efflux and increased influx elevate cytosolic calcium, promoting vascular smooth muscle contraction and elevated BP.

2.5.4 *Elevated sympathetic nervous system activity*

In salt-sensitive patients with essential hypertension (245), inappropriately high SNS activity during high salt intake may raise BP and cause sodium retention (Section 2.4.2). This may account for the inadequate decrease in vascular resistance, which in turn may be responsible for the rise in BP elicited by an increase in sodium

intake (246,247). The pressor response to NA infusions during high salt intake is greater in salt-sensitive compared with salt-resistant normotensive (248) and hypertensive (249) subjects. An increased reactivity to the pressor actions of NA may contribute to the maintenance of hypertension in salt-sensitive subjects.

In salt-sensitive subjects, the mechanisms underlying enhanced SNS activity and responsiveness during high salt diet are uncertain. Gavras (250) speculated that salt-induced increases in SNS activity are due to salt-induced diminished affinity of α_2 -adrenergic receptors in the central nervous system. Since α_2 -adrenergic tone inhibits sympathetic outflow, this decrease in affinity will increase the systemic SNS activity. Trimarco et al. (251) have shown that high salt diet potentiates cardiopulmonary baroreceptor reflexes in salt-resistant but not in salt-sensitive hypertensive patients. The inadequate reduction in sympathetic discharge after salt loading may be secondary to a dysfunction of compensatory baroreceptor reflex mechanisms.

2.5.5 *Alteration of calcium and potassium metabolism*

Excess dietary sodium may generate calcium and potassium deficiencies leading to altered hormone balances, vascular smooth muscle contraction and elevated BP (252).

Excess dietary salt intake enhances urinary calcium excretion. If this is prolonged, the calcium deficit generates defects in cellular calcium pump activity, thus increasing intracellular calcium and vascular constriction (253). The associated increases in circulating parathyroid hypertensive factor and 1,25-dihydroxyvitamin D levels may increase cellular calcium influx in vascular smooth muscle cells and stimulate BP rises (254).

Krishna (255) has reviewed the aetiological role of potassium deficiency in hypertension. Epidemiological studies from different regions have shown an inverse relationship between potassium intake and prevalence of hypertension. Potassium supplementation in patients with hypertension lowers BP. Amelioration of diuretic-induced hypokalaemia with potassium supplementation enhances the BP response. Deficient potassium intake elevates BP in both normotensive and hypertensive subjects. Potassium depletion may be accompanied by sodium retention and calcium depletion.

Suter (256) has reviewed the possible mechanisms by which dietary potassium can lower BP. These include effects on natriuresis, baroreflex sensitivity, vasodilatory actions, and the central nervous system, as well as improvement of glucose tolerance and modulation of the RAS. Potassium deficiency due to high sodium intake (which increases urinary potassium loss) and poor intake may elevate BP.

2.5.6 *Changes in membrane cation transport*

Changes in membrane cation transport have been implicated as a possible mechanism underlying the haemodynamic and BP changes during high salt intake (257). The role of the Na^+, K^+ -ATPase, $\text{Na}^+, \text{K}^+, \text{Cl}^-$ cotransport, Na^+, H^+ antiport, erythrocyte Na^+, L^+ countertransport have all been assessed. It remains unclear which membrane function is primarily affected by sodium loading, what mechanisms are responsible and whether altered membrane transport is secondary to another as yet undefined cellular disturbance evoked by sodium loading.

2.5.7 *Reduced insulin sensitivity*

Recent evidence indicates that the cellular effects of insulin can underlie the increase in total body sodium and BP in salt-sensitive hypertensive subjects (258). Chronic infusion of insulin causes a sustained increase in BP in rats (259). Acute and chronic infusion of insulin may cause sodium retention (260), stimulate the SNS (261), alter the membrane cation transport (262) and stimulate hypertrophy of smooth muscle cells (263). High salt intake reduces insulin sensitivity in young normotensive salt-sensitive subjects with a genetic predisposition to developing hypertension but not in salt-resistant subjects (264). There is an association between hyperinsulinaemia and salt-sensitivity in blacks with normal BP or borderline hypertension (265).

2.5.8 *Suppression of nitric oxide synthesis*

Previous studies showed that NO production in response to high salt intake may be impaired in patients with essential hypertension. Higashi et al. (266) showed that salt loading attenuates the conversion of L-arginine to NO in the endothelium of the renal vasculature in patients with salt-sensitive essential hypertension. Campese et al. (267) showed that high salt intake decreases plasma NO concentration in black hypertensive patients. Fujiwara et al. (268) showed that plasma NO level in these patients is decreased after salt loading and reversed after salt restriction. However, plasma NO and BP of normotensive subjects and patients with salt-resistant hypertension are unaffected by the change in salt intake (268).

The suppressant effect of high salt intake on NO synthesis could occur in susceptible subjects by several mechanisms, including altered transport of L-arginine through the endothelium membrane, decreased activity of the enzyme eNOS, and an

increased breakdown or excretion of NO (268). Asymmetrical dimethyl-L-arginine (ADMA) is produced by endothelial cells and blood vessels (269) and is present in plasma and urine of humans (270), suggesting that ADMA may be an endogenous inhibitor of NOS in vivo. ADMA also inhibits L-arginine uptake into endothelial cells. Its plasma level is increased after high salt intake and decreased after salt restriction (268). Hence, a change in ADMA may be one mechanism for modulating NO synthesis during salt loading.

2.6 The clinical relevance and predictors of salt sensitivity

From both a clinical and public health perspective, the phenomenon of salt sensitivity in normotensive and hypertensive subjects is important. Normotensive salt sensitivity is possibly a common precursor of hypertension (271,272), but the phenomenon may be susceptible to dietary intervention, which may prevent or delay its progression to hypertension (*vide infra*). An association between salt sensitivity and a greater propensity to develop renal failure has been described in certain groups of hypertensive patients, such as blacks, the elderly and diabetic patients (273). Salt-sensitive hypertensive patients are more likely than salt-resistant subjects to have left ventricular hypertrophy, microalbuminuria, metabolic abnormalities and vascular endothelial damage that may predispose them to cardiovascular diseases (273,274). Salt restriction in hypertensive patients may lower BP (275) and augment the efficacy of antihypertensive drugs (276).

2.6.1 *Methods and criteria to diagnose salt sensitivity*

One should realise that the changes in BP that occur with sodium loading follow a Gaussian distribution. Questions arise when one tries to define the boundaries of the 'salt-sensitive'. How large a rise in BP is necessary? How much sodium should be used to elicit a response? The lack of uniformity in the criteria and methods used to diagnose salt sensitivity is well illustrated in Table 2b. However, it is reasonable to regard a 10% change in MAP as significant.

Table 2b. Examples of methods and criteria to diagnose salt sensitivity.

Authors	Protocol	Subjects	Criteria (Δ MAP)
Weinberger et al. (8)	0.9% saline infusion (2 litres/4 h), 10 mmol sodium (1 day) plus frusemide 40 mg (3 doses/8 h) ≤ 80 mmol sodium for 90 days	NT, HT NT	10mg Hg >10%
Campese et al. (245)	10 mmol Na for 7 days, 100 mmol sodium for 7 days, 200 mmol sodium for 7 days, random order	HT	>10%
Sharma et al. (264)	220 mmol sodium for 7 days, 20 mmol sodium for 7 days	NT	>3 mm Hg
Kawasaki et al. (277)	109 mmol sodium for 7 days, 9 mmol sodium for 7 days, 240 mmol sodium for 7 days	HT	>10%
Sullivan et al. (278)	Ad libitum for 1 day, 10 mmol sodium for 4 days, ad libitum for 2 days, 200 mmol sodium for 4 days	HT	>5%

MAP = mean arterial pressure. NT = normotensive, HT = hypertensive.

2.6.2 *Subject groups with a higher prevalence of salt sensitivity*

Weinberger et al. (8) studied the changes in MAP in normotensive and hypertensive subjects after ECF volume expansion and contraction. In both groups, salt-sensitive subjects were older and had lower baseline PRA than the salt-resistant subjects. Factors related to changes in MAP after changes in ECF volume included baseline BP and age. The hypertensive subjects were much more likely to be salt-sensitive than the normotensive subjects (51 vs 26%). Luft et al. (21) used an identical protocol to compare the changes in MAP in blacks and whites. They found that 32% of normal black subjects and 30% of normal whites were salt-sensitive. Conversely, 73% of hypertensive blacks were salt-sensitive compared with 55% of hypertensive whites. Salt-sensitive subjects in both the normotensive and hypertensive subject groups were older.

Skrabal et al. (248) studied the effects of salt restriction (reduced from 200 to 50 mmol/day) over two weeks in 52 young healthy subjects. Twenty-two subjects (42%) showed a significant fall in ambulatory BP. Compared to 30 subjects without significant changes in BP, these 22 subjects showed a 2.5-fold higher incidence of a positive family history of hypertension and a significant higher baseline BP.

Salt sensitivity also appears to be more common in subjects with obesity, diabetes mellitus and renal failure (279,280).

2.6.3 *Markers of salt sensitivity*

Various groups have attempted to identify markers of salt sensitivity, thus avoiding the inconvenience of studies involving salt loading and/or salt depletion over a period of several days.

Salt-sensitive normotensive subjects display several features found in patients with hypertension, including increased vascular reactivity to pressor substances, increased forearm vascular resistance, decreased venous compliance, low PRA, decreased α_2/β_2 adrenoreceptor ratio and insulin resistance (281).

Abnormal erythrocyte Na^+,Li^+ countertransport, a genetically determined trait, may serve as a marker of salt sensitivity (282). The haptoglobin phenotype Hp 1 is associated with salt sensitivity, whereas the Hp 2 phenotype is associated with salt resistance (283).

Giner et al. (284) evaluated the association between genetic polymorphisms of the RAS and salt-sensitive hypertension. They reported that Spanish patients with II and DI genotypes have significantly higher prevalence of salt sensitivity than DD hypertensives.

Adducin, an α/β heterodimeric protein found in the renal tubule is thought to regulate renal sodium transport through changes in the actin cytoskeleton. Cusi et al. (285) reported a significant linkage of the α -adducin locus in essential hypertension and greater sensitivity to changes in sodium balance in Caucasian subjects. An association between the α -adducin locus and essential hypertension is not seen in Japanese subjects (286).

Chapter 3 Dopamine – an important intrarenal natriuretic factor

Overall the past 30 years, the pioneer work of several workers has helped establish that DA is an important intrarenal natriuretic, vasodilatory substance in the body. Much of this work has been summarised elsewhere (10,240,287-302).

3.1 Evidence in support of renal DA being an important natriuretic factor

The idea that renal DA is important for the regulation of sodium homeostasis is based on the observations summarised here.

3.1.1 *Dopamine in the urine is out of proportion to other catecholamines*

It has long been known that the amount of free DA excreted in human urine is 5-20 times greater than that of NA (303). In patients with impaired renal function, urinary free DA output declines with falling GFR (see Section 4.2), whereas free NA excretion falls only at the stage of advanced renal failure (198). These observations suggest that free DA in the urine is derived from the kidney.

The kidney is the likely source of urinary free DA

Initially, the assumption was that the increased amount of DA in the urine reflects the filtered load of DA and is thus derived from the plasma. When a sensitive assay for plasma free DA became available, Da Prada & Zürcher (304) reported a mean level in man of 0.74 nmol/L. Assuming a GFR of 120 ml/min, this should result in a free DA excretion rate of 130 nmol/day. In fact, Crout (305) reported values of 650-2285 nmol/day. Subsequently, Ball et al. (306) determined

the plasma and urine levels of free DA simultaneously in six healthy subjects. They calculated a urinary clearance rate of $1,996 \pm 185$ ml/min (range 402-3,844 ml/min), which is greatly in excess of the GFR and, in most individuals, greater than the calculated renal plasma flow. Therefore, DA must be synthesised in the kidney with the bulk of urinary free DA originating from the kidney.

Two other hypotheses were put forward to explain the large amounts of free DA in the urine. Both are now considered very unlikely for the reasons summarised by Lee (288). Unger et al. (307) proposed that DA, derived from the adrenal gland, is rapidly conjugated as sulphate and glucuronide. The conjugated DA circulates to the kidney, where deconjugase enzymes act on the compound to release free DA, first into the renal tissue and then, after secretion, into the urine. However, Ball & Lee (15) showed that carbidopa greatly reduces urinary free DA output, suggesting that L-AAAD is necessary for the intrarenal production of DA and L-dopa from the plasma is the renal source of DA (see Section 3.1.10). Moreover, Ball et al. (306) showed in rats that both urinary free and conjugated DA increase in parallel after salt loading. If the 'deconjugation hypothesis' were correct, a rise in urine free DA should be accompanied by a corresponding decrease in its conjugates. Akpaffiong (308) showed that rats with their adrenal glands removed still exhibit a marked increase in urinary DA after oral salt loading. Studies using the renal pro-drugs, γ -glutamyl-L-dopa (gludopa) and γ -glutamyl-DA suggested that the former is a more effective source of urinary free DA (see Section 3.1.5).

DA-containing neuronal elements are present in animal kidneys (309). Since rapid, effective mechanisms exist for the re-uptake and inactivation of DA and other catecholamine neurotransmitters as soon as they are released into the synaptic cleft,

it is doubtful if DA released from renal dopaminergic nerve endings can contribute in a material way to the excess of DA in the urine (288).

Sources and physiological significance of DA in human plasma and urine

In humans, DA in the plasma occurs mainly as DA sulphate, a biologically inactive metabolite, with the free (unconjugated) DA accounting for <1% of the total DA (310). Plasma free DA is mainly derived from the peripheral sympathetic nerve terminals and the released DA is converted to conjugated DA and to DA metabolites (311). Plasma DA sulphate originates from dietary intake as well as synthesis and the metabolism of endogenous DA, especially in the gastrointestinal tract (312). The former may reflect a gut-blood barrier to detoxify catecholamines derived from the diet and the latter a third catecholamine system, in which sulphoconjugation delimits the actions of DA as an autocrine-paracrine factor. Conjugated DA can also serve as a reservoir of active DA (195,313). It was suggested that free DA can be generated from this pool through deconjugation whenever a need for it arises (313).

Elchisak (314) reported that free DA accounts for 75% of the total DA in the kidney. This finding is consistent with the intrarenal synthesis of DA.

Free DA accounts for 16-20% of the total DA in the urine (315,316), but the measured proportion is higher if the urine samples are acidified to a lower pH which may result in hydrolysis of conjugated DA (317). Other evidence supporting the kidney (proximal tubules) as the source of urinary free DA will be discussed later in this chapter.

Sulphated and non-sulphated conjugates of DA account for 80-84% of the total DA in the urine (315,316) and are derived from the filtrated load at the glomeruli (311).

3.1.2 *Dopamine receptors are present in the kidney*

Cardiovascular and renal actions of DA are mediated through the interaction with specific DA receptors. Receptor-ligand binding, receptor autoradiography and, recently, molecular biology techniques have been used to examine the subtypes and distribution of DA receptors in the target organs (291,296). These peripheral DA receptors were traditionally divided into DA₁ and DA₂ subtypes, whereas DA receptors in the brain were divided into D₁ and D₂ subtypes. Advances in molecular biology have afforded the identification of at least five subtypes of DA receptors. These newly described receptors correspond pharmacologically and biochemically to the classic 'D₁-like' (D₁ and D₅) and 'D₂-like' (D₂, D₃ and D₄) receptors based on their ability to stimulate or inhibit adenylyl cyclase, respectively. The pharmacological properties of DA₁ receptors approximate to those of D₁ and D₅ receptors, whereas those of DA₂ receptors approximate to those of D₂ receptors (Table 3a).

Within the kidney, the DA₁ receptors are present on the smooth muscle of the renal arteries, the juxtaglomerular apparatus and the renal tubules (Table 3a). The DA₂ receptors are found in the intimal layer of the renal vasculature, glomeruli, sympathetic nerve terminals and the renal tubules (296). The tubular DA₁ receptors are present in higher density in the proximal tubules than the distal parts of the nephron, as well as the renal vasculature. DA₁ receptors and DA₂ receptors are also located on both basolateral and brush border membranes of the proximal tubules.

The adrenal gland is another peripheral target of DA that is also linked to the ECF volume and BP homeostasis (302). In adrenal cortex, DA inhibits primarily aldosterone secretion, whereas in the adrenal medulla, it is involved in the regulation of catecholamine release from chromaffin cells.

Table 3a. Peripheral DA receptors.

Subtype	Location	Response
DA ₁ (‘D ₁ -like’)	Blood vessels Renal tubules Juxtaglomerular cells	Vasodilation Natriuresis, diuresis Renin release
DA ₂ (‘D ₂ -like’)	Postganglionic sympathetic nerves Sympathetic ganglia Adrenal cortex (zona glomerulosa) Adrenal medulla Renal tubules	Vasodilation, bradycardia Inhibition of transmission Inhibition of aldosterone release Inhibition of catecholamine release ?

Adapted from Hussain & Lokhandwala (296).

3.1.3 Dopamine is a potent natriuretic agent

Our knowledge of this subject stems from the pioneer work of Leon Goldberg over a period of 25 years (16, 287). He and his colleagues first showed that DA is a potent natriuretic and diuretic agent in man (11). They also showed in dogs that intrarenal infusion of DA results in greater increases in renal blood flow, GFR and sodium excretion in the infused side compared with the non-infused kidney (318).

In the 1980s, it was uncertain whether DA’s natriuretic effect is secondary to an increase in renal blood flow and GFR (287) or a redistribution of intrarenal blood flow and/or a direct action on tubular sodium transport (319). Indirect evidence for a tubular site of action came from the studies of Wassermann et al. (320) who showed that the natriuretic effect of DA in the cat is independent of changes in renal blood flow and GFR.

DA is a potent natriuretic agent even at so-called 'renal doses' (0.5-2.0 $\mu\text{g}/\text{kg}$ body weight/min) (321). In salt-repleted (i.e. usual salt intake prior to study with or without 0.9% saline infusion during the study) and hydrated (i.e. water to drink during the infusion) subjects, DA infusions produce dose-dependent increases in natriuresis and diuresis with increases in renal blood flow and the GFR (11,322-325). The renal actions of DA are well illustrated by the studies summarised in Table 3b.

Table 3b. Natriuretic and diuretic effects of DA in healthy subjects.

Authors	DA dose ($\mu\text{g}/\text{kg}/\text{min}$) / study design	Main findings
McDonald et al., 1964 (11)	2.6-7.1 i.e. the largest dose without \uparrow MAP	Sodium excretion 171 \rightarrow 571 $\mu\text{mol}/\text{min}$ Renal plasma flow 507 \rightarrow 798 ml/min GFR 109 \rightarrow 136 ml/min Cardiac output \uparrow , no changes in heart rate
Levinson et al., 1985 (322)	0.03-3.0 vs 5% dextrose for 2 h on 4 occasions	Sodium excretion 188-390 vs 168 $\mu\text{mol}/\text{min}$ Urine volume 3.8-5.6 vs 3.2 ml/min Heart rate 64 vs 61/min at dose of 3.0 $\mu\text{g}/\text{kg}/\text{min}$ No changes in BP
Olsen et al., 1990 (323)	3 for 3 h	Clearance of sodium 1.10 \rightarrow 2.51 ml/min/1.73 m ² Fraction excretion of sodium 1.04 \rightarrow 2.13% Absolute distal reabsorption rate of sodium 42% \uparrow Proximal fractional reabsorption 13% \downarrow Fractional distal reabsorption of sodium 96.5 \rightarrow 94.5% GFR 108 \rightarrow 118 ml/min Clearance of lithium (an index of proximal tubular outflow of sodium and water) 32 \rightarrow 46 ml/min/1.73 m ² Urine volume 13 \rightarrow 16 ml/min Effective renal plasma flow 498 \rightarrow 711 ml/min MAP 92 \rightarrow 88 mmHg

Studies employing different DA antagonists have helped characterise the specific intrarenal DA receptors mediating the actions of DA (324,325).

It is equally important to realise that the renal effects of DA can be attenuated during sodium and water depletion. Bughi et al. (325) showed that the increase in renal blood flow in healthy subjects during DA infusions is attenuated by a low salt diet. Agnoli et al. (326) showed that DA 'loses' its vasodilatory, natriuretic effects in ECF volume depletion induced by low dietary salt intake and diuretic treatment. It is likely that in these situations, the renal effects of DA are overcome by those of antinatriuretic and vasoconstrictor systems (Section 2.4). In contrast, prior ECF volume expansion by 0.9% saline infusion greatly enhances the natriuretic and diuretic actions of DA in pre-operative cardiac surgery patients (327).

At higher doses (2-5 $\mu\text{g}/\text{kg}$ per min), DA also binds to β -adrenergic receptors (321). Above 5 $\mu\text{g}/\text{kg}/\text{min}$, α -adrenergic receptors are also activated. However, at low or higher doses, there is overlap in receptor activation, depending on the clinical state of the subject such as the BP and ECF volume status (328). Olsen et al. (329) suggested that in healthy salt-repleted, water-loaded subjects, the natriuretic effect of low doses of DA is primarily caused by attenuation of the increase in distal sodium reabsorption normally seen after an increase in proximal tubular flow. Pressor doses of DA further increase sodium excretion, suggesting the presence of pressure-natriuresis at these high doses.

3.1.4 *Other DA₁ agonists are also potent natriuretic agents*

With the availability of highly selective DA₁ agonists, the role of renal DA in sodium homeostasis can be characterised better.

Fenoldopam

Fenoldopam is a selective DA₁ receptor agonist without significant α - or β -adrenoceptor activity (330). In salt-repleted, hydrated healthy subjects, fenoldopam increases renal blood flow, decreases renal vascular resistance and produces dose-related natriuresis and diuresis (Table 3c). It also produces peripheral vasodilation via stimulation of DA₁ receptors in blood vessels.

Table 3c. Renal and other effects of fenoldopam in healthy subjects.

Authors	Dose	Main findings
Stote et al., 1983 (331)	1 dose of 25, 50 or 100 mg by mouth	After 100 mg, changes seen in hour 1, peak in hour 2 fractional excretion of sodium 0.84 → 1.64% urine volume 13 → 21 ml/min renal plasma flow 671 → 1,012 ml/min free water clearance 10 → 16 ml/min Smaller changes after 25 mg and 50 mg
Allison et al., 1987 (332)	i.v. 0.025, 0.05 or 0.1 μ g/kg/min for 2 hours	Dose-dependent increases: urine volume 14 → 17-25 ml/min renal plasma flow 609 → 748-1,047 ml/min GFR 110 → 123 ml/min clearance of water 11 → 13-19 ml/min Fractional excretion of sodium (at 0.5 mg) 0.8 → 3.0% Dose-dependent \uparrow in renal vascular resistance and DBP
Huges et al., 1988 (333)	i.v. 0.05 μ g/kg/min	Sodium excretion 150 → 320 μ mol/min Urine volume 13 → 17 ml/min Renal plasma flow 344 → 481 ml/min Fractional excretion of sodium 1.3 → 2.7% Filtration fraction 25 → 16 ml/min Distal sodium load \uparrow Distal sodium reabsorption \downarrow No changes in GFR, BP or heart rate

In healthy subjects, infusions of fenoldopam 0.025-0.5 $\mu\text{g}/\text{kg}/\text{min}$ produce small decreases in DBP. In hypertensive patients, fenoldopam 0.1-0.9 $\mu\text{g}/\text{kg}/\text{min}$ decreases SBP (baseline >180 mmHg) by 18.5-28% and DBP (baseline >120 mmHg) by 22-31.5% (330).

Dopexamine

Dopexamine, a synthetic DA analogue developed to improve myocardial and renal function in patients with low cardiac output states, has the renal effects of DA and haemodynamic effects of dobutamine. It predominantly stimulates DA_1 and β -adrenergic receptors (334). It has renal and vasodilatory effects (Table 3d). It has no α -adrenergic activity and has less agonist activity at DA_2 receptors.

Table 3d. Renal and other effects of dopexamine in healthy subjects.

Authors	Dose	Main findings
Mousdale et al., 1988 (335)	1, 2, 4 $\mu\text{g}/\text{kg}/\text{min}$ for 30 min each	Dose-related \uparrow renal plasma flow, max 22% \uparrow SBP, max 15% \downarrow DBP, max 38% Dose-related \uparrow heart rate, max 65%
Olsen et al., 1993 (336)	1 $\mu\text{g}/\text{kg}/\text{min}$ for 2 hours	Sodium clearance 1.3 \rightarrow 1.7 ml/min/1.73m ² Fractional excretion of sodium 1 \rightarrow 1.3% Urine flow 13 \rightarrow 16 ml/min Renal plasma flow 558 \rightarrow 619 ml/min/1.73m ² 30% \uparrow cardiac output SBP 121 \rightarrow 139 mmHg Heart rate 52 \rightarrow 62 beats/min Peripheral resistance 21.6 \rightarrow 17.9 mmHg.min/L No changes in DBP

In pre-operative high-risk surgical patients, dopexamine can improve the cardiac output and allow greater increases in oxygen delivery than dobutamine (337). It appears safe and effective in patients with low cardiac output syndrome after coronary artery bypass graft surgery (338). It produces beneficial renal and haemodynamic effects in patients with severe congestive heart failure (339).

3.1.5 *Dopamine pro-drugs are also potent natriuretic agents*

Pro-drugs are pharmacologically inert. They must undergo biotransformation in the body before they can elicit a pharmacological effect (340). Such an approach is used with several objectives in mind: (a) to increase the efficacy of these drugs; (b) to target these drugs to a specific organ or site; (c) to decrease the toxicity of these drugs.

The DA pro-drugs include L-dopa, gludopa, ibopamine and docarpamine. The enzymes required for biotransformation are L-AAAD (see Section 3.1.10) for L-dopa, γ -glutamyl transferase for gludopa, esterases for ibopamine and hydroxylases for docarpamine.

L-dopa

After ingestion or intravenous administration as an infusion, L-dopa is converted to free DA by L-AAAD in the kidney. DA can then act in a paracrine manner and induces renal vasodilatation and natriuresis (341-343). Hence, the renal effects of L-dopa in humans are very similar to those produced by DA infusion (Table 3e).

Table 3e. Renal and other effects of L-dopa in healthy subjects.

References	Dose	Main findings
Brown & Dollery, 1981 (341)	1 dose of 250 mg by mouth	At baseline, positive correlation between plasma dopa and urine DA ($r=0.85$, $p<0.01$) Plasma dopa \uparrow 98-fold, urine DA \uparrow 93-fold, but plasma DA \uparrow by 26-fold only, calculated clearance of plasma dopa by renal decarboxylation 114 ml/min
Shigetomi et al., 1991 (342)	1 dose of 500 mg by mouth	Sodium clearance 0.9 \rightarrow 2.7 ml/min Plasma dopa (area under curve) \uparrow 792-fold Glomerular dopa load \uparrow 427-fold Urine free DA \uparrow 891-fold Positive correlation between glomerular dopa load and urine free DA ($r=0.59$, $p<0.02$) Positive correlation between urine sodium and urine DA excretion ($r=0.52$, $p<0.02$) SBP/DBP \downarrow 4.1/6.7 mmHg
Worth et al., 1988 (343)	i.v. 7 μ g/kg/min for 2 hours	Sodium excretion 103 \rightarrow 195 μ mol/min Urine volume 2.7 \rightarrow 5 ml/min ($p>0.05$) Urine free DA 0.08 \rightarrow 31.7 μ mol/h 24% \uparrow effective renal plasma flow c.f. placebo 6% \uparrow GFR ($p>0.05$) c.f. placebo DBP 74.5 \rightarrow 69.5 mmHg

These studies provided further evidence that plasma dopa is the precursor of urinary free DA. At the baseline, there was a positive correlation between plasma dopa and free DA excretion (341). After the administration of L-dopa, plasma dopa and urinary free DA both increased by a similar proportion and the increase was much greater than that of plasma DA (341,342). There was a positive correlation between glomerular dopa load and urinary free DA (342). The increase in plasma dopa, but not plasma DA, can account for the increase in urinary free DA (341,342).

Gludopa

Wilk et al. (344) showed that gludopa is more renally specific DA pro-drug than L-dopa or γ -glutamyl DA. In gludopa, glutamic acid is substituted on the γ -nitrogen of L-dopa. It becomes a substrate, first for γ -glutamyl transferase in the brush border of proximal tubular cells (345) to generate L-dopa, and then for L-AAAD in proximal tubular cells to generate DA.

In healthy subjects with adequate hydration, gludopa given as an intravenous infusion (12.5 or 25 $\mu\text{g}/\text{kg}/\text{min}$) produces increases in urine sodium and free DA that outlast the time of infusion, presumably because of a prolonged conversion to DA in the kidney (Table 3f).

Table 3f. Renal and other effects of gludopa in healthy subjects.

Authors	Dose	Main findings
Worth et al., 1986 (346)	12.5 $\mu\text{g}/\text{kg}/\text{min}$ for 2 hours	Sodium excretion 80 \rightarrow 118 $\mu\text{mol}/\text{min}$ Urine volume 3 \rightarrow 5 ml/min Effective renal plasma flow \uparrow 8% ($p>0.05$) GFR \uparrow 17% ($p>0.05$)
MacDonald et al., 1988 (347)	25 $\mu\text{g}/\text{kg}/\text{min}$ for 2 hours	Effects \uparrow with the duration of infusion Sodium excretion 190 \rightarrow 520 $\mu\text{mol}/\text{min}$ GFR 95.6 \rightarrow 112.5 ml/min Effective renal plasma flow 588 \rightarrow 738 ml/min Filtration fraction 0.17 \rightarrow 0.16% Urine free DA \uparrow several hundred-fold
Jeffrey et al., 1988 (348)	25 $\mu\text{g}/\text{kg}/\text{min}$ for 2 hours	Sodium excretion 120 \rightarrow 340 $\mu\text{mol}/\text{min}$ (330-290 for 2 h after infusion) Urine free DA \uparrow 900-fold (640 to 260-fold \uparrow for 2 h after infusion) Carbidopa inhibited DA excretion by 97% and abolished the natriuretic response

In contrast, an equimolar dose of γ -L-glutamyl-L-tyrosine (glutyrosine) (25 $\mu\text{g}/\text{kg}/\text{min}$ intravenously for two hours) does not produce a natriuresis (349). This is not surprising since tyrosine delivered to the kidney is not the substrate for renal DA synthesis. [As discussed in Section 3.2, the substrate for renal DA synthesis is L-dopa supplied to the proximal tubular cells.]

Ibopamine (di-isobutyryl-*N*-methyldopamine)

The need for parenteral administration limits DA's application in clinical practice. Ibopamine, active after oral administration, has been introduced, mainly for the treatment of congestive heart failure (350). It is de-esterified in the gut and liver to release epinine (*N*-methyldopamine) into the circulation. Epinine stimulates DA_1 and DA_2 receptors and, to a lesser degree, β - and α -adrenoreceptors. It induces natriuresis with an increase in creatinine clearance in both healthy subjects and patients with renal impairment (351,352).

Ibopamine improves the symptoms of patients with congestive heart failure. The haemodynamic effects (increased cardiac index, increased stroke volume and decreased systemic vascular resistance) probably can be ascribed to the stimulation of DA_1 and DA_2 receptors, whereas its neurohormonal effects (decreased plasma aldosterone and NA) are produced by DA_2 receptors (350). However, for some unexplained reasons, ibopamine seems to increase the risk of death among those with moderate to severe heart failure (353). On the basis of these results, the indication for ibopamine in some countries was restricted to patients with mild heart failure.

Like DA, ibopamine has been used in the treatment of chronic renal failure (354). In DA-dependent patients, intravenous DA infusion can sometimes be switched to ibopamine (355).

Docarpamine [N,(N-acetyl-L-methionyl)-O,O-bis(ethoxycarbonyl) dopamine]

Docarpamine is well absorbed after oral administration (356,357). It is easily hydrolysed to the de-ethoxycarbonyl intermediate and then DA, mainly in the liver but also in the small intestine. DA is released into the circulation.

Not surprisingly, docarpamine exhibits natriuretic, diuretic and positive inotropic effects in experimental animals after enteral administration (356,357). Increases in renal blood flow and GFR may also be seen.

Docarpamine has been used in patients with refractory ascites (358) and low cardiac output states (359). It has been used in post-cardiac surgery patients once their haemodynamics are stabilised and DA therapy can be weaned off (359,360). However, its use in cardiac patients may be limited by the risk of cardiac arrhythmias (359).

3.1.6 *Positive correlation between urine sodium and urine free DA*

If DA is an important intrarenal natriuretic hormone and dietary sodium is the major determinant of its intrarenal synthesis, there should be a positive correlation between urinary sodium and free DA outputs. Indeed, this is seen in some but not all ethnic groups (Table 3g).

In ethnic groups with, traditionally, a high salt diet (Caucasians, Japanese, Thais and Zimbabweans), there is a positive correlation between the 24-hour urinary sodium and free DA outputs. The x coefficient of the regression line is the highest among the Thais. The x coefficient or the 'slope' of the regression line is the factor by which urinary free DA output is elevated in proportion to an increase in sodium output.

Table 3g. Correlation between 24-hour urinary sodium and free DA outputs.

Authors	Subjects	Sodium (mmol)	DA (nmol)	x coef.	r	P value
Critchley et al., 1989 (361)	Caucasians (U.K.)					
	57 M, 26 F	156	1,480	3.19	0.40	<0.001
	23 ± 0.7 y	± 6.5	± 52			
	Zimbabweans					
	21 M, 3 F	163	1,820	5.1	0.64	<0.001
	24 ± 0.2 y	± 14	± 112			
	Thais					
	51 M, 23 F	114	1,510	5.93	0.53	<0.001
	22 ± 0.7 y	± 6.5	± 73			
Patrick et al., 1989 (362)	Caucasians (UK)					
	40 M	173	1,572	-	0.51	<0.001
	26 (20-39) y	± 8.5	± 72			
Saito et al., 1986 (363)	Japanese	188	1,815	-	0.52	<0.01
	36 M	± 13	± 104			
	20 ± 0.2 y					
Critchley et al., 1989 (361)	W. Africans (UK)					
	16 M, 9 F	169	1,780	-0.08	0.01	NS
	36 ± 1.2 y	± 12.2	± 104			
	Iranians					
	28 M, 11 F	169	1,830	0.2	0.03	NS
	26 ± 1.6	± 8.8	± 88			

In contrast, a significant renal sodium-DA relationship is not seen in West Africans and Iranians, ethnic groups from areas with intense heat and, traditionally, a low salt diet (see Section 1.3 for possible explanation).

Unlike urban subjects in northern Venezuela who have a high salt intake, Piaroa Amazonic Indians do not use salt in their regular food intake. Romero-Vecchione et al. (364) reported that urinary excretion of sodium (210.7 ± 24.5 vs

12.6 ± 5.2 mmol/day) and DA (5,227 ± 387 vs 2,055 ± 262 nmol/day) differed markedly between these urban subjects and the Piaroa Amazonic Indians. They commented that indigenous tribes may require less renal DA synthesis to excrete the very small amounts of salt they consume in their regular diet.

In Japanese subjects, the urinary excretion of water, sodium and free DA shows circadian variations with higher rates during daytime and lower rates at night (365). Urinary sodium and water correlate positively with urinary free DA. These results suggest that endogenous DA plays a role in the circadian variations of sodium and water metabolism.

A positive correlation between urinary sodium and free DA has also been reported in healthy Caucasian subjects under constant sodium intake (15), after changes in sodium intake (see Section 3.1.7) or after inhibition of DA synthesis with a subsequent fall in both urine sodium and free DA (15).

On changing from a supine to an upright posture, a decrease in natriuresis and diuresis is seen in healthy subjects, possibly due to a decrease in renal DA synthesis but an increase in SNS and RAS activities (366). A positive correlation between the urinary excretion of sodium and free DA is seen in both supine and upright posture.

3.1.7 *Oral salt loading increases urinary excretion of both sodium and free DA*

If intrarenal DA regulates the urinary excretion of sodium, the natriuretic response to oral salt loading should be accompanied by an increase in urinary free DA excretion. Indeed, this has been shown convincingly in humans (Table 3h and Table 3i) and experimental animals (Table 3j).

Studies in humans

Table 3h. Increased urinary free DA excretion during the natriuretic response to oral sodium loading in healthy Caucasian subjects.

Authors	Study design / daily Na intake	Main findings
Alexander et al., 1974 (12)	6 M, 1 F, 19-29 y* Na (9 mmol D1-8, 259 mmol D9-18) frusemide 40 mg/d on D1-3	Sodium excretion 9 (mean of D6-8) → 259 (mean of D15-18) mmol/d Free DA excretion 888 → 1,273 nmol/d Free NA excretion 221 → 125 nmol/d GFR 92 → 105 ml/min
Oates et al., 1979 (13)	6 M* Na (100 mmol D1-3, 20 mmol D4-8, 220 mmol D9-13)	Sodium excretion ↑ from D5, max on D7 Free DA excretion 1,200 (low Na) → max 1,800 nmol/d (D5) Plasma free DA unchanged Free DA clearance in a pattern similar to free DA excretion PRA ↑ and ↓ with low and high Na intake
Casson et al., 1983 (367)	5M, 43 ± 2.7 y* Na (40 mmol D1-3 220 mmol D4-12)	Sodium excretion 24 (D3) → 92 (D4), 157 (D5) and 266 mmol/d (D6) Free DA excretion 830 (D3) → 855 (D4), max 1,150 (D8) nmol/d PRA ↓ with high Na intake
Harvey et al., 1984 (368)	6M awaiting minor surgery, 45 ± 5 y* Na (20 mmol D1-7 220 mmol D8-14)	Sodium excretion 22 (D7) → max 248 (D10) Free DA excretion 820 (D7) → 830 (D8), max 1,090 (D11) nmol/d PRA ↓ with high Na intake
Gill et al., 1988 (369)	2M, 3F, 20-62 y* Na (109 mmol D1-7, 9 mmol D8-14, 249 mmol D15-21)	Free DA excretion 882 (D13-14) → 1,371 (D21-22) nmol/d PRA and plasma aldosterone ↓ with Na loading No changes in MAP, plasma/urine free NA
Critchley et al., 1990 (370)	20 subjects 200 mmol Na on D1	Sodium excretion 142 → 257 mmol/d Free DA excretion 2,030 → 2,740 nmol/d
Rudberg et al., 1997 (371)	16 subjects, 14-29 y* Na (normal, then high on D1-3)	Sodium excretion 107 → 206 mmol/d Free DA excretion 1,476 → 1,750 nmol/d Aldosterone excretion 6.4 → 4.1 nmol/12 h

Table 3h. (cont'd)

Goldstein et al., 1989 (372)	10 subjects* 20-24 y Na (109 mmol D1-7, 9 mmol D8-14, 249 mmol D15-21)	About 10 times as much free DA excreted as dopa throughout the study Free DA excretion 1,104 (D1), 895 (D4) → 686 (D12), 816 (D14), → 1,313 (D19), 1,123 (D21) nmol/d Dopa excretion 15.2 (D1), 14.2 (D4) → 9.2 (D12), 10.8 (D14) → 21.0 (D19), 18.1 (D21) µg/d Dopa clearance 4.6 (109 mmol Na), 3.9 (9 mmol Na), 7.8 (249 mmol Na) ml/min per 100 ml GFR Changes in dopa excretion parallel almost exactly changes in free DA excretion Plasma free NA 141 (D14) → 91 (D21) pg/ml
Gill et al., 1991 (373)	4 M, 6 F, 20-24 y* Na (9 mmol D1-7, 249 mmol D8-14)	Free DA excretion 912 (D1), 838 (D7) → 1,332 (D12), 1,141 (D14) nmol/d Dopa excretion 65 (D1), 51 (D7) → 103 (D12), 79 (D14) nmol/d Free DA excretion ↑ 60% and dopa excretion ↑ 75% between D6-7 and D13-14 Dopa clearance 4.15 → 7.46 ml/min per 100 ml GFR Plasma free NA ↓ during the high Na intake Free NA excretion 105 (D1), 107 (D7) → 75 (D9), 96 (D14) nmol/d
Wolfowitz et al., 1993 (374)	6M, 6F, 24-55 y* Na (40 mmol D1-7 unrestricted D8-14 340 mmol D15-21) Sequence of diets randomised L-dopa infusion to ↑ plasma dopa by about 10-fold	Free DA excretion 1,483 (D7) → 1,872 (D21) nmol/d Dopa excretion 115 (D7) → 202 (D21) → 202 (D21) nmol/d No changes in BP, plasma dopa or renal plasma flow Strong positive correlations among urinary excretory rates of dopa, free DA and dihydroxyphenylacetic acid (DOPAC) L-dopa infusion → no changes in plasma DA, but ↑↑↑ dopa and free DA excretion ↑ rate of entry (spillover) of L-dopa into the circulation cannot explain ↑ dopa and free DA excretion during Na loading

*All Caucasian subjects?

From these studies of renal DA response to dietary salt loading, several important observations should be highlighted:

- (a). As already discussed in Section 3.1.1, if urinary free DA simply reflects the filtered load of DA and were derived from plasma free DA, a daily excretion rate of about 130 nmol is expected. The actual urinary free DA output is 6-15 times as much before salt loading. The figure is even higher (8-21 times as much) after salt loading. The calculated clearance values (indices of delivery of plasma free DA to the kidney) also far exceed renal blood flow, indicating that free DA is formed within the kidney (13).
- (b). The increase in urinary free DA excretion, in the absence of changes in plasma free DA after increased oral salt loading, implies that free DA is formed within the kidney in response to salt loading (13,373,374).
- (c). Free DA excretion appears to lead, rather than follow, sodium excretion, suggesting renal DA needs to be mobilised first before a natriuretic response to oral salt loading is seen (13).
- (d). The proportionate changes in dopa excretion during dietary manipulations parallel almost exactly the proportionate changes in free DA excretion. Both increase during salt loading and decrease during salt restriction. These results imply that: (i) increases in free DA excretion during salt loading can be accounted by increases in delivery of dopa to renal tubular fluid; (ii) urinary free DA is derived from circulating dopa (372-374).
- (e). Under basal conditions, the strong positive correlations among urinary excretion rates of dopa, free DA and its metabolite, dihydroxyphenylacetic acid (DOPAC), are consistent with the renal synthesis and metabolism of DA after the uptake and decarboxylation of circulating dopa (374).

- (f). By administrating L-dopa to healthy subjects taking a low or high salt diet, Wolfovitz et al. (374) calculated that renal uptake and decarboxylation of circulating dopa accounted for virtually all the urinary excretion of free DA. They also suggested that dietary salt loading may enhance the sodium-coupled uptake of dopa from the plasma, thereby increasing the excretion rates of dopa and free DA.

Increases in urinary excretion of free DA during the acute natriuretic response to oral salt loading have also been reported in other ethnic groups (Table 3i).

Table 3i. Increased urinary free DA excretion during the natriuretic response to oral sodium loading in healthy Thai and black subjects.

Authors	Study design / daily Na intake	Main findings
Critchley et al., 1990 (370)	Thais, 20 subjects Usual diet + 200 mmol Na on D1	Sodium excretion 118 → 223 mmol/d Free DA excretion 1,714 → 2,314 nmol/d
Sowers et al., 1988 (375)	Blacks, 14 subjects 44 ± 6 y Na (40 mmol D1-14 180 mmol D15-28)	Sodium excretion 40 → 185 mmol/d Free DA excretion 1,521 → 1,887 nmol/d Free NA excretion 943 → 124 nmol/d No changes in urinary free DA/NA ratio No correlations between urinary free DA/NA ratio and sodium output Plasma aldosterone 0.58 → 0.28 nmol/L No changes in PGE ₂ excretion MAP ↓ 5-10 mmHg in all subjects when Na intake changed from normal to 40 mmol/d MAP ↑ 6 ± 0.2 mmHg (7 ± 0.2%) when Na intake changed from 40 to 180 mmol/d

Critchley et al. (370) also studied 13 Ghanaians (baseline 24-hour sodium output 141 ± 4.2 mmol), 29 Iranians (160 ± 10.0 mmol) and 15 Thai vegetarians (60 ± 5.4 mmol). Both the Ghanaians and Iranians showed no significant changes in urinary free DA output after salt loading (see Section 1.3 for the evolutionary benefit of their inability to mobilise renal DA). They proposed that the DA response among the Thai vegetarians with a very low salt intake may have become 'uncoupled' in order to conserve salt. Alternatively, there is a concealed positive DA response which is sluggish and delayed due to disuse.

It is worth noting that blacks are particularly salt-sensitive. Salt restriction or modest salt loading induces an obvious change in MAP (375). One possible reason for this is the lack of an efficient renal DA system among blacks. On salt loading, they either show no changes (370) or relatively small increases (375) in free DA excretion. The 'natriuretic index' (urinary free DA/NA ratio) does not change after salt loading and there is no correlation between this ratio and the sodium output.

However, Barendregt et al. (376,377) in the Netherlands reported that urinary free DA excretion in healthy Caucasian subjects is not affected by the changes in their sodium intake from 50 to 250 or from 50 to 150 and 300 mmol/day. Luippold et al. (378) in Germany reported that urinary free DA excretion in healthy volunteers do not change during low sodium (<85 mmol/day), normal sodium (normal food ad libitum) and high sodium (normal food plus 1.7 mmol/kg/day) intake. However, the dietary intakes of other nutrients could not be entirely controlled (376-378).

Studies in experimental animals

An increase in urinary free DA excretion during oral salt loading has also been shown in the rats (Table 3j).

Table 3j. Increased urinary free DA excretion during the natriuretic response to oral sodium loading in rats.

Authors	Study design	Main findings
Ball et al., 1978 (306)	Wistar rats Low Na diet D1-7 + NaCl, NaHCO ₃ , KCl or NH ₄ Cl on D5-7	Free DA excretion (nmol/d), D3 → D6 NaCl, 7.28 → 14.24 NaHCO ₃ , 8.12 → 5.59 KCl, 6.7 → 8.76 NH ₄ Cl, 8.28 → 12.76
Yamazaki et al., 1986 (379)	Wistar rats Basal (0.26%) or high (3.14%) Na diet for 4 weeks	High vs basal Na diet, D28 Free DA excretion 19.7 vs 9.1 nmol/d Free NA excretion 12.8 vs 4.2 nmol/d Free adrenaline excretion 2.0 vs 0.7 nmol/d PRA 0.3 vs 3.8 ng/ml/h Renal free DA content 22.6 vs 15.5 ng/g No differences in renal NA content
Yoshimura et al., 1987 (380)	Wistar rats Basal (0.26%) or high (3.14%) Na diet	Renal denervated vs sham-operated rats Basal Na diet → higher sodium excretion, lower free NA excretion, no differences in free DA/PGE excretion High Na diet → higher sodium excretion, lower free NA/PGE excretion, no differences in free DA excretion Rats on high vs low Na diet Free DA excretion 44.0 vs 20.5 nmol/d PGE excretion 35.6 vs 12.4 ng/d No differences in free NA excretion Bromocriptine → sodium excretion ↑, PGE excretion ↑, free NA excretion ↓ Carbidopa → free DA/PGE excretion ↓, renal DA content ↓, no changes in sodium/free NA excretion and renal NA content
Grossman et al., 1990 (381)	Dahl SS & SR rats Low (0.1%, w/w) or high (0.8%) Na diet	SS and SR did not differ in urinary responses Free DA and dopa excretion ↑ 6-fold Renal denervation blunted the urinary free DA and dopa responses for 5 days Dopa spillover (from steady-state clearance of i.v. radioactive dopa) and free DA excretion ↑ to the same extent Radioactive dopa/DA ratio in arterial plasma 300 times that in urine

Table 3j. (cont'd)

Hayashi et al., 1991 (382)	Wistar rats Low or high (0.9% saline) Na diet	Free DA excretion ↑ in both innervated and denervated high salt group L-AAAD activity of proximal tubules ↑ Denervation → ↓↓ renal NA content in all, slight ↓ renal DA content in high-salt group
Jadhav et al., 1991 (383)	Sprague-Dawley rats Drank 0.9% saline or water	High salt group → urinary excretions of water, sodium, free DA and DOPAC ↑, ANP ↑ No changes in free NA excretion No changes in renal DA/NA contents Transient ↑ DA ₁ receptor binding sites
Mühlbauer et al., 1993 (384)	Sprague-Dawley rats Controls ± carbidopa high Na ± carbidopa high Na + deoxycorticosterone ± carbidopa	Sodium excretion → ↑ 5-6 fold in high salt ± DOCA groups, ↓ in high salt + DOCA + carbidopa group, no changes in control + carbidopa group Free DA excretion → 27% higher in high salt than in low salt group, ↓ 57-67% in all rats
Sharif et al., 1995 (385)	Sprague-Dawley rats 0.33% (normal Na diet), 0% (low) or 7.8% (high) NaCl for 4 weeks	High salt vs normal salt diet → free DA excretion ↑, plasma aldosterone ↓, no changes in free NA or DA/NA ratio Plasma aldosterone ↑ in low salt diet Diuresis during high salt diet ↓ by carbidopa or SCH 23390, but not domperidone Cortical/medullary DA ₁ and DA ₂ sites ↓ by low salt diet and cortical DA ₁ binding sites ↓ by high salt diet
Ho et al., 1994 (386)	Sprague-Dawley rats Tap water, then 18 g/l NaCl to → drink	Sodium excretion → peak ↑ on D2 Free DA excretion → peak ↑ on D4 (49%) STI (sodium transport inhibitor) excretion → peak ↑ on D5 (6.7-fold) STI excretion parallels sodium excretion, but free DA excretion lags behind
Wang et al., 1997 (387)	Sprague-Dawley rats Normal (0.28% NaCl) or high (4% NaCl) Na diet Response to gludopa i.v. infusion	High salt diet → natriuresis and free DA excretion ↑, but ↓ renal interstitial fluid (RIF) DA Gludopa → natriuresis with ↑↑ free DA excretion than RIF DA Prior bilateral renal denervation did not affect basal DA excretion/RIF DA or gludopa induced natriuresis

Table 3j. (cont'd)

Ho et al., 1997 (388)	Sprague-Dawley rats Low Na/high Na ± carbidopa →	High salt diet → natriuresis, ↓ OLS (ouabain- like substance/Na ⁺ ,K ⁺ -ATPase inhibition Carbidopa ↓ natriuresis during high salt, but not normal salt diet Carbidopa ↑ OLS during low salt diet
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Several important observations from these studies should be highlighted:

- (a). In rats initially maintained on a low salt diet and then given equimolar dietary supplements of NaCl, NaHCO₃, KCl or NH₄Cl (306), urinary free DA excretion increased in rats given NaCl, KCl and NH₄Cl, but decreased in rats given NaHCO₃. These findings argue against a simple effect of ECF volume expansion by sodium ion and suggest that the renal DA response is not specific to the sodium ion. However, since free DA becomes unstable in alkaline urine, the fall in urinary free DA after NaHCO₃ may well be an *in-vitro* phenomenon.
- (b). Renal DA content was higher in rats maintained on a salt diet than in rats on a normal sodium diet (379). This observation is compatible with increased DA synthesis within the kidney during salt loading. Failure of suppression of SNS activity during salt loading contributed to the increase in BP, which could be prevented by ganglionic blockade using hexamethonium.
- (c). Renal denervation has no effects on the increase in urinary free DA excretion in response to oral salt loading (380,382). However, urinary excretion of NA is decreased (380). In addition, salt loading is associated with an increase in L-AAAD activity in the proximal convoluted tubules. These results suggest

that the increase in urine free DA excretion during high salt intake is mainly caused by the enhancement of extra-neuronal DA production by the kidney.

- (d). The increases in urinary free DA excretion associated with salt loading can be accounted for by the increased spillover of dopa into the bloodstream (381). Renal nerves may contribute to the early DA excretory responses since renal denervation can delay the urinary DA and natriuretic responses. There is substantial intrarenal conversion of dopa to DA since the ratio of radioactive dopa to radioactive DA is much more higher in arterial plasma than in urine.
- (e). Quantitative autoradiographic studies have shown that chronic salt loading is associated with a decrease in the number of DA₁ receptors in the renal cortex (385). This finding is compatible with the observations that a high salt diet increases the production of renal DA and that this then causes the observed down-regulation of the cortical DA₁ receptors.
- (f). Using a novel in-situ interstitial microdialysis technique, Wang et al. (387) have characterised the intrarenal DA production and distribution in response to chronic salt loading. DA produced in the kidney is released preferentially into the tubule lumen, rather than peritubular space, and exerts a direct tubular effect on the control of sodium excretion.

3.1.8 *Urinary free DA excretion decreases during salt restriction*

In contrast to oral salt loading, a fall in urinary free DA and sodium output is seen in humans during salt restriction (372,373,389,390). Urinary free DA excretion remains lower than the baseline during the salt restriction period (373,390).

Piaroa Amazonic Indians living in the Amazons Forrest have a very low sodium intake (12.6 mmol/day) compared with urban subjects (210.7 mmol/day) in

northern Venezuela (Section 3.1.6). Consistent with a role for renal DA in sodium homeostasis is the presence of a much lower free DA output (2,055 vs 5,227 nmol/day).

3.1.9 *Intravenous saline infusion increases the urinary excretion of both sodium and free DA*

Intravenous saline infusion increases the urinary excretion of both sodium and free DA. This has been demonstrated both in humans (Table 3k and Table 3l) and experimental animals (Table 3m and Table 3n).

Studies in humans

Table 3k. Increased urinary free DA excretion during the natriuretic response to acute saline infusion in healthy Caucasian subjects.

Authors	Study design / 0.9% NaCl i.v.	Main findings
Alexander et al., 1974 (12)	7 F, 19-23 y* 59 mmol Na D1-3 5% dextrose → NaCl 900/min	Sodium excretion 6.4 → 21.5 mmol/h Free DA excretion 42.4 → 54.9 nmol/h No changes in free NA + adrenaline excretion, GFR or renal blood flow
Castellano et al., 1986 (391)	4 M, 4F, 19-57 y* 120 mmol Na D1-5 2 L/2h	Free DA excretion 128 → 150 nmol/mol cr Free NA excretion 17 → 14 nmol/mol cr
Jeffrey et al., 1989 (392)	9 M, 20-38 y* 20 ml/kg/h for 3h (at 2-5 h) Carbidopa 100 mg or placebo at 0 and 5 h Hydrated	Sodium excretion ↑ 5-fold, peak in 5-6 h, 26% of total Na excreted during study Free DA excretion ↑ 26% in the 5-6 h and lasted until the end of study (10-11 h) PRA ↓, plasma AII ↓, plasma ANP ↑ Carbidopa ↓↓ free DA excretion without effect on sodium excretion

Table 3k. (cont'd)

Stenvinkel et al., 1991 (393)	9 M, 31-40 y* 25 ml/kg for 2 h Hydrated	Sodium excretion ↑ 46% Free DA excretion ↑ 12.8% in 2nd and 3rd h, 16.2% in 4th h, 9.5% in 5th h PRA ↓, plasma ANP ↑, GFR ↓ No changes in renal plasma flow
Stenvinkel et al., 1992 (394)	4 M, 6F, 21-49 y* 0.9% NaCl vs 5% dextrose 25 ml/kg for 2 h Hydrated	Fractional excretion of sodium progressively ↑ from 1st h, max 85.7% (4th h) Free DA excretion ↑ 16% (2nd h), 16.5% (3rd h) and 15.6% (4th h) Free NA excretion ↓ 21.4% (4th h) Plasma ANP ↑ 59% (2nd h) and 27% (4th h) PRA ↓, GFR ↓ in 1st and 2nd h 5% dextrose → fractional excretion of sodium and free DA excretion did not change, free NA excretion ↑ 33.3% (4th h), plasma ANP ↑ 44.3% (2nd h), PRA transiently ↓
Stokes et al., 1993 (395)	8 M, 4 F* 100 mmol Na 2 L/3 h plus carbidopa 100 mg, indomethacin 50 mg or placebo Urine collections (90 min) x 5 (U1-5) starting at -90 min (U1)	27% of 300 mmol Na excreted in U1, 65.7% by U2 and 111% by U3 Urinary free DA/NA ratio 9.2 → 34.1 Plasma ANP ↑ up to 86.3% (U4) No changes in PGE ₂ and kallikrein excretion Carbidopa → ↓↓ sodium excretion in 2 of 6 subjects, urinary DA/NA ratio ↓ 1.6 (U1), 1.1 (U2), 0.9 (U3) and 3.4 (U4) Indomethacin → 3 of 6 subjects with ↓↓ sodium excretion, urinary DA/NA ratio 15.3-19 (U1-3), PGE ₂ excretion ↓ (U1)
Stenvinkel et al., 1997 (396)	15 subjects, 34 ± 1 y* 12.5 mg/kg/h for 2 h	Free DA excretion ↑ 14%
Stokes et al., 1997 (397)	14 M, 6 F, 28 ± 3 y* 100 mmol Na diet 2 L/3 h plus Carbidopa 100 mg, indomethacin 50 mg or placebo Hydrated (1st collection) Hydrated	Sodium excretion ↑, urinary DA/NA ratio ↑, plasma albumin ↓, plasma ANP ↑, PRA ↓, plasma aldosterone ↓, no changes in urinary PGE ₂ Carbidopa → urinary DA/NA ratio ↓↓, no effects on other parameters Indomethacin → natriuresis ↓, ↑ distal sodium reabsorption, PGE ₂ ↓ transiently, no effects on other parameters

Table 3k. (cont'd)

Stokes et al., 1997 (398)	6 M,4 F, mean 27 y* 100 mmol Na diet 2L/3 h or 200 ml/3h 4% dextrose- 0.18% saline plus carbidopa 100 mg or placebo Urine collections (90 min) x 5 (U1-5) starting at -90 min (U1) Hydrated Free DA measured in aliquots and , analysed under pH 1-1.5 and 2-2.5	Saline infusion → natriuresis, urine free DA (at pH 2-2.5) ↑ in U1-4, but no significant changes overall, free DA (57 ± 23%) and conjugated DA (1,622 ± 497%) ↑ U1-5, no changes in free/conjugated NA, free DA/NA ratio 6.1 (U1) and 8.2 (U5), conjugated DA/NA ratio 6.1 (U1), 58.3 (U5), free DA (at pH 1-1.5) ↑ 225 ± 97% U1-5, ↓ plasma albumin, PRA, aldosterone, Carbidopa → p=NS for ↓ natriuresis U3-4, and ↓ cumulative sodium excretion (6.3%), free DA excretion ↓ U1-5, no changes in conjugated DA, free DA/NA ratio 76-98% (U1-4) and 210% (U5), conjugated DA/NA ratio 88-122% (U1-5)
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*All Caucasian subjects?

Hydrated = water was given at regular intervals to ensure adequate urine flow rates.

Several important observations from these studies should be highlighted:

- (a). After intravenous saline infusion, the magnitude of increases in urinary free DA excretion is considerably small (up to 34%) compared to the changes in sodium excretion (up to 5-fold).
- (b). In the study of Jeffrey et al. (392), free DA excretion increased after saline infusion and remained elevated at the end of the study, at a time when more than 70% of the sodium load remained to be excreted. In the studies of Stenvinkel et al. (393,394), free DA excretion increased during the second hour of infusion and remained elevated for two hours post-infusion. It is conceivable that renal DA assumes greater significance during the later stages of the natriuretic response.

- (c). The preliminary observations made by Stokes et al. (395) suggest that some subjects can be identified as having renal DA- or prostaglandin-dependent mechanisms in the early phase of natriuretic response to saline infusion.
- (d). Stokes et al. (397,398) subsequently showed that the natriuretic response to saline infusion is associated with an increase in the 'natriuretic index' (urinary free DA/NA ratio). Although there was an increase in free DA excretion during the infusion (398), the change did not reach statistical significance by repeated measures of analysis of variance. Carbidopa failed to attenuate the natriuretic response despite a fall in urinary free DA excretion (in contrast to the studies summarised in Table 3m on page 80 and Section 3.1.10).
- (e). Stokes et al. (398) showed that conversion of conjugated DA to free DA can occur *in-vitro* if urine aliquots are acidified to a pH of 1.0-1.5 instead of 2.0-2.5. To prevent unwanted cardiovascular and renal effects, excessive DA may be conjugated (Section 3.1.1 and Section 3.2.4) as soon as it is formed. Thus, there is a surge in excretion of conjugated DA after saline infusion.

Increased urinary free DA excretion is also seen in healthy Japanese subjects during the natriuretic response to intravenous saline infusion (Table 3l). However, Barendregt et al. in the Netherlands (376,377) could not confirm an increase in free DA excretion during the natriuretic response to saline infusion in Caucasian subjects. During the low sodium intake period (50 mmol/day), a sodium load (38 mmol) was given as 500 ml glucose 2.5%/NaCl 0.45% over two hours. During the high sodium intake period (250 mmol/day), a sodium load (154 mmol) was given as 1,000 ml of 0.9% NaCl over two hours. On both diets, the saline infusions enhanced sodium excretion but did not increase free DA excretion.

Table 3l. Increased urinary free DA excretion during the natriuretic response to acute saline infusion in healthy Japanese subjects.

References	Study design / 0.9% NaCl i.v.	Main findings
Kawabe et al., 1991 (399)	4 M, 3 F, 49 ± 3 y 100 mmol Na D1-10 500 ml/h for 3 h Hydrated	Sodium excretion ↑ 151% Free DA excretion ↑ 34% PRA and plasma aldosterone ↓ Plasma ANP ↑, GFR ↑ No changes in plasma/urine free NA/adrenaline
Kawabe et al., 1992 (400)	5M, 3F, 46 ± 3 y 100 mmol Na D1-10 500 ml/h for 3 h Hydrated	Sodium excretion ↑ 47% Free DA excretion ↑ 64% PRA and plasma aldosterone ↓ Plasma ANP ↑, GFR ↑

Studies in experimental animals

Table 3m. Increased urinary free DA excretion during the natriuretic response to acute saline infusion in anaesthetised dogs.

References	Study design / 0.9% NaCl i.v.	Main findings
Faucheux et al., 1977 (401)	1 ml/kg/min for 20 min, then 0.5, 25% albumin 6.5 ml/kg over 20 min or no treatment	Saline → sodium excretion ↑ 840%, fractional sodium excretion ↑ 811%, urine volume ↑ 270%, free DA ↑ 811%, fractional clearance of DA ↑ 138%, % ↑ DA excretion related to fractional sodium excretion (r=0.41, p<0.05), plasma free NA + adrenaline ↓ 53.8%, free NA + adrenaline excretion ↓ 60.9%, no changes in plasma DA or GFR, renal plasma flow ↑ 19% Albumin → plasma NA + adrenaline ↓ 51.2%, free NA + adrenaline excretion ↓ 58.2%, renal plasma flow ↑ 20%, no changes in urine sodium/free DA, plasma DA or GFR

Table 3m. (cont'd)

Cuche et al., 1983 (402)	<p>Hypotonic (0.45% NaCl or dextrose 2.5%) 2 for 45 min → 1 ml/kg/min</p> <p>Isotonic saline 1 for 45 min → 0.5 ml/kg/min</p> <p>Hypertonic (1.8% NaCl or Na SO₄) 0.5 for 45 min → 0.25 ml/kg/min</p>	<p>Comparable ↓ plasma protein and ↑ GFR/renal plasma flow</p> <p>Plasma sodium/osmolality ↓ in hypotonic, ↑ in hypertonic, no changes in isotonic</p> <p>Fractional excretion of sodium ↑ 30-fold in hypotonic, 7-fold in isotonic and 16-fold in hypertonic groups</p> <p>Free DA excretion ↑ 53.4% in isotonic group, ↑ 31.3% in hypertonic group, no change in hypotonic group</p> <p>Free NA excretion ↓ 52.9% in hypotonic, no changes in isotonic or hypertonic groups</p>
Sowers et al., 1984 (403)	<p>30 ml/kg/h x 2 h with or without carbidopa</p>	<p>Sodium excretion ↑ 415% (during infusion) and 289% (post infusion)</p> <p>Free DA excretion ↑ 267% (during infusion) and 106% (post infusion)</p> <p>Correlation between urinary sodium and free DA during infusion ($r=0.72$, $p<0.025$)</p> <p>Urine volume ↑ 253% (during infusion) and 107% (post infusion)</p> <p>Free NA excretion ↓ 72.3% (during infusion) and 57.2% (post infusion)</p> <p>No changes in MAP, GFR or renal plasma flow</p> <p>Carbidopa → no increases in free DA and sodium excretion during saline infusion</p>
McClanahan et al., 1985 (404)	<p>30 ml/kg/h x 2 h with or without carbidopa</p>	<p>Sodium excretion ↑ during (1st h 190%, 2nd h 621%) and post (279%) infusion</p> <p>Free DA excretion ↑ during (1st h 24.2%, 2nd h 227%) infusion</p> <p>Correlation between urinary sodium and free DA during infusion ($r=0.72$, $p<0.025$)</p> <p>Urine volume ↑ during (2nd h 346%) and after (146%) infusion</p> <p>Free NA excretion ↓ during (1st h 40.6%, 2nd h 73%) and post (57.2%) infusion</p> <p>PRA and plasma aldosterone ↓</p> <p>No changes in MAP, GFR or renal plasma flow</p> <p>Carbidopa → sodium excretion ↓ 40% (in 2nd h of infusion c.f. control), sodium load excreted during infusion ↓ 35%, free DA excretion ↓ during infusion (57% in 1st and 86% in 2nd h)</p>

Table 3m. (cont'd)

Boren et al., 1980 (405)	5% of body weight over 30 min	Sodium excretion ↑ 933%, clearance of sodium ↑ 1,003%, free DA excretion ↑ 56% DA clearance ↑ 53.6%, fractional clearance of DA ↑ 27.1% No changes in renal blood flow, GFR, plasma DA, plasma/urinary NA or filtered DA
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Several important observations are summarised here:

- (a). During the natriuretic response to saline infusion, the SNS activity (urinary free NA and adrenaline output) decreased quite rapidly, whereas the renal DA response was more sluggish (401). Both the renal and fractional clearance of free DA increased in the absence of changes in plasma DA, suggesting that the increase in urine DA after salt loading is of renal origin. The positive correlation between increases in urine free DA and the fractional excretion of sodium suggests that renal DA regulates sodium excretion. An increase in urinary DA excretion occurred after saline infusion but not after albumin infusion despite a similar reduction in NA and adrenaline excretion. This observation argues in favour of a specific effect of sodium or sodium chloride on intrarenal DA synthesis and against a 'spillover' of DA from SNS neurons.
- (b). Cuche et al. (402) could show a dissociation between urinary sodium and free DA excretion using hypotonic saline infusion. This finding does not provide support for a physiological role of renal DA during ECF volume expansion.
- (c). Carbidopa eliminates the increase in urinary free DA excretion and markedly attenuates the natriuretic response to saline infusion (403,404). It appears that renal production of DA is an important mechanism mediating the natriuretic response to acute ECF volume expansion with saline infusion.

Table 3n. Increased urinary free DA excretion during the natriuretic response to acute saline infusion in anaesthetised rats.

Authors	Study design / 0.9% NaCl i.v.	Main findings
Hansell et al., 1988 (406)	Sprague-Dawley rats Ringer solution 2ml/ 0.1 kg over 1 h (2% of BW per h) with or without haloperidol	Sodium excretion ↑ 17-fold Free DA excretion ↑ 37.9% DOPAC excretion ↑ 29.9% Plasma ANP ↑ Urine volume ↑ 123%, GFR ↑ 19.8% No changes in free NA excretion or BP Haloperidol → greater ↑ in plasma ANP and free DA/DOPAC excretion, ↓ natriuretic/diuretic responses by 45%
Hegde et al., 1989 (407)	Sprague-Dawley rats 20.4 ml over 1 h with or without SCH-23390	Sodium excretion ↑ during (max 60-fold) and after (38 → 18-fold) infusion Urine volume ↑ during (max 20-fold) and after (11 → 4-fold) infusion Free DA excretion ↑ during (max 4.7-fold) and after (6.2 → 3.5-fold) infusion SCH-23390 ↓ the natriuresis/diuresis
Chen et al., 1991 (408)	Sprague-Dawley rats Volume expansion, modest (9 ml = 2.5% BW), moderate (18 ml = = 5% BW) or severe (36 ml = 5% BW) over 1 h	Modest → sodium excretion ↑ 31-fold, free DA excretion ↑ 26%, urine volume ↑ 14-fold, correlation in urine sodium/ DA pre/during infusion ($r=0.63$, $p<0.05$) Moderate → sodium excretion ↑ 60-fold, free DA excretion ↑ 48%, urine volume ↑ 23-fold, correlation in urine sodium/ DA pre/during infusion ($r=0.82$, $p<0.01$) Large → sodium excretion ↑ 123-fold, free DA excretion ↑ 57%, urine volume ↑ 23-fold, correlation in urine sodium/ DA pre/during infusion ($r=0.82$, $p<0.01$)
Bass et al., 1991 (409)	Sprague-Dawley rats Volume expansion, small ($2 \pm 0.1\%$ ↑ BW) or large ($17.9 \pm 0.6\%$ ↑ BW)	Small → sodium excretion ↑ 155% (69% after SCH 23390), urine volume ↑ 59%, free DA excretion ↑ 25% Large → sodium excretion ↑ 735%, urine volume ↑ 1,026%, no changes in free DA

Table 3n. (cont'd)

Hegde et al., 1992 (410)	Sprague-Dawley rats 20.4 ml (6% of BW) over 1 h With or without intact vagi or renal nerves ± SCH 23390	Rats with intact vagi vs vagotomised rats similar ↑ in sodium excretion/urine volume, free DA excretion ↑ in rats with intact vagi, SCH 23390 ↓ natriuresis/diuresis in rats with intact vagi Rats with innervated vs denervated kidneys denervated kidney showed similar sodium excretion/urine volume and similar ↑ urine free DA but greater ↑ in sodium excretion/urine volume after infusion SCH 23390 ↓ natriuresis and diuresis in denervated kidneys
Hansell et al., 1996 (411)	Sprague-Dawley rats ± adrenalectomy NaCl (5% of BW)	Urine sodium ↑ >13-fold and free DA ↑ 42% (>14-fold and 36% in adrenalectomised rats) Plasma dopa, adrenal > systemic before (62%) and after (42%) infusion in both groups Dopa excretion was similar in both groups
Reddy et al., 1998 (412)	Wistar rats Normal or high salt diet + ANP infusion, saline infusion (8 ml/h), both or none	Sodium excretion ↑ by high salt diet Fractional excretion of sodium ↑ by both ANP infusion and saline infusion Synergistic effect of ANP/saline infusion on sodium excretion during normal salt intake Free DA excretion ↑ with a high salt diet Free DA excretion ↑ further after saline infusion during a normal or high salt diet Free DA excretion ↑ even further after saline and ANP infusion during a normal or high salt diet Benserazide to inhibit DA formation ↓ free DA and sodium excretion

Several important observations from studies in rats are highlighted here:

- (a). The natriuretic and diuretic responses to acute ECF volume expansion with saline infusion are accompanied by increased free DA excretion and are markedly attenuated by DA receptor blockade with haloperidol or SCH-23390 (406,407). These findings provide strong support for a role of renal

DA in mediating the natriuretic and diuretic responses to acute ECF volume expansion.

- (b). Chen & Lokhandwala (408) showed that the relative contribution of renal DA to acute ECF volume expansion-induced natriuresis is related to some extent to the degree of volume expansion. With modest to moderate increments in the ECF volume (2.5 to 5% body weight), the kidney can adjust the renal production of DA, as evidenced in the finding that moderate ECF volume expansion produces further increase in free DA excretion in comparison with modest expansion (48 vs 26%). However, with further increase in the degree of ECF volume expansion (10% body weight), the kidney is unable to produce more DA. Under this condition, other factors probably play a more important role in the natriuretic response.
- (c). Bass & Murphy (409) showed that renal DA contributes to the natriuresis produced by small (2% of body weight), but not large (18% of body weight), increases in ECF volume induced by saline infusion. Under the former but not the latter condition, a concomitant increase in free DA excretion is seen and the natriuresis is attenuated by DA₁ antagonist. Hedge & Lokhandwala (410) also showed that the effects of DA receptor blockade on natriuresis produced by saline infusion is dependent on the degree of hypervolaemia. The renal DA system appears to be relatively more important in promoting natriuresis at the lower (physiological) range of hypervolaemia, whereas in the higher range, other factors probably have a greater impact.
- (d). Hedge & Lokhandwala (410) tried to identify the mechanism whereby an intravenous sodium load and/or ECF volume expansion stimulate the renal production of DA. Unlike rats with intact vagi, bilaterally vagotomised rats

did not show an increase in urinary DA excretion after saline infusion. DA₁ receptor blockade also attenuated the natriuretic and diuretic responses in rats with intact vagi, but failed to do so in vagotomised rats. The results suggest that in rats renal DA contributes to the natriuretic and diuretic responses to ECF volume expansion through a reflex involving the vagus nerve. Vagal afferents originating in the cardiopulmonary region may relay to the central nervous system information regarding the ECF volume status. However, even without the contribution from renal DA, vagotomised rats were equally capable of handling a salt load. It has been speculated that activation of vagal afferents during ECF volume expansion leads to the release of a humoral substance which in turn mediates the increase in endogenous DA production. Alternatively, a decrease in the circulating level of a substance having a tonic inhibitory effect on DA synthesis could be responsible.

- (e). During 0.9% saline infusion, urinary sodium and free DA excretion increased similarly in both intact and adrenalectomised rats. Hansell et al. (411) therefore concluded that the adrenal glands are only minor suppliers of plasma L-dopa and minor sources of urinary free DA.
- (f). Reddy et al. (412) compared the renal mechanisms of natriuresis in acutely and chronically ECF volume-expanded rats. Circulating ANP did not play a significant role in the natriuresis of chronic salt loading. The synergism between ECF volume expansion and ANP did not reside in changes of proximal tubular sodium transport but was a property of more distal nephron segments where both ANP and DA, induced by chronic salt loading, may share a common intracellular pathway to inhibit tubular sodium reabsorption.

3.1.10 *Studies using inhibitors of L-AAAD*

The DA-synthesising enzyme L-AAAD has been identified in proximal tubule cells of the kidney by immunohistochemistry (413,414), biochemical (382) and in-situ hybridization (415) methods.

Studies using inhibitors of L-AAAD have provided further evidence that intrarenal DA plays an important role in regulating renal excretion of sodium:

- (a). Ball & Lee (15) studied the effect of interference with renal DA synthesis on urinary sodium excretion in healthy subjects receiving their usual diets. They found that carbidopa causes a fall not only in urinary free DA excretion but also in urinary sodium excretion.
- (b). Carbidopa markedly reduces the urinary free DA output and attenuates the natriuretic response to salt loading (Section 3.1.7 and Section 3.1.9). However, because of the abundance and redundancy of compensatory systems determining sodium balance, the effects of inhibition of endogenous DA synthesis (and of any other systems) may be transient and small.
- (c). Some healthy subjects in the early phase of natriuresis after an intravenous saline load can be identified as having a DA-dependent mechanism for sodium excretion. Carbidopa reduces the renal excretion of both free DA and sodium in such subjects (Section 3.1.9).
- (d). Bertorello et al. (414) reported that the proximal tubules from rats maintained on a high sodium diet (8.25 mmol/day) for 10 days showed the expected decrease in $\text{Na}^+\text{-K}^+\text{-ATPase}$ activity compared to rats maintained on a normal salt diet. This difference was abolished by pretreatment with the L-AAAD inhibitor, benserazide.

- (e). Protein ingestion leads to increased urinary excretion of free DA (22-50%), sodium (36-125%) and water (24-31%) in healthy subjects aged 20-43 years (416,417). Suppression of DA excretion by carbidopa markedly attenuates the natriuretic and diuretic responses to protein ingestion. The concordant responses of both DA and sodium to protein ingestion (416,417) and to carbidopa (416,417) and the correlation between urinary sodium and DA (416) support a role for DA in the regulation of sodium excretion.
- (f). Carbidopa inhibits the increase in free DA excretion and abolishes the renal actions of gludopa in healthy subjects (Section 3.1.5).

3.1.11 *Studies using DA receptor antagonists*

Pharmacological evidence supporting the importance of renal DA in sodium homeostasis is provided by studies involving the use of non-selective DA antagonists (metoclopramide, haloperidol and *cis*-flupenthixol), selective DA₁ antagonists (SCH-23390 and (+)-sulpiride) and selective DA₂ antagonist (domperidone).

- (a). Infusion of SCH-23390 into the renal artery of uninephrectomized dogs (418) and rats (419) produces a dose-dependent reduction in urine volume, urinary sodium excretion and fractional excretion of sodium. Rebound natriuresis and diuresis occur after the drug is stopped (418).
- (b). In healthy subjects, metoclopramide attenuates the natriuretic response to ECF volume expansion produced by saline infusion (17,420).
- (c). In rats, prior treatment with haloperidol (406,421), SCH-23390 (407,409,410) or *cis*-flupenthixol (422) attenuates the natriuretic response to ECF volume expansion produced by saline infusion.

(d). In healthy subjects, (+)-sulpiride attenuates the natriuresis and the acute fall in PRA seen with gludopa (347). Domperidone inhibits the fall in PRA but does not affect the natriuretic response to gludopa infusion (346).

Hansell & Fasching (421) emphasised that the effect of DA blockade on natriuresis is dependent on the degree of hypervolaemia, with the renal DA system being relatively more important in promoting natriuresis at the lower range of hypervolaemia. This may be the reason why metoclopramide has no effects in rats on the natriuretic response to saline infusion equal to 10% rise in body weight (423).

However, Bradley et al. (424) were unable to block the natriuretic response to saline infusion in dogs using either SCH-23390 or domperidone.

3.1.12 *Evidence from studies involving water immersion*

Head-out water immersion (WI) produces a prompt cephalad redistribution of circulating blood volume. The increase in central blood volume produces natriuresis and diuresis. This physiological manipulation provides a model to investigate the mechanisms governing volume homeostasis during acute hypervolaemia.

In healthy subjects submitted to WI, the natriuresis and diuresis is accompanied by an increase in plasma ANP and endogenous inhibitor Na^+, K^+ -ATPase and a decrease in SNS activity, PRA and plasma aldosterone (425,426). The natriuresis associated with a normal sodium intake (100 mmol/day) is significantly greater than that of a low sodium intake (20 mmol/day) (425).

The natriuresis and diuresis produced by WI can be blunted by DA blockade with metoclopramide (427,428) or domperidone (428). This finding suggests that the DA system contributes to the natriuretic response produced by WI.

3.1.13 Evidence from *in-vitro* studies

Rat kidney slices can synthesise DA when incubated with its precursor, L-dopa, or the DA pro-drug, gludopa. This has been shown by histofluorescence (429) and biochemical studies (430-432). The DA formed is histologically extraneuronal, intracellular and localised in segments S1 and S2 of proximal tubules in innervated as well as in denervated kidneys (429). However, DA fluorescence is not seen in the presence of a L-AAAD inhibitor (benserazide) or in the absence of sodium (429), suggesting that the synthesis of DA by kidney tubules is dependent on the presence of both L-AAAD and sodium.

In isolated rat kidney preparations, perfusion with L-dopa or gludopa results in an increased release of free DA into both the urine and the perfusate (431). Both L-dopa and gludopa also induce a concentration-dependent renal vasodilation, which can be abolished by the DA₁ antagonist, SCH-23390.

Renal tubular Na⁺,K⁺-ATPase, which regulates sodium transport, is inhibited by DA (18,414,432-435). The inhibitory effect of DA on proximal tubule Na⁺,K⁺-ATPase activity is enhanced by a high salt diet (414) or when the intracellular sodium concentration is increased (432). In rats, uninephrectomy results in increased renal DA activity and DA-sensitive enhanced natriuresis (435). Apart from in the proximal tubule, DA inhibits Na⁺,K⁺-ATPase activity in the cells of the distal nephron (434). The inhibition of sodium transport in renal proximal tubules by DA is also exerted via the Na⁺-H⁺ exchanger at the luminal or brush border membrane (see Section 3.2).

In-vitro studies have also helped to identify the putative DA₁ receptor-mediated signal transduction pathways (see Section 3.2). This action is mediated via DA₁ receptors.

3.2 The formation, actions and inactivation of intrarenal DA

3.2.1 The formation of DA at the proximal tubule

The synthesis of DA at the proximal tubule is summarised in Figure 3a.

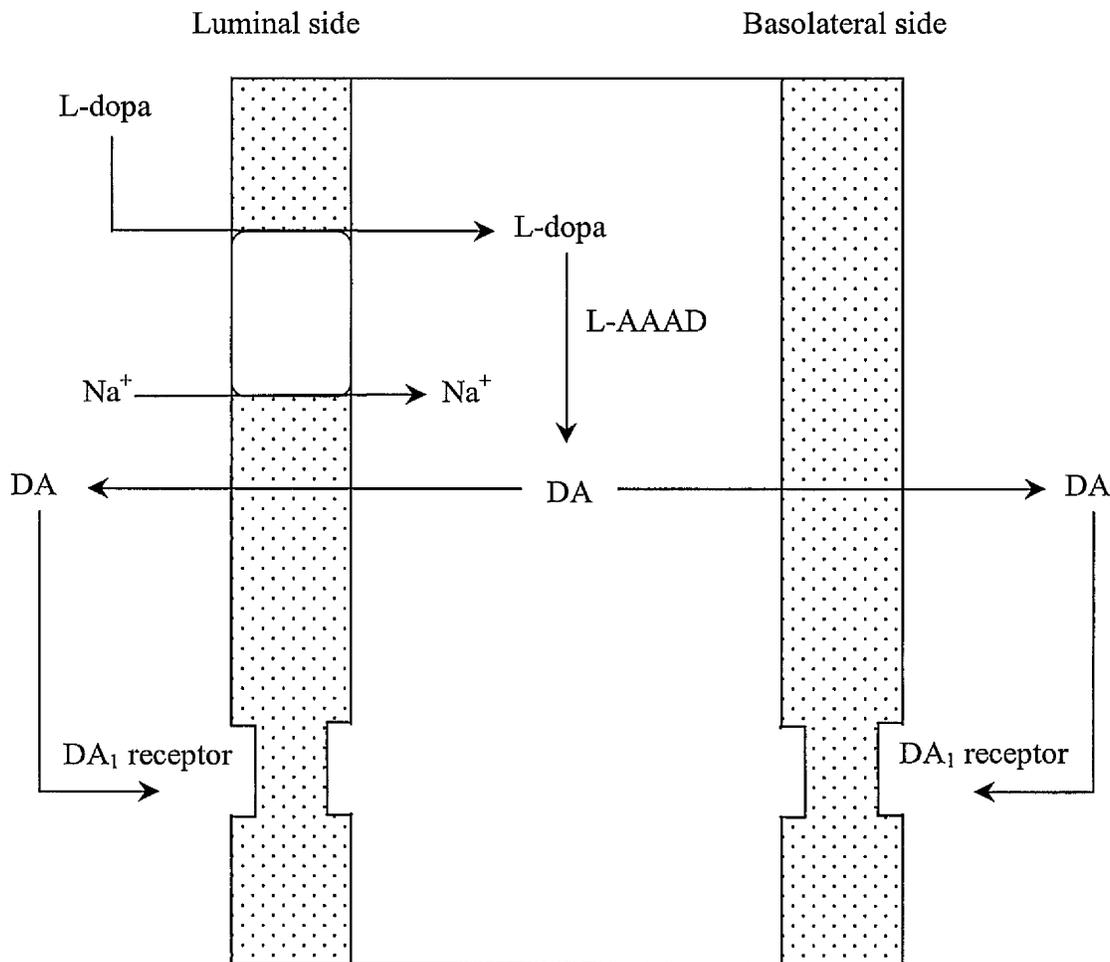


Figure 3a. A schematic representation depicting the formation and actions of DA at the level of the proximal tubule.

As discussed in the text, filtered L-dopa present in the tubular lumen can be taken up by the proximal tubule and converted into DA. This DA can exit the cells and acts as an autocrine or paracrine factor. The stimulation of DA₁ receptors in the renal tubules will produce a natriuresis; the stimulation of DA₁ receptors in the renal vasculature will produce vasodilation and increase the GFR.

Adapted from Lokhandwala & Amenta (291).

L-dopa from the plasma is filtered at the glomerulus

The proximal tubule cells lack tyrosine hydroxylase, the rate-limiting enzyme for catecholamine synthesis in neuronal tissue which is responsible for hydroxylating tyrosine to L-dopa (436). L-dopa necessary for DA production must, therefore, be supplied to the proximal tubular cells from the glomerular filtrate (or the blood from the peritubular space).

L-dopa is taken up into the proximal tubular cells by active transport

Using microperfusion and capillary perfusion techniques, Chan (437) has shown in the rat that L-dopa is absorbed from the proximal renal tubule very avidly. This process is not affected by L-AAAD inhibitors, suggesting that it does not depend on intratubular decarboxylation mechanisms. Cyanide can block this uptake process, suggesting that this is an energy-dependent process.

L-dopa transporters are located both in the apical and basolateral membranes (291). L-dopa enters proximal tubule cells via a sodium cotransporter (Figure 3a).

The active transport of L-dopa is stimulated by the sodium ion

As with most transport systems for amino acids, the transport of L-dopa across the apical and basolateral membranes of the proximal tubular cells is sodium-dependent and stimulated by sodium ions (438).

L-dopa inside the proximal tubular cells is converted by L-AAAD to free DA

Once within the proximal tubular cells, the L-dopa will be exposed to the high concentrations of L-AAAD that exist there and will be rapidly converted to DA.

Baines & Chan (439) injected L-dopa into the proximal tubule or peritubular space of innervated and denervated rat kidneys. They reported a rapid conversion of L-dopa to urinary free DA. There were no important differences between the innervated and denervated kidneys. They estimated that at least 30% of urinary free DA is derived from circulating L-dopa. When L-tyrosine was injected into the proximal tubule, they found very little conversion to urinary free DA even in the innervated kidney.

Rat kidney slices can synthesise DA when incubated with its precursor, L-dopa (Section 3.1.13). The synthesis of DA by kidney tubules is dependent on the presence of both L-AAAD and sodium. The latter may facilitate the uptake of L-dopa into proximal tubular cells (Section 3.2.2).

In the isolated rat kidney perfused with L-dopa, increased release of free DA into urine and perfusate is seen (Section 3.1.13).

cGMP may be involved in the regulation of DA synthesis, probably through the control of the entry of L-dopa and sodium into the proximal tubular cells (440,441). In rat kidney slices incubated with exogenous L-dopa, the addition of cGMP phosphodiesterase inhibitors (which prevent the degradation of cGMP) to the incubation medium decreases DA synthesis.

DA so formed is transported out of proximal tubular cells before it can effect a natriuresis and vasodilation

In order to be active, DA formed in proximal tubular cells has to leave the compartment where the synthesis has occurred, since the DA receptors are believed to be located on the external surface of the cell membrane. The outflow of newly formed DA in both dog and rat kidney slices loaded with exogenous L-dopa follows

Michaelis-Menten kinetics with a saturable component and a non-saturable one, the latter assuming importance only at higher concentrations of DA (442).

A substantial fraction of DA formed in the proximal tubule will be secreted into the luminal space where it may act, via the DA₁ receptors, as a paracrine or autocrine mediator of renal haemodynamic and/or tubular function (443). DA may also be secreted into the peritubular space. From the peritubular space, DA can be transported via the capillary network and act as a paracrine factor on the basolateral membrane of tubular cells.

In the proximal tubule, where the most significant amount of natriuresis is induced, DA exerts its action via inhibition of the two principal sodium transporters: the basolateral Na⁺-K⁺-ATPase (Section 3.1.13) and apical Na⁺-H⁺ exchanger (444).

The outward transfer of DA in renal tissues seems to involve the activation of a carrier-mediated process (442) and probably involves changes in the activity of the Na⁺-H⁺ exchanger (445). While the flux of sodium ions across tubular epithelial cells is largely dependent on Na⁺-K⁺-ATPase activity, the Na⁺-H⁺ exchanger on the luminal membrane plays a major role in the transport of sodium ions into these cells. This luminal Na⁺-H⁺ exchanger is thought to be responsible for the bulk of active sodium reabsorption in the proximal tubule. In rat kidney slices loaded with L-dopa, inhibition of the Na⁺-K⁺-ATPase results in a considerable reduction in the outflow of newly formed DA, whereas activation of the Na⁺-H⁺-exchanger facilitates the outflow of DA (445).

Under certain conditions (e.g. positive sodium balance and ECF volume expansion), renal DA₂ receptors may enhance the ability of DA₁ receptors to inhibit sodium transport both in the renal proximal tubule and in more distal nephron segment by synergistic effects on signal transducers (297).

3.2.2 *The regulation of renal DA synthesis*

Sodium intake is the most important factor regulating renal DA synthesis.

The extracellular sodium

The active reabsorption of L-dopa and other amino acids from the proximal tubule is a carrier-mediated process stimulated by sodium ions (438). This may be one of the mechanisms whereby oral (Section 3.1.7) and intravenous (Section 3.1.9) sodium loading and hence the local sodium concentration in the kidney can stimulate the renal production of free DA.

As already discussed in Sections 3.1.7 and Section 3.1.9, salt loading results in an increased delivery of L-dopa to sites of uptake by proximal tubular cells and hence an increase in free DA synthesis.

Production of free DA by renal tissues under *in-vitro* conditions also appears to be dependent on the sodium chloride concentration in the medium (431,446).

The transtubular reabsorption of sodium

If tubular sodium regulates intrarenal DA synthesis, inhibition of sodium reabsorption should reduce free DA production from L-dopa by the proximal tubule. In fact, ANP has been reported to produce a marked reduction in the synthesis of DA by rat kidney slices loaded with L-dopa (441). This natriuretic agent inhibits tubular sodium reabsorption following the activation of specific receptors. These receptors are coupled to guanylate cyclase, the activation of which results in an increase in either tissue cGMP levels or its urinary excretion (447). In rat kidney slices incubated with L-dopa, the addition of cGMP phosphodiesterase inhibitors or a cGMP derivative decreases DA synthesis (440,441). These data suggest that

increased intrarenal accumulation of endogenous cGMP modulates tubular sodium reabsorption and may restrict the uptake of L-dopa by tubular epithelial cells.

Ouabain is an inhibitor of $\text{Na}^+\text{-K}^+\text{-ATPase}$, which control the net transtubular transport of sodium in proximal renal tubules. In rat kidney slices incubated with L-dopa, ouabain produces a concentration-dependent inhibition of the formation of DA (446), suggesting that the tubular transport of L-dopa is sodium-dependent.

The intrarenal production of DA is dependent on the integrity of the tubular transport of sodium, namely on the association between the actin cytoskeleton and $\text{Na}^+\text{-K}^+\text{-ATPase}$ in tubular epithelial cells. Damage of the actin cytoskeleton leads to a modification in the localisation of $\text{Na}^+\text{-K}^+\text{-ATPase}$ in the cell wall which ultimately results in a decrease in the tubular reabsorption of sodium (448). In rat kidney slices loaded with L-dopa, cytoskeleton disrupter compounds inhibit the formation of DA (449).

Protein intake and feeding

In healthy subjects, protein ingestion leads to an increase in urinary free DA excretion and natriuresis, both of which are markedly attenuated by carbidopa (416,417). A protein meal provides an amino acid load including tyrosine, which is converted to dopa via tyrosine hydroxylase predominantly in peripheral tissues that contain catecholamine-synthesising cells such as sympathetic nerve terminals and chromaffin-containing tissues. Urinary DA is then produced by the action of L-AAAD on dopa.

Rats fed a high protein meal also show an increase in sodium and free DA excretion (450,451). Another study, however, has shown that feeding, but not salt loading, is the dominant factor controlling urinary free DA excretion in rats (452).

Potassium intake

In normotensive elderly subjects, potassium chloride (40 mmol/day) given intravenously increases the urinary excretions of sodium, DA and potassium (453). Spironolactone given orally (75 mg/day) increases sodium excretion but decreases the excretion of potassium and DA. In rats maintained on a low salt diet, free DA excretion increases after dietary supplements of potassium chloride (306).

Calcium intake

Dazai et al. (454) studied the effects of oral calcium supplementation (1 g/day for one week) on renal DA activity in patients with mild to moderate hypertension. Urinary excretion of free DA, sodium clearance and fractional clearance of sodium were increased. Sodium clearance and fractional excretion of sodium showed significant correlations with urinary free DA. These results suggest that oral calcium supplements induce natriuresis partly through augmentation of renal DA activity.

Phosphate intake

Berndt et al. (455) showed in rats that increasing dietary phosphate intake augments the urinary excretions of free DA and its metabolite DOPAC. They also showed that this increase in free DA excretion occurred whether or not the renal nerves were present (456).

3.2.3 *Cellular signalling processes coupled to DA₁ receptors*

The messenger systems coupled to renal DA₁ receptors are outlined in Figure 3b.

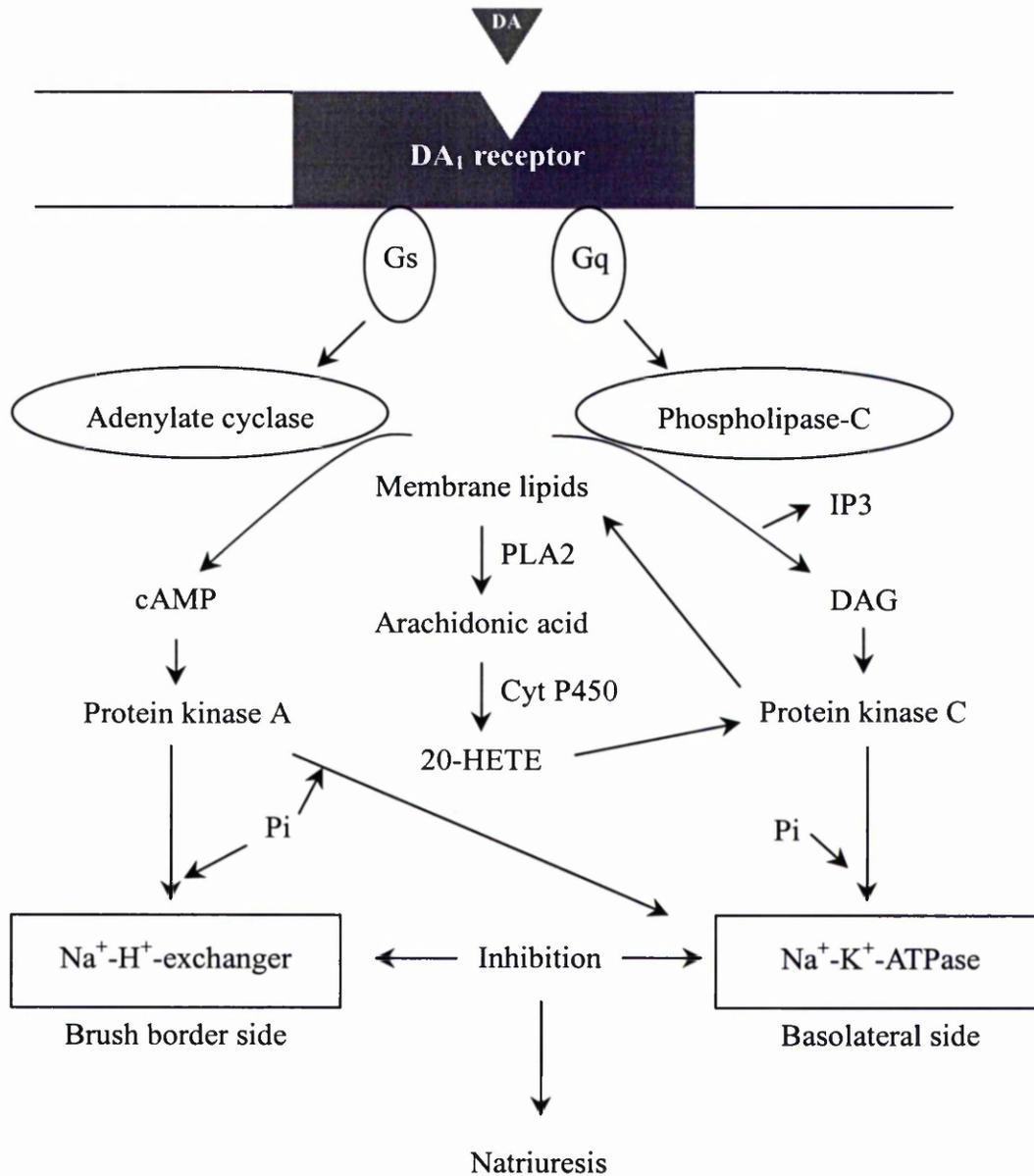


Figure 3b. A schematic presentation of DA₁ receptors and associated cellular signalling systems in the nephron that mediate the inhibition of sodium-transport proteins and thereby increase in natriuresis. The two signalling pathways, adenylate cyclase and phospholipase C, are linked to DA₁ receptors through the coupling of G proteins. IP₃, inositol triphosphate; DAG, diacylglycerol; PLA₂, phospholipase A₂; Cyt P450, cytochrome 450 monooxygenase; Pi, phosphorylation; 20-HETE, a metabolite of arachidonic acid. Adapted from Hussain & Lokhandwala (296).

In the renal proximal tubular cells, DA₁ receptors are coupled to both adenylate cyclase (457) and phospholipase-C (458). Activation of adenylate cyclase following stimulation of DA₁ receptors is followed by inhibition of the Na⁺-H⁺ exchanger located in the brush border membrane, whereas activation of phospholipase-C upon stimulation of DA₁ receptors is followed by an inhibition of Na⁺,K⁺-ATPase located in the basolateral membrane presumably via protein kinase C mediated phosphorylation (296). Therefore, it is likely that both pathways act synergistically to produce DA-mediated inhibition of tubular sodium reabsorption.

Decreased sodium reabsorption in the proximal convoluted tubule tends to be compensated for by increased sodium reabsorption in the downstream segments, and the final regulation of the amount excreted in the urine takes place in the collecting duct. In the rat cortical collecting duct and in Madin-Darby canine kidney cells (a cell line derived from the dog distal nephron), both DA and fenoldopam inhibit Na⁺,K⁺-ATPase activity (434). That this action of DA is mediated via the DA₁ receptors is further supported by several other studies. This action is completely abolished by the selective DA₁ antagonist, SCH-23390. The effect of DA or fenoldopam on Na⁺,K⁺-ATPase is closely paralleled (in a reciprocal fashion) by their stimulation of adenylate cyclase. A role for adenylate cyclase stimulation and the consequent increase in cAMP in Na⁺,K⁺-ATPase inhibition is also supported by the finding that phosphodiesterase inhibitors inhibit the pump to a degree similar to that from DA stimulation.

Direct evidence that DA₁ receptor activation causes inhibition of Na⁺,K⁺-ATPase via stimulation of protein kinase C is provided by the work of Kansra et al. (459). DA and fenoldopam produce concentration-dependent increases in protein kinase C activity, which is blocked by SCH-23390. A high salt intake also increases

phospholipase C activity in rat renal cortex tissue (459), and this increase is reduced by SCH-23390.

3.2.4 *The inactivation of renal DA*

DA is metabolised by degradation (Figure 3c) or conjugation (see page 101).

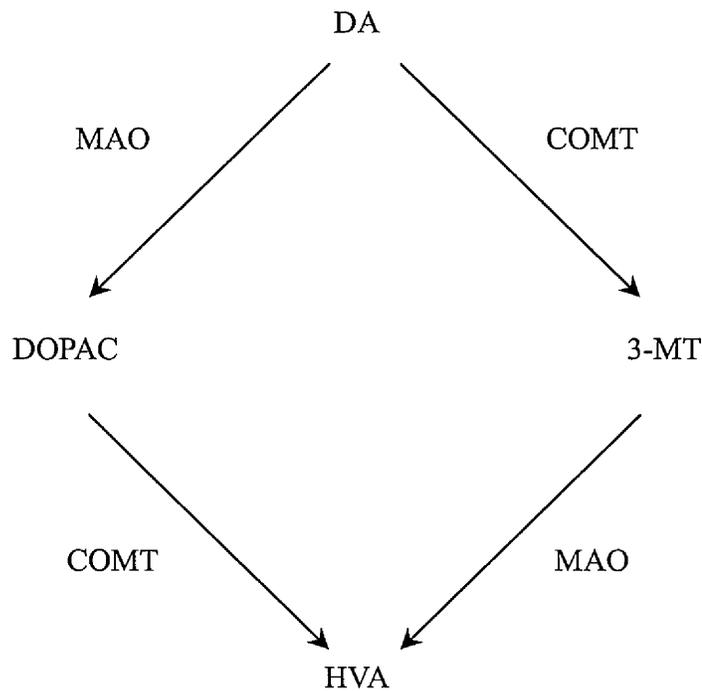


Figure 3c. Major pathways for renal DA metabolism. Renal DA can be metabolised both by deamination via monoamine oxidase (MAO) and by methylation via catechol-O-methyl-transferase (COMT). The common end-metabolite is homovanillic acid (HVA), the predominant DA metabolite in the urine. Thus, the physiological significance of renal DA ultimately depends on its capacity to be regulated. DOPAC, dihydroxyphenylacetic acid; 3-MT, 3-methoxytyramine. Adapted from Eklöf *et al.* (460).

The MAO activity in renal tissue is high (461). The COMT activity in renal tissue is also very high (462). These enzymes may participate in the regulation of renal DA availability. In rats, nitecapone, an inhibitor of peripheral COMT, induces a dramatic natriuresis, which is associated with inhibition of $\text{Na}^+\text{-K}^+\text{-ATPase}$ activity in both the proximal convoluted and proximal straight tubules (463). Both effects are completely abolished by SCH-23390. Nitecapone and gludopa have an additive effect on the natriuretic response. Other nitrocatechol COMT inhibitors (entacapone and tolcapone) also markedly increase urinary sodium excretion (464). In opossum kidney cells, all three nitrocatechol derivatives increase cAMP accumulation and reduce $\text{Na}^+\text{-K}^+\text{-ATPase}$ and $\text{Na}^+\text{-H}^+$ exchanger activities, this being prevented by SCH-23390 (464). Others have shown that inhibition of COMT results in increased RIF DA, urinary DA and absolute excretion of sodium (239). Combined inhibition of COMT and MAO increased RIF DA, urinary DA and urinary 5-HT (Section 2.4.6), which is accompanied by increases in urine flow rate and, absolute and fractional excretion of sodium. COMT plays an important role in determining renal DA availability and hence the natriuretic effects of the renal DA system.

To prevent any unwanted effects, the excess DA from intrarenal synthesis may also be inactivated by conjugation with sulphate (see Section 3.1.1) or glucuronic acid (465).

3.3 Interactions of renal DA with other neurohormonal systems

The natriuretic and vasodilatory effects of renal DA are in part related to its interactions with other vasoactive systems.

3.3.1 *Bi-directional regulation of tubular sodium reabsorption*

The natriuretic (Section 2.3) and antinatriuretic (Section 2.4) neurohormones appear to regulate sodium excretion through a common pathway that involves reversible activation or deactivation of renal tubular Na^+, K^+ -ATPase (463). Aperia et al. (18) reported in 1987 that DA inhibits the activity of proximal tubular Na^+, K^+ -ATPase. Since then, this enzyme as a target for the bi-directional regulation of sodium transport, has been extensively studied. Other agents with a well established natriuretic effect, such as ANP (466), PGE_2 (467) and nitric oxide (468), also inhibit the activity of Na^+, K^+ -ATPase. Agents with an antinatriuretic effect, such as NA (469,470), AII (471), aldosterone (472), serotonin (473), insulin (474) and neuropeptide Y (469), stimulate the activity of this key enzyme.

3.3.2 *The natriuretic agents*

Atrial natriuretic peptide

In rats, the natriuretic and diuretic effects of ANP are attenuated or blocked by DA antagonists (475,476) or carbidopa (476). During ANP infusion, there are no changes in the urinary excretion of free DA. The response to carbidopa suggests that, at least, background levels of endogenous DA are necessary for the full effects of administered ANP. Other workers reported that carbidopa or benserazide attenuates the natriuretic effect of ANP in ECF volume-expanded rats (477). The natriuretic response to ANP is restored by small doses of DA, which do not in themselves have a natriuretic action.

The situation is less clear in man. Wilkins et al. (478) reported that carbidopa partially blocks the natriuretic effect of ANP in normal subjects. However, Lewis et al. (479) were unable to confirm this finding. Moreover, the studies by Freestone et

al. (480) using (+)-sulpiride and Allen et al. (481) using domperidone suggested that neither DA₁ nor DA₂ activation is involved in the natriuretic effect of ANP.

Studies trying to determine whether DA infusions affect ANP synthesis or release yielded conflicting results. Neither the studies of Shenker et al. (482) in healthy subjects nor Tulassay et al. (483) in premature infants showed any effects of DA infusions on ANP levels. However, Fontana et al. (484) could demonstrate a differential effect of DA infusions on plasma ANP levels in healthy subjects (an increase) and patients with congestive heart failure (a decrease). Hirata et al. (485) have shown that the effects of ANP and DA on GFR and natriuresis are additive in healthy subjects and in patients with essential hypertension. In contrast with DA, ANP increases efferent arteriolar resistance and urinary cGMP excretion, suggesting that the renal effects of ANP are different from those of DA.

Brismar et al. (486) recently summarised the mechanisms by which renal DA and ANP interact to regulate sodium excretion. They suggested that the renal tubular DA₁ receptors are, under basal conditions, mainly located intracellularly. ANP and its second messenger, cGMP, cause a rapid translocation of DA₁ receptors to the plasma membrane.

Renal prostaglandins

Gullner et al. (487) studied the effect of inhibition of prostaglandin synthesis on urinary free DA excretion in six healthy women. Indomethacin (2 mg/kg/day) for seven days produced a transient decrease in urinary sodium excretion, averaging 80 mmol over three days. Urinary free DA excretion during the control period was not different from that during indomethacin treatment.

Intravenous frusemide is a known stimulus for both DA and prostaglandin production. Jeffrey et al. (488) reported that a single dose of indomethacin 100 mg had no effect on the urinary DA response to frusemide in healthy males.

Yeyati et al. (489) reported that intravenous DA (6 $\mu\text{g}/\text{kg}/\text{min}$) produces renal vasodilation and natriuresis in healthy subjects. These effects could be reversed by intravenous indomethacin (2 mg/kg), suggesting that the renal effects of DA may depend on normal prostaglandin production. Horton et al. (490) reported that DA infusion (1 $\mu\text{g}/\text{kg}/\text{min}$) increases the renal blood flow and renal production of PGI_2 in healthy subjects. These renal effects could be blocked by both metoclopramide and cyclo-oxygenase inhibitors. However, the increase in GFR and natriuresis after fenoldopam infusion (Section 3.1.4) could not be blocked by cyclo-oxygenase inhibitors. In contrast, Jeffrey et al. (491) reported that healthy subjects given gludopa showed no consistent increases in urinary PGE_2 output. However, urinary PGE_2 measurements are notoriously unreliable (RL Jones, personal communication). Prior treatment with a single dose of indomethacin 100 mg did not alter the natriuresis produced by gludopa.

Yoshimura et al. (380) studied the role of renal nerves and DA on urinary excretion of PGE in rats with or without salt loading. In rats with renal denervation, urinary excretion of PGE decreased during salt loading, but urinary excretion of PGE and sodium was enhanced by bromocriptine, a DA_2 agonist. Urinary excretion of PGE and free DA was suppressed by carbidopa, a L-AAAD inhibitor. These observations suggest that renal adrenergic nerves and tubular DA in the kidney play an important role regarding the release of PGE, particularly during salt loading.

In isolated rat glomeruli and cultured rat mesangial cells, Barnett et al. (492) found that DA does not affect the rate of mesangial cell production of PGE_2 . Huo &

Healy (493) reported that rat inner medullary collecting duct cells express DA₂ receptors. When these cells are incubated with DA, there is increased production of PGE₂. This increase can be blocked by domperidone, a DA₂ antagonist.

Renal kallikrein-kinin system

Little is known about the interaction between intrarenal DA and the renal kallikrein-kinin system. Shimamoto et al. (494) have shown an augmented response of urinary kallikrein-kinin and PGE₂ to DA infusion in Japanese subjects with a family history of hypertension. Similar findings have been reported in patients with essential hypertension (495).

Nitric oxide

Haynes et al. (496) studied the physiological role of NO in regulation of renal function in humans. L-NMMA, an inhibitor of NO synthase, significantly reduced GFR, urine flow rate, sodium and free water excretion. Urinary excretion of nitrate (an index of NO release) and free DA also decreased.

Venkatakrishnan et al. (497) investigated the effect of locally generated NO on renal function and its potential influence on the renal responses to DA₁ receptor agonists. The intrarenal infusion of a NO synthase inhibitor, L-NAME, in rats produced significant decreases in urine volume, urinary sodium excretion, GFR and fractional sodium excretion. The natriuresis and diuresis produced by intravenous infusion of fenoldopam or DA were associated with increases in urinary nitrate excretion. These renal effects and increases in nitrate excretion due to DA₁ receptor stimulation were attenuated by L-NAME.

These results indicate that intrarenal NO plays an important role in regulating renal sodium excretion (Section 2.3.5) and that an intact renal NO system is required for the full expression of the natriuretic and diuretic responses seen during DA₁ receptor activation.

3.3.3 *The antinatriuretic agents*

Renin-angiotensin system

With its β_1 -adrenoreceptor or α -adrenoceptor agonist effects, DA in higher doses is expected to stimulate renin release (290). Furthermore, by interacting with the vascular DA₁ receptors, DA can vasodilate afferent arterioles, stimulate the renal baroreceptors and release renin. In contrast to fenoldopam, gludopa, which increases DA synthesis within the kidney, lowers PRA (290). These observations suggest that intrarenally generated DA, but not exogenous DA, can inhibit renin release from the juxtaglomerular apparatus.

Eadington et al. (498) have shown that infusion of AII in sub-pressor doses in man reduces urinary free DA output. This reduction in DA output may contribute to the antinatriuresis, since the prior administration of carbidopa does not affect the antinatriuretic effect of AII.

Systemic and/or locally produced AII stimulates salt and water reabsorption in the renal proximal tubule. DA may serve as a counterregulatory hormone to AII's acute actions on the renal proximal tubule. Cheng et al. (499) tried to find out if DA modulates AT₁ receptor expression in cultured proximal tubule cells expressing DA₁ receptors. They concluded that DA, acting through DA₁ receptors, decreases AT₁ receptor expression in the proximal tubule, an effect likely to be mediated by increased intracellular cAMP levels. Local production of DA leads to decreased AT₁

receptor expression, suggesting DA may reset the sensitivity of the proximal tubule to AII.

Pharmacological, functional and clinical evidence indicates a role for DA in the activity of the adrenal cortex, primarily by regulating aldosterone secretion (500). In fact, DA and DA₂ agonists inhibit aldosterone secretion from the adrenal cortex. These effects are abolished by DA receptor antagonists such as metoclopramide and sulpiride. Metoclopramide increases plasma aldosterone levels.

In patients with mild to moderate hypertension, bromocriptine administered at a single dose of 2.5 mg reduced the SBP and DBP by 4.5% and 2.2%, respectively (501). At the same time, a 41.5% reduction in PRA and a 8.2% reduction in plasma aldosterone were seen. All these actions of bromocriptine could be blocked by metoclopramide.

Sympathetic nervous system

The role for endogenous DA in modulating SNS activity has been reviewed (502,503). DA₁ receptor stimulation-induced vasodilation may activate the SNS and RAS. Activation of DA₂ receptors, which are found prejunctionally on sympathetic nerve endings and on chromafin cells, causes an inhibition of NA release from sympathetic nerve terminals and adrenaline release from the adrenal medulla. An inhibitory effect of the non-selective DA agonist ibopamine on the rise in plasma NA levels during exercise is found in normal man and in patients with severe congestive heart failure.

Infusions of DA 1 µg/kg/min, but not 3 µg/kg/min, were found to reduce the rise in plasma NA levels associated with cold pressor test or exercise in normal man (503,504). This effect was abolished by pretreatment with domperidone. With the

lower dose DA infusion, the inhibitory effects of presynaptic DA₂ receptor or α_2 -adrenoceptor stimulation on plasma NA concentration predominate. With the higher dose DA infusion, the inhibitory effects may be counteracted by enhanced synthesis and release of NA.

Mannelli et al. (505) studied the effects of presynaptic DA₂ receptor blockade on the sympathetic-adrenal response to graded exercise in healthy subjects. Graded exercise caused significant increases in SBP, MAP, plasma NA plasma adrenaline and PRA. Pretreatment with domperidone caused a greater increase in plasma NA at the end of exercise and an overall increase in plasma adrenaline. These results may suggest that endogenous DA, by activating DA₂ receptors, limits catecholamine release during sympathetic-adrenal stimulation induced by exercise.

In patients with essential hypertension, dihydroergotoxine, a DA₂ receptor agonist, has been shown to reduce SBP and DBP, heart rate and plasma NA levels (506). The fall in BP correlates significantly with the decrease in plasma NA levels.

In rabbits, arterial NA concentration and total and renal NA spillover rates are markedly decreased in a dose-related manner by gludopa infusions (507).

In isolated proximal renal tubular cells, stimulation of β_2 -adrenergic receptors decreases the number of high-affinity L-dopa uptake sites resulting in reduction of the uptake of L-dopa and the production of DA (508).

Chapter 4 Pathophysiological significance of renal DA in common diseases

Since renal DA may play an important role in ECF volume and BP homeostasis in man, any dysregulation of this endogenous natriuretic factor may contribute to sodium and fluid retention and their consequences such as hypertension and the exacerbation of cardiac failure.

4.1 Hypertension

As summarised in Chapter 3, the physiological effects of endogenous DA are 'protective' against sodium retention and hypertension. Abnormalities in the renal DA system may lead to salt-sensitive hypertension.

4.1.1 *Human essential hypertension*

There are reports of a deficiency in the intrarenal synthesis of DA in various forms of human hypertension. This subject has recently been reviewed by Hussain & Lokhandwala (296), O'Connell & Aherne (297), Soares-da-Silva et al. (298) Murphy (301) and Amenta et al. (302).

Urinary free DA output is reduced in established hypertension but may be increased in the early phases of hypertension

Suppressed urinary free DA excretion has been reported in: (a) Caucasian patients with essential hypertension compared with normotensive subjects (509-511); (b) Japanese patients with salt-sensitive hypertension compared with normotensive subjects and patients with salt-resistant hypertension (512); and (c) Japanese patients

with low-renin hypertension compared with healthy subjects and patients with normal-renin hypertension (513). For example, Kjeldsen et al. (509) in Norway reported that 24-hour urine free DA output was 7.4% lower in hypertensive patients (aged 50 ± 1 years, BP $169 \pm 3 / 113 \pm 1$ mmHg) compared with normotensive subjects of similar age. In Japanese patients with low-renin hypertension, the depressed intrarenal DA synthesis is often associated with increased renal vascular resistance, decreased renal plasma flow and expansion of ECF volume (514).

In contrast to the findings in older patients (aged 41.6 ± 2.2 years) with mild to moderate hypertension (513), young Japanese patients (aged 18-27 years, SBP >150 mmHg or DBP >90 mmHg) had higher urinary free DA ($1,920 \pm 80$ vs $1,520 \pm 130$ nmol/day, $p < 0.001$) and NA (216 ± 11 vs 179 ± 12 nmol/day, $p < 0.05$) outputs compared with age-matched normotensive subjects (515).

By measuring DA and its metabolites in plasma (DA sulphate) and urine (homovanillic acid) as well as its response to an intravenous injection of frusemide 40 mg, Kuchel (516) recognised two distinct patterns in Caucasian patients with essential hypertension: a hyperdopaminergic state in borderline (labile) hypertension (BP $<140/90$ mmHg following four days of bed rest without medications) and a renal DA deficiency in stable hypertension (BP $>140/90$ mmHg). The borderline essential hypertension patients were 10-15 years younger than the stable hypertension group. Approximately 20% of borderline hypertensive patients became stable hypertensives within 12 years. Re-investigation of these patients indicated that their renal DA indices changed from those typical of borderline hypertension to those typical of stable hypertension. It was hypothesised that the increased intrarenal DA synthesis in borderline hypertension may represent an early antihypertensive compensatory mechanism.

Iimura et al. (517) reported that normotensive Japanese subjects with a family history of hypertension have a lower urinary free DA excretion compared to those without a family history. The study was performed in young healthy subjects (aged 20-30 years) after a standard diet (120 mmol sodium and 75 mmol potassium) for three days. On day 4, the 1-hour urinary free DA output was 18.5% lower in the subjects with a family history. This observation suggests that renal DA activity is already suppressed at the prehypertensive stage and the renal 'defect' is genetic, since it precedes the onset of hypertension.

In other studies, the 24-hour urinary free DA output in normotensive subjects with a family history of hypertension (363) or patients with borderline hypertension (365) was found to be similar to that of normotensive subjects without such a family history. However, unlike normotensive Japanese subjects without a family history of hypertension (aged 20.2 ± 0.2 years, DA output $1,815 \pm 104$ nmol/day), those with a positive family history (aged 19.7 ± 0.2 years, DA output $1,881 \pm 118$ nmol/day) did not show a positive correlation between their 24-hour urinary sodium and free DA outputs (Table 3g), suggesting that their renal DA system is altered even in the prehypertensive stage.

Absence of a renal DA response to salt loading

Unlike healthy subjects (see Table 3h), patients with essential hypertension generally do not show an increase in urinary free DA excretion following oral sodium loading. For example, Harvey et al. (368) in the U.K. studied eight hypertensive patients (aged 42 ± 5 years, BP $162 \pm 9 / 103 \pm 4$ mmHg) over two weeks during which oral sodium intake was increased from 20 to 220 mmol/day. A paradoxical

fall in urine free DA output followed by a return to baseline values was seen. These hypertensives tended to retain more sodium and have a greater increase in BP.

Others reported that urinary free DA output does not change after salt loading in salt-sensitive patients with hypertension, but an increase in urine DA is still seen in non salt-sensitive patients (369,373,512). Gill et al. (369) in the U.S. classified 19 patients with normal-renin essential hypertension as salt-sensitive (7 whites and 1 black, aged 61 ± 2.8 years) or salt-resistant (9 whites and 2 blacks, aged 41 ± 3.5 years), depending whether their MAP did or did not increase by $\geq 8\%$ when salt intake was increased. The responses of the two subsets and five normal subjects to sodium intakes of 9, 109 and 249 mmol/day each given for seven days were as follows. The salt-sensitive patients retained more sodium than normal subjects did (427 ± 33 vs 256 ± 44 mmol, $p < 0.01$); plasma or urinary free NA did not decrease and urinary free DA did not increase after salt loading. The salt-resistant patients excreted sodium normally; their plasma and urinary NA was decreased when they were given a high salt intake; urinary DA did not change after salt loading. Shikuma et al. (512) in Japan classified 18 patients with mild hypertension as salt-sensitive ($n=10$, aged 53.5 ± 6.3 years) and salt-resistant ($n=8$, aged 60 ± 5.3 years), depending whether their MAP increased by $>10\%$ when salt intake was increased from 34-51 mmol/day for seven days to 341-376 mmol/day for seven days. The mean increase in MAP after salt loading was 16% in the salt-sensitive patients and 2% in the salt-resistant patients. Urinary free NA decreased in both groups. Urinary excretion of free DA increased in the salt-resistant patients, but did not change in the salt-sensitive patients.

Unlike the situation with dietary salt loading, an increase in urinary free DA after an intravenous 0.9% saline infusion is seen in Japanese patients with essential

hypertension (399,400) and Caucasian patients with borderline (391,518,519) or stable (391) hypertension. Kawabe et al. (399) in Japan studied 12 patients (aged 52 ± 2 years, DBP >95 mmHg) 10 days after a regular diet (100 mmol of sodium and 60 mmol of potassium per day). The increase in urinary DA output after an intravenous 0.9% saline infusion (1,500 ml over three hours) was similar to that of age-matched controls (Table 3k). Castellano et al. (391) in Italy studied nine patients (aged 23-58 years) with borderline hypertension (WHO criteria) and 11 patients with established hypertension (aged 30-55 years) after a standard diet (120 mmol of sodium and 80 mmol of potassium per day) for five days. Urine analysis was performed on two separate days: under basal conditions and after a saline infusion (2,000 ml over two hours). The mean increase in urinary free DA output was 14% in patients with borderline hypertension and 29% in patients with established hypertension; these changes were not statistically different from those seen in normal subjects (Table 3k). The decrease in urinary free NA output was significantly less in patients with stable hypertension (6% vs 23% for patients with borderline hypertension and 18% for normal subjects, $p < 0.05$). Borghi et al. (519) in Italy studied 16 patients with borderline hypertension (aged 23 ± 1 years, DBP >90 and <90 mmHg on two of three occasions) after a constant daily sodium intake of 100 mmol for seven days. They all had a normal PRA (according to a standard nomogram relating upright PRA to 24-hour urinary sodium excretion). An intravenous 0.9% saline infusion (0.4 ml/min/kg for 45 minutes, followed by 0.15 ml/min/kg for 75 minutes) resulted in a 110% increase in urinary free DA output.

Unlike healthy Caucasian subjects without a family history of hypertension, those with a family history tend to show reduced natriuretic and renal DA responses to intravenous 0.9% saline infusion. This was seen after these subjects were on a

low (20 mmol/day) or medium (170 mmol/day) sodium diet for the previous week (520). No differences were seen between those with and without a family history of hypertension following a high (340 mmol/day) sodium diet.

Decreased renal decarboxylation of L-dopa

The work of Gill et al. (369,373) helped define the nature of the defect in DA mobilisation in Caucasian patients with normal-renin essential hypertension. Sixteen patients were on a constant diet containing 9 mmol/day of sodium for seven days, followed by the same diet but containing 249 mmol/day of sodium for seven days. Salt-sensitivity was defined as an increase in MAP of 8 mmHg between the diets; on this basis, nine patients were salt-sensitive and seven, salt-resistant. The rate of urinary excretion of dopa was significantly higher in the salt-sensitive patients than in the salt-resistant patients throughout the study. When dietary salt intake was increased to 249 mmol/day, urinary dopa excretion increased more in salt-sensitive patients than salt-resistant patients. At the end of the high salt diet, DA excretion was significantly attenuated in the salt-sensitive patients, despite higher rates of dopa excretion. Thus, the urinary ratio of free DA to dopa was decreased in salt-sensitive patients, regardless of salt intake. These results suggest that salt-sensitive patients have decreased renal uptake or decarboxylation of dopa and this deficiency is associated with enhanced delivery of dopa to renal uptake sites during dietary salt loading. A higher rate of urinary excretion of dopa and a low urinary free DA/dopa ratio appear to be markers of salt-sensitive hypertension in humans.

Aoki et al. (521) studied the conversion of L-dopa to DA in the kidneys in normotensive and hypertensive Japanese subjects. Plasma dopa concentrations, creatinine clearance and urinary sodium and free DA outputs were measured before

and after the single oral administration of L-dopa 400 mg in the two groups of subjects while on a constant diet. Dopa administration caused increases in plasma dopa concentrations, urinary free DA output and the fractional excretion of sodium. No significant differences were found in the plasma dopa concentration and the product of plasma dopa x creatinine clearance (which reflects the dopa delivery at the renal proximal tubule) between the normotensive subjects and the patients with normal-renin or low-renin hypertension. However, the ratio of urinary free DA to the product of plasma dopa x creatinine clearance (which indicates the conversion of dopa to DA in the kidney) was lower in the patients with essential hypertension (especially those with low-renin hypertension) than that in the normotensive subjects. These results suggest that a reduced intrarenal conversion of dopa to DA may contribute to the attenuated natriuresis as well as renal DA activity in low-renin hypertension.

Impaired intrarenal conversion of dopa to DA is also seen in Caucasian patients with stable essential hypertension. After the administration of a single dose of dopa (500 mg orally), hypertensive patients showed a greater reduction in BP, a lower creatinine clearance, a higher fractional excretion of sodium and a lower PRA than healthy subjects (522). The hypertensive patients also had higher plasma dopa concentrations, ratios of plasma dopa to DA and urine DA metabolite output. Despite an augmented glomerular load of dopa, the excretion rates of free DA and its metabolites remained comparable to those in the control subjects. These observations suggest that in patients with hypertension, less exogenous dopa is decarboxylated to DA and DA is metabolised more rapidly.

Iimura et al. (517) in Japan studied 20 young healthy subjects with (n=10, aged 23.0 ± 0.5 years) or without (n=10, aged 23.5 ± 0.9 years) a family history of

hypertension. After three days on a standard diet with 120 mmol/day of sodium and 75 mmol/day of potassium, renal clearance was measured during 1-hour clearance periods before and after a DA infusion ($3 \mu\text{g}/\text{kg}/\text{min}$). Blood samples were taken at the midpoint of the 1-hour renal clearance urine collection periods. The following parameters were determined: renal plasma flow, MAP, plasma dopa, urinary free DA, creatinine clearance and fractional excretion of sodium. Compared to subjects without a family history, those with a family history had a significantly larger urine volume (132%), urinary sodium excretion (106%) and fractional excretion of sodium (168%) in response to the DA infusion. No significant differences were found in the product of plasma dopa x renal blood flow and the product of plasma dopa x creatinine clearance (which reflect the dopa delivery at the renal proximal tubule). However, the ratio of urinary free DA to the product of plasma dopa x creatinine clearance (which indicates the conversion of dopa to DA in the kidney) was lower in those with a family history. These findings suggest that a reduction in the intrarenal conversion of L-dopa to DA already exists in the prehypertensive stage.

Consistent with a renal defect in the conversion of L-dopa to DA is the finding by Clark et al. (510) that Caucasian patients with essential hypertension have a decreased urinary free DA excretion in response to an increase in dietary precursors for DA supplied by a protein load (see Section 3.2.2). They studied six hypertensive men (aged 48-67 years, BP 140-180/90-100 mmHg) and seven healthy men (aged 44-68 years) after two different meals: 60 g protein and a non-caloric electrolyte-equivalent broth. In the normotensive subjects, there was a 23% increase in urinary free DA output after protein feeding. In hypertensive subjects, there was no change in urinary free DA. In the normotensive subjects, there was a 74% increase in sodium excretion after the protein meal, but no significant change was seen in

hypertensive subjects. There were no differences in renal plasma flow, GFR and plasma dopa between the groups before and after the protein meal.

However, features of accelerated, rather than diminished, renal DA synthesis are seen when patients with borderline hypertension are given L-dopa. This has been demonstrated by Shigetomi et al. (523) in Canada. Ten patients aged 24-51 years with borderline hypertension (BP returned to normal after four days of bed rest) and 10 healthy subjects aged 19-29 years were studied while receiving a diet containing 150 mmol sodium, 100 mmol potassium and 60 g protein per day. Basal and L-dopa-induced (500 mg orally) changes in BP and pulse rate as well as in 3-hourly plasma and urine samples were measured. They found that hypertensive patients, compared with controls, (a) showed a greater dopa-induced decrease of SBP; (b) had an accelerated plasma DA sulphate and urinary DOPAC excretion (see Section 3.2.4) in response to dopa; and (c) eliminated comparable quantities of DA in urine despite a lower rise in the glomerular dopa load. Furthermore, dopa-induced natriuresis was greater in the borderline hypertensive patients. Urinary sodium excretion after dopa administration in the control subjects was significantly correlated with urinary free DA excretion ($r=0.52$, $p<0.02$). Such a correlation was not seen in the hypertensive patients and the dopa-induced hypernatriuresis exceeded the urinary DA increase.

In patients with hypertension, the impairment of intrarenal conversion of L-dopa to DA may be due to a defect in L-AAAD (510,516), the enzyme that catalyses this conversion, or a decrease in the renal tubular uptake of L-dopa (369,373).

Up-regulation of DA receptors and/or a change in receptor affinity

Several observations provide support for the hypothesis of an up-regulation of the DA receptors and/or a change in the receptor affinity in patients with essential

hypertension, secondary to decreased endogenous intrarenal DA production (524). First, the natriuretic response to DA infusion (2-3 $\mu\text{g}/\text{kg}/\text{min}$) is significantly greater in both Caucasian and Japanese patients with essential hypertension than in normotensive subjects (513,524-527). DA infusions generally lower the MAP and increase the heart rate in hypertensive patients but not in normotensive controls (525,526). DA infusions also produce greater increases in GFR in hypertensive patients (525,526). Secondly, the increase in urinary cAMP output (which correlates with sodium excretion) in response to tubular DA receptor stimulation by DA infusion is more pronounced in Caucasian patients with hypertension than in healthy subjects (524). Thirdly, a greater increase in the urinary excretion of kallikrein and PGE_2 is seen in Japanese patients with hypertension compared with healthy subjects (527). Fourthly, the antinatriuretic response to injected metoclopramide is greater in Japanese patients with low-renin hypertension, when compared with normal-renin hypertensive patients and normotensive subjects (513).

Japanese patients with low-renin hypertension show greater increases in urinary excretion of sodium (525,527), kallikrein and PGE_2 (527) to DA infusions, when compared with patients with normal-renin hypertension and healthy subjects. After the oral administration of L-dopa, BP falls but sodium excretion and GFR increase in both normotensive and hypertensive subjects; these responses are greater in borderline hypertensive patients than healthy subjects (523) and in salt-sensitive hypertensive patients compared to salt-resistant patients (528).

Augmented responses to a DA infusion (3 $\mu\text{g}/\text{kg}/\text{min}$) are also seen in healthy Japanese subjects with a family history of hypertension when compared to subjects without a history (494,517,527). Both the natriuretic response and the increase in urinary kallikrein activity are greater.

A recent study by O'Connell et al. (529) also provides support for the theory that DA₁ (D₁-like) receptor up-regulation occurs in patients with essential hypertension. The up-regulation of DA₁ receptor function takes place in the distal tubules and not in the proximal tubules. Such an up-regulation of the DA₁ receptor in the distal tubules offsets the defect in the proximal tubules, leading to natriuresis and diuresis in response to fenoldopam.

A defect in the coupling of the DA₁ receptor and its messenger system, adenylyl cyclase (Section 3.2.3) has recently reported in the primary cultured cells of hypertensive human proximal tubules (530). This defect is receptor-specific. A DA₁ receptor/adenylyl cyclase defect in human proximal tubular cells is similar to the defects found in the proximal tubules of some animal models of genetic hypertension (see Section 3.1.4).

4.1.2 *Hypertension associated with obesity*

Salt sensitivity in obese subjects may be due to the combined effects of hyperinsulinaemia, hyperaldosteronism, increased SNS activity and impaired renal DA activity (531,532).

In obese Japanese patients with essential hypertension, the body mass index (BMI) correlates positively with the MAP, plasma volume, ECF volume and total exchangeable sodium, and negatively with plasma NA concentration and PRA (532). The fractional excretion of sodium, which reflects renal tubular reabsorption of sodium, is significantly lower in obese patients than in normal weight patients with essential hypertension. The hypotensive effect of salt restriction and the natriuretic response to DA infusions are more pronounced in overweight than in normal weight patients. Urinary free DA excretion correlates positively with the BMI in normal

weight patients. In contrast, there is a negative correlation between BMI and urinary free DA excretion in overweight patients. In obese patients with essential hypertension, sodium retention and ECF volume expansion may be due to attenuated renal DA activity.

4.1.3 *Association of dopamine receptor gene polymorphism with hypertension*

The human DA D1 receptor (DRD1) gene has been localised to chromosome 5 at q35.1 (533). A polymorphism, A-48G, has been identified at -48 bp of the 5' untranslated region (534). Sato et al. (535) performed an association study in 131 patients with essential hypertension and 136 age-matched normotensive controls. Polymerase chain reaction was used to amplify the A-48G polymorphic site in the DRD1 gene, and restriction analysis of the polymerase chain reaction product was used to score the *A* and *G* alleles. *G* allele was observed more frequently in patients with hypertension (16% vs 8%, $p=0.01$). DBP was higher in the *A/G* patients than in the *A/A* patients (115 ± 15 vs 106 ± 11 mmHg, $p<0.01$). The A-48G polymorphism in the 5' untranslated region of the DRD1 gene was associated with essential hypertension in Japanese subjects.

My colleagues have studied the association between DA D2 receptor (DRD2) gene *TaqI* polymorphism, BP and obesity in Hong Kong Chinese (536). They recruited 209 non-diabetic hypertensive and 174 gender-matched normotensive subjects. The hypertensives had increased BP ($149 \pm 18 / 89 \pm 11$ vs $113 \pm 9 / 66 \pm 9$ mmHg, $p<0.001$), increased dyslipidaemia, and a greater degree of obesity (BMI 25.8 ± 3.8 vs 23.3 ± 3.6 kg/m², $p<0.001$). The A1 and A2 alleles of the DRD2R gene *TaqI* polymorphism were identified using a polymerase chain reaction-based restriction fragment length polymorphism protocol. The A1 allele frequency was

decreased in the hypertensives compared with controls (42 vs 52%, $p=0.006$). In the combined population, SBP, DBP and MAP were 6, 5 and 6 mmHg lower, respectively, in subjects with A1A1 genotype relative to the A2A2 genotype ($p<0.05$), whereas skinfold thickness was increased at the iliac ($p<0.001$) and triceps ($p<0.03$) sites.

4.1.4 *Rat models of hypertension*

Studies using Dahl salt-sensitive rats and SHR also provide evidence for a defect in the renal DA system having an aetiological role in salt-sensitive hypertension (296).

Dahl salt-sensitive rats excrete sodium poorly in response to a sodium load. The kidney DA content is decreased in salt-sensitive rats fed a high salt diet (537). Urinary DA excretion does not increase in salt-sensitive rats subjected to acute ECF volume expansion (538). Urinary DA and cAMP excretions are reduced in salt-sensitive rats compared with Wistar rats fed a normal salt diet (514).

A defect in the DA₁ receptor has also been reported in the proximal tubules of Dahl salt-sensitive rats. In normotensive rats, when endogenous DA is allowed to accumulate through the use of a DA β -hydroxylase inhibitor, which inhibits DA conversion to NA, down-regulation of proximal tubular DA₁ receptors and complete ablation of DA₁ receptor mediated stimulation of adenylyl cyclase activity occurs (538,539). The DA₁ receptors in salt-sensitive rats are resistant to such effects. A high salt intake for 10 days down-regulates Na⁺-K⁺-ATPase activity in the proximal tubules of salt-resistant rats and this is reversed by the L-AAAD inhibitor, benserazide (540). In contrast, a high salt intake does not affect Na⁺-K⁺-ATPase activity in salt-sensitive rats apparently because of a defective DA₁ receptor mediated

cellular signaling mechanism. DA₁ agonists are unable to stimulate adenylyl cyclase in the proximal tubules from salt-sensitive rats, whereas direct stimulation of the enzyme by forskolin has the same effects in salt-sensitive and salt-resistant rats (541).

In SHR, DA production and excretion is normal or even increased (542). In spite of normal DA receptor density, DA and fenoldopam-mediated natriuretic responses are diminished under normal conditions, as well as during acute ECF volume expansion (543,544).

DA₁ agonists stimulate adenylyl cyclase activity to a lesser extent in the proximal tubules of SHR compared with normotensive Wistar rats (545). Further studies revealed that the defect resides in the coupling of the receptor with adenylyl cyclase. The defect is nephron segment-specific (only in the proximal part) and organ-specific (only in the kidney) (26). The stimulation of another signaling system, phospholipase C (Section 3.2.3), by DA₁ agonists is also reduced in SHR.

Thus, in SHR, in spite of normal renal production of DA and DA receptor density, there is defective transduction of the DA₁ receptor signal in the proximal tubules, resulting in decreased inhibition of sodium transport (Na⁺-K⁺-ATPase and Na⁺-H⁺-exchanger activity) by DA.

In obese hypertensive rats, reduction in DA₁ receptor numbers and a defect in receptor-G protein coupling may account for the inability of DA to activate DA₁ receptor-coupled signal transduction pathway and cause inhibition of Na⁺-K⁺-ATPase (546).

4.1.5 *The role of DA receptors in the pathogenesis of hypertension*

Albrecht et al. (547) studied the role of the DA_{1A} receptor in the pathogenesis of genetic hypertension in the F2 generation from female Wistar-Kyoto rat and male spontaneously hypertensive rat crosses. A DA₁ agonist inhibited Na⁺-H⁺-exchanger activity in brush border membrane samples from normotensive F2s but not hypertensive F2s. A DA₁ agonist, when infused into the renal artery, increased sodium excretion in normotensive F2s but was inactive in hypertensive F2s. SBP was higher in the homozygous and heterozygous rats compared to normal controls. Moreover, the rats lacking one or both DA_{1A} alleles developed diastolic hypertension. The co-segregation with hypertension of impaired DA₁ receptor regulation of renal sodium transport and the development of elevated SBP and DBP in rats lacking one or both DA_{1A} alleles suggests a causal relationship of the DA_{1A} receptor gene with hypertension.

Asico et al. (548) studied the cardiovascular consequences of the disruption of the D₃ receptors (a member of the family of DA₂ receptors) expressed in renal proximal tubules and juxtaglomerular cells. Mice lacking the D₃ receptors (mutant mice) were generated by target mutagenesis. Both the SBP and DBP was higher in heterozygous and homozygous than in wild-type mice. Saline infusions increased urine flow rate and sodium excretion to a similar extent in wide-type and heterozygous mice, but these responses were attenuated in homozygous mice. PRA was much higher in homozygous than in wild-type mice; values for the heterozygous mice were intermediate. Blockade of AT₁ receptors decreased SBP for a longer duration in mutant than in wide-type mice. Thus, disruption of the D₃ receptor increases renal renin production and produces renal sodium retention and renin-dependent hypertension.

4.2 Chronic renal diseases

In chronic renal diseases, sodium retention and hypertension are common and may be due to abnormalities in intrarenal DA synthesis. Pestana et al. (549) have recently reviewed renal dopaminergic mechanisms in renal parenchymal diseases and hypertension.

Chronic renal parenchymal diseases are accompanied by a progressive loss of tubular units endowed with the ability to synthesise DA from L-dopa. Therefore, large reductions in the urinary excretion of free DA are seen in patients with chronic renal failure (550-553). There is a strong, negative correlation between the urinary excretion of free DA and the GFR (551-553). Urine free DA excretion is similar in patients with glomerular or tubulo-interstitial diseases (551). Urinary free DA excretion increases markedly in renal failure patients following renal transplantation (552). The recovery of renal function in renal transplant recipients is accompanied by an enhanced ability to synthesise DA and inactivate it to DOPAC and HVA (554). Casson et al. (367) noted that patients with chronic renal diseases and varying degrees of renal impairment do not show the expected increase in urinary free DA excretion after oral salt loading. Smit et al. (555) reported that the response to DA infusions (0.25-8 $\mu\text{g}/\text{kg}/\text{min}$) is blunted in patients with renal failure (GFR 34-85 ml/min).

Pestana et al. (553) showed that the reduced urinary free DA output in renal failure is not attributable to the enhanced metabolism of renal DA. The daily urinary outputs of L-dopa, free DA and its metabolites, DOPAC and HVA, in conditions of controlled sodium, potassium and phosphate intakes were compared in two groups of patients (average GFR in group 1 and group 2 is 39 and 139 ml/min/1.73 m²). Group 1 showed a 60% reduction in the urinary excretion of L-dopa, DA and DOPAC and a

40% reduction in HMA excretion. A positive linear relationship was seen in the two groups between the GFR and daily urinary outputs of L-dopa, DA, DOPAC and HVA. The urinary DA:L-dopa and DOPAC:DA ratios were similar in both groups. Hence, the reduced urinary free DA output in renal failure is attributable to decreased intrarenal DA synthesis.

Kuchel & Shigetomi (556) suggested that in patients with hypertension and moderate chronic renal failure, renal DA deficiency may have a potential role in perpetuating renal failure via glomerular hypertension.

The beneficial effects of dopaminergic drugs in the long-term treatment of patients with progressive renal failure have been confirmed (354).

In autosomal dominant polycystic kidney disease (ADPKD), hypertension is often observed before the loss of renal function. The role of intrarenal DA in relation to sodium homeostasis was studied by Barendregt et al. (557). ADPKD patients with borderline hypertension and near normal renal function and matched healthy subjects were studied at three levels of daily sodium intake: 150, 50 and 450 mmol. At all levels of sodium intake, cumulative sodium balance was similar, but daily urinary outputs of DA and dopa were higher in ADPKD patients. Renal vascular resistance, filtration fraction and BP were also higher in ADPKD patients. During the 450 mmol sodium intake period, the effects of a L-dopa infusion ($7 \mu\text{g}/\text{kg}/\text{min}$) were studied. The L-dopa infusion appeared to 'normalise' the patients' renal haemodynamics resulting in a stimulation of natriuresis similar to that of the controls. These results suggest that: (a) increased intrarenal DA production, as a mechanism to maintain sodium homeostasis, occurs in borderline hypertensive ADPKD patients; and (b) DA agonists, by 'normalising' renal haemodynamics, may be beneficial to these patients.

In patients with IgA nephropathy and near normal renal function, the changes in 24-hour mean BP when going from low to high sodium intake (from 20 to 350 mmol/day) correlated negatively with the daily excretion of DA ($r=0.77$, $p<0.01$) (558). The urinary excretion of L-dopa and DA in salt-sensitive patients was lower than in salt-resistant patients, irrespective of their daily sodium intake. The rise in urinary DA output during salt loading (from 20 to 350 mmol/day) was greater in salt-sensitive patients than in salt-resistant patients ($21.2 \pm 2.5\%$ vs $6.3 \pm 1.4\%$ increase, $p<0.05$).

Cyclosporin A is a potent immunosuppressant used for organ and tissue transplantation to prevent graft rejection and graft-versus-host disease. This drug is markedly nephrotoxic and may cause hypertension. Administration of cyclosporin A to rats produces an increase in BP, a reduction in urinary excretion of sodium, free DA, DOPAC and HVA (559). These reductions appear to result from a fall in the amount of L-dopa made available to the kidney and do not involve changes in tubular L-AAAD and DA metabolism.

4.3 Congestive heart failure

As cardiac performance declines in patients with congestive heart failure, various neurohormonal systems are progressively stimulated in an attempt to maintain sufficient blood flow to peripheral organs (560). This may result in further progression of the heart failure with sodium and water retention (561). The inability to excrete ingested sodium is a well recognised feature of congestive heart failure (562).

When the daily sodium intake in patients with mild, asymptomatic heart failure is increased from 100 to 250 mmol, both the left ventricular end-diastolic and end-systolic volume increases, whereas the ejection fraction and stroke volume fail to increase and the total peripheral resistance fails to decrease (563). These patients also have a higher cumulative sodium balance than healthy subjects.

An interesting finding of Lang & Kaufmann (564) on the urinary excretion of DA in patients with New York Heart Association (NYHA) classes II, III and IV heart failure suggests the possibility of a compensatory role of DA in mild heart failure, the efficiency of which may be reduced in severe cases. The mean urinary excretion of DA was increased to 12,407, 9,142 and 6,530 nmol/day in class II, III and IV patients, respectively. It returned to the normal range of 2,612-5,224 nmol/day when the heart failure was treated adequately. Similar results have been reported by Asakura et al. (565) in classes III and IV patients (free DA excretion falling from 15,855 to 3,454 nmol/day following treatment). They also found a marked increase in urinary free DA within 24 hours after the onset of the symptoms of heart failure.

Hayashi et al. (566) studied the urinary free DA excretion, the delivery of L-dopa to the renal proximal tubules (plasma L-dopa x creatinine clearance) and the intrarenal conversion of L-dopa to DA (the ratio of urinary free DA to the product of plasma dopa x creatinine clearance in 30 patients with congestive heart failure and 12 normal controls. With increases in NYHA functional class, urinary free DA, plasma L-dopa, delivery of L-dopa and intrarenal DA production fell progressively. Urinary sodium excretion ($r=0.46$, $p<0.05$) and creatinine clearance ($r=0.54$, $p<0.01$) correlated positively with urinary free DA. Linear correlations were seen between left ventricular ejection fraction and urinary free DA ($r=0.57$, $p<0.01$), plasma NA ($r=0.50$, $p<0.01$) or plasma L-dopa ($r=0.42$, $p<0.05$). These results suggest that in

patients with heart failure, both the delivery of L-dopa to the proximal tubules and its conversion to DA within the kidney are suppressed.

Ferreira et al. (567) studied post-acute myocardial infarction patients with or without asymptomatic left ventricular systolic dysfunction (ejection fraction <40%). Patients with left ventricular systolic dysfunction had lower urinary excretion of L-dopa (66.8 ± 10.1 vs 115.3 ± 21.9 nmol/day, $p=0.04$). Urinary free DA was similar in the two groups ($1,124 \pm 172$ vs $1,049 \pm 146$ nmol/day), resulting in higher urinary DA/L-dopa ratios in patients with left ventricular systolic dysfunction (20.4 ± 3.0 vs 9.9 ± 0.8 , $p<0.001$). Ejection fraction was negatively correlated with urinary DA/L-dopa ratios. Renal DA synthesis is well preserved in patients with asymptomatic left ventricular systolic dysfunction, despite reduced urinary excretion of its precursor. This suggests that renal uptake and/or decarboxylation of L-dopa is enhanced in this condition, as a compensatory mechanism, contributing to preservation of urinary sodium excretion.

There are good theoretical reasons for the use of dopaminergic drugs in the treatment of congestive heart failure (Section 3.1.3, Section 3.1.4 and Section 3.1.5). Although the prognoses of these patients have not been shown to improve after the long-term use of ibopamine (Section 3.1.5), these drugs are still very valuable in the acute management of heart failure because of their beneficial effects on renal and systemic haemodynamics and sodium and water excretion. In particular, low-dose DA may have a renal-protective effect during vigorous diuretic therapy for congestive heart failure associated with mild or moderate renal insufficiency (568). As already mentioned earlier (Section 3.1.3), low-dose DA is not always natriuretic, depending whether the antinatriuretic systems are 'overactive' after the development of hypovolaemia. That may be the reason why DA infusions are not particularly

useful in elderly heart failure patients already receiving high dose loop diuretics (569).

4.4 Diabetes mellitus

Diabetes mellitus and hypertension often coexist. In our surveys of adults attending specialist clinics at the Prince of Wales Hospital, about half of Chinese diabetic patients (predominantly NIDDM) have hypertension (570,571). In contrast to western countries where coronary artery disease is the main cause of mortality in diabetic patients, renal failure and strokes are the leading causes of death in Hong Kong Chinese diabetics (572).

Diabetes mellitus is characterised by a tendency to sodium retention. An increase in total exchangeable sodium has been shown in both normotensive and hypertensive diabetic patients (573,574). The natriuretic response to salt loading is impaired in diabetic patients (575,576). Diabetic patients, particularly those with nephropathy, are often salt-sensitive (279,576). Diabetic patients with hypertension respond well to moderate restrictions of salt intake (576,577). Cardiovascular pressor responsiveness to NA and AII may be exaggerated in diabetic patients (578). Thus, the hypertension accompanying diabetes mellitus involves abnormalities in at least two major BP-regulating systems: the body sodium-fluid volume state and cardiovascular reactivity.

Since IDDM patients given an intravenous saline infusion have a higher GFR but a lower sodium excretion than healthy controls, enhanced tubular reabsorption of sodium rather than impaired filtration is suspected (575). It was subsequently confirmed that some IDDM patients have increased sodium reabsorption at the

proximal renal tubule (579), which is also the site of action of intrarenal DA and may reflect a deficiency of DA synthesis and action. The renal sodium-DA relationship in diabetic patients has since been extensively investigated.

NIDDM patients

Murabayashi et al. (580) in Japan studied the urinary excretion of free DA in 21 NIDDM patients (aged 54.8 ± 1.9 years) and six normal subjects (aged 48.8 ± 4.8 years) after receiving a standard diet (150 mmol sodium and 50 mmol potassium per day). Diabetic patients with a poor control (2 hour postprandial blood glucose >13.8 mmol/L), obesity (BMI >27 kg/m²) or hypoalbuminaemia (serum albumin <30 g/L) were excluded. Twelve diabetic patients had nephropathy (albumin excretion >200 mg/day). SBP was higher in diabetics with nephropathy (155.0 ± 2.4 mmHg) than in diabetics without nephropathy (117.6 ± 9.4 mmHg) and healthy subjects (122.0 ± 1.0 mmHg). Urinary free DA output was lower in diabetics with nephropathy (342 ± 58 nmol/day) than in diabetics without nephropathy (859 ± 108 nmol/day) and in healthy subjects ($1,173 \pm 101$ nmol/day). Multiple regression analysis revealed that the 24-hour urinary excretion of DA correlated significantly with the creatinine clearance as well as the SBP and DBP. The results indicate that renal DA synthesis may decrease as diabetic nephropathy progresses.

My colleagues (581) at the Chinese University of Hong Kong studied the relationships between plasma ANP, urinary DA, BP and albuminuria in 165 Chinese patients with NIDDM treated by diet or oral hypoglycaemic drugs. In group 1, 88 patients had normoalbuminuria (urinary albumin excretion ≤ 30 mg/day). In group 2, 48 patients had microalbuminuria (30-300 mg/day). In group 3, 29 patients had macroalbuminuria (≥ 300 mg/day). Supine BP was higher in patients with abnormal

albuminuria (140.9 ± 2.9 mmHg in group 1, 158.1 ± 3.8 mmHg in group 2, 166.7 ± 4.4 mmHg in group 3, $p < 0.001$). The 24-hour urinary sodium output was similar in the three groups. Mean plasma ANP concentrations increased with increasing proteinuria (33.3 pg/ml in group 1, 39.1 pg/ml in group 2, 50 pg/ml in group 3, $p < 0.01$). Mean 24-hour urinary free DA output was inversely related to proteinuria ($1,292$ nmol in group 1, $1,142$ nmol in group 2, 983 nmol in group 3, $p < 0.05$). In patients with albuminuria >30 mg/day, plasma ANP was negatively correlated with urinary DA output ($r = -0.25$, $p < 0.02$). It was hypothesised that, in patients with NIDDM, defective DA mobilisation in the renal tubules may cause impaired natriuresis with increased BP and a compensatory rise in plasma ANP.

Segers et al. (582) in Belgium studied the renal sodium-DA relationship in 72 NIDDM patients without proteinuria (aged 55 ± 1.4 years, HbA_{1c} $10.0 \pm 0.2\%$, BMI 30.0 ± 0.6 kg/m²). All had a GFR ≥ 90 ml/min. Basal urinary free DA excretion during a 200 mmol sodium diet was reduced in the diabetic males (non-insulin treated 992 ± 26 , insulin treated 866 ± 24 nmol/g creatinine) and females (non-insulin treated $1,328 \pm 27$, insulin treated $1,070 \pm 22$ nmol/g creatinine) compared with the normal ranges for males ($1,196 \pm 28$ nmol/g creatinine) and females ($1,438 \pm 26$ nmol/g creatinine). No correlation was present between urinary free DA and the BMI, age, duration of diabetes, HbA_{1c} or the presence or absence of hypertension. As in healthy controls, a significant correlation between urinary free DA and fractional excretion of sodium was seen in the non-insulin treated patients ($r = 0.30$, $p < 0.04$) but not in diabetics treated by insulin. In diabetic patients, an intravenous infusion of 0.9% saline (120 ml over 5 minutes, then 600 ml over one hour) induced a progressive natriuresis that was comparable to that of the healthy subjects. In healthy subjects, urinary free DA excretion increased up to $136 \pm 12\%$. However, in

the NIDDM patients, no increase in urinary free DA was seen. The insulin treated patients actually showed a reduction in urinary free DA 30 minutes after the saline infusion. DA infusion ($3 \mu\text{g}/\text{kg}/\text{min}$) for one hour induced an increase in sodium excretion that was less pronounced in the diabetic patients than in the controls. The natriuretic response was the lowest among the insulin-treated patients. These results suggest that NIDDM patients display a derangement of their renal DA system, which is accentuated by insulin treatment.

Shigetomi et al. (583) in Japan studied the cardiovascular and renal DA responses to the ingestion of 100 g protein in NIDDM patients. Both patients and healthy controls showed an increase in GFR, but the expected increase in fractional excretion of sodium was seen only in the controls. Since the increase in urinary L-dopa was blunted in the patients, the impaired natriuretic response to protein feeding may result from a decreased intrarenal DA synthesis.

IDDM patients

In their study of 48 IDDM patients (aged 25-38 years), Patrick et al. (362) in the U.K. found no evidence of a defect in the mobilisation of renal DA prior to the development of hypertension or overt nephropathy. All 48 patients had a creatinine clearance $>80 \text{ ml}/\text{min}$, but 11 had microalbuminuria (albumin/creatinine ratio $>3 \text{ g}/\text{mol}$). The 24-hour urinary sodium output in diabetic patients with (albumin excretion 44-248 mg/day) or without (3-22 mg/day) microalbuminuria did not differ from the 40 healthy controls (165 ± 16 and 160 ± 10 vs $173 \pm 9 \text{ mmol}$, $p=\text{NS}$). Their 24-hour urinary free DA output was also similar ($1,450 \pm 89$ and $1,632 \pm 72$ vs $1,572 \pm 72 \text{ nmol}$, $p=\text{NS}$). Moreover, both diabetic patients and healthy subjects showed a

significant correlation between urinary free DA and sodium ($r=0.80$, $p<0.01$ and $r=0.44$, $p<0.01$ vs $r=0.51$, $p<0.001$; $p=NS$ for within group comparison).

Madacsy et al. (584) in Hungary reported that IDDM children with (albumin excretion 101-288 mg/day) or without (<29 mg/day) incipient nephropathy had lower mean urinary excretion of free DA (165.2 and 430.3 nmol/day) and sodium (98.4 and 206.2 mmol/day) than age-matched healthy controls (478.6 nmol/day and 198.1 mmol/day). BP was higher in the children with incipient nephropathy than in controls. These results suggest that a decrease in endogenous DA can play a role in sodium retention which may in turn lead to the development of hypertension in children with incipient diabetic nephropathy.

Segers et al. (585) in Belgium also found that the 24-hour urinary free DA output was lower in IDDM patients compared with controls. A significant correlation between urinary free DA and sodium excretion was present in normoalbuminuric patients and in controls, but not in patients with microalbuminuria. Patients with a relatively short duration of diabetes (<15 years) had a comparable DA infusion-induced increase in fractional excretion of sodium as normal controls. However, patients with a longer duration of diabetes (>15 years) or microalbuminuria displayed no significant changes in sodium output during DA infusion.

Rudberg et al. (371) in Sweden studied the renal DA response to a high salt diet (usual intake with additional 103 mmol/day for three days) in 16 IDDM adolescents with normal BP and albumin excretion rates. The average increase in sodium excretion tended to be lower than in healthy controls, implying a sluggish response and a degree of sodium retention. A 4.4% increase in SBP was seen in the patients, but not in the controls. Furthermore, free DA excretion rose significantly in

the control subjects (Table 3h), but not in the IDDM patients. Thus, a positive correlation between the changes in DA and sodium output was found in the controls, but not in the patients. This suggests an impairment in the renal DA system in IDDM patients despite the absence of nephropathy or hypertension.

Stenvinkel et al. (393) in Sweden studied the renal DA response to a 2 hour 0.9% NaCl infusion (25 ml/kg) in eight men (aged 27 ± 1 years) with uncomplicated IDDM. The maximum increase in sodium excretion was reduced in the patients compared with healthy controls (47.6 vs 92.2%). The fractional proximal tubular sodium reabsorption decreased in the controls but not in the patients. Fractional distal tubular reabsorption decreased similarly in both groups. Urinary free DA excretion increased by about 15% in the controls (Table 3h) but did not change in the patients. The mean urinary DA excretion above baseline was greater in the controls (8.4 ± 2.1 nmol/h) than in the patients (-2.2 ± 2.1 nmol/h). Plasma ANP also increased in the controls but not in the patients. These results suggest that IDDM patients have a blunted natriuresis in response to ECF volume expansion with saline infusion. This abnormality seems mainly to be due to impaired inhibition of proximal tubular sodium reabsorption, which may be the result of defective intrarenal DA mobilisation.

Since ACE inhibitors can facilitate renal sodium excretion and AII infusions may reduce urinary free DA excretion in IDDM patients, Stenvinkel et al. (394) in Sweden studied the effects of ramipril on the natriuretic and renal DA responses to a 2-hour 0.9% NaCl infusion (12.5/kg/h) in nine patients (aged 28 ± 3 years, HbA_{1c} $8.4 \pm 0.7\%$, BP $127 \pm 4/84 \pm 3$ mmHg) with early nephropathy (249 ± 56 mg/day) and 15 healthy controls (Table 3h). The diabetic patients had a higher basal renal perfusion pressure than the controls. Following two days of ramipril treatment, a

decrease in basal renal perfusion pressure was seen. ACE inhibition was claimed to improve natriuretic responses due to a normalisation in proximal tubular sodium handling. A blunted increase in urinary free DA excretion was seen in IDDM, which tended to normalise following ACE inhibition. These results suggest that IDDM patients with early nephropathy display abnormalities in renal haemodynamics, natriuresis and urinary DA mobilisation in response to a sodium load, which can be reversed by short-term inhibition of ACE (394).

Chapter 5 Renal sodium-DA relationships in normotensive and hypertensive Chinese

5.1 Background of the study and main objectives

If DA is an important intrarenal natriuretic hormone and dietary sodium is the major determinant of its intrarenal synthesis, there should be a positive correlation between 24-hour urinary sodium and free DA output (Section 3.1.6). Indeed, this has been reported in Caucasians, Japanese, Thais and Zimbabweans (Table 3g).

A direct relationship between 24-hour urinary sodium and free DA outputs is not seen in West Africans and Iranians, ethnic groups from regions with intense heat and, previously, a salt-scarce environment (Section 3.1.6). The evolutionary benefit of diminished salt excretion from an inability to mobilise renal DA is obvious (Section 1.3). However, the genes that previously protected against salt depletion are a disadvantage in modern times when salt is readily available. Individuals with an inability to mobilise renal DA in response to increases in salt intake (Section 3.1.7) will be prone to salt retention and even hypertension (Section 2.5).

Chinese have been living in a salt-abundant environment for centuries and subjects living in Hong Kong have a higher salt intake now than in previous times (Section 1.6). It is interesting and important to find out whether they have an efficient renal DA system. Hypertensive subjects and their first-degree relatives are more likely to be salt-sensitive (Section 2.6), possibly due to a deficient renal DA system (Section 4.1.1). Whether this is also the case in Chinese was not known.

In the two cross-sectional studies now described, my main objective was to determine if there is a positive correlation between 24-hour urinary sodium and free DA outputs in normotensive Chinese subjects with or without a family history of

hypertension and patients with essential hypertension. These studies were performed during 1989-1991 and 1996-1998. There was a need for the second study because of the changes in classification of hypertension (586) and the recommended choice of BP measurement device (Section 5.2.2) since the first study. Also, our assay could now measure urinary DA and kallikrein simultaneously (Section 5.2.4). The greater number of subjects recruited in the second study also enabled me to investigate the interrelationships between urinary cations and free DA and kallikrein outputs in both normotensive subjects and patients with hypertension.

5.2 Subjects and methods

These cross-sectional studies had been approved by the Clinical Research Ethics Committee of the Chinese University of Hong Kong.

5.2.1 Subjects

All the healthy subjects and patients with hypertension were recruited during the cooler months of 1989-1991 and 1996-1998. The hot summers were avoided because of the problem of increased sodium loss from sweating and uncertain effects of high environmental temperature on the stability of catecholamines during urine collections (587). Informed consent was obtained from the participants after the nature and purpose of the study were explained.

Study one. As part of my study of the association between BP and sodium and potassium intakes (29), 96 healthy Chinese subjects aged 20-65 years were randomly recruited mainly from the community, but also from the staff of the Prince of Wales Hospital and medical students. They all had BP below 140/90 mmHg. In

addition, 24 patients with essential hypertension but no other diseases were recruited from our medical clinics at the Prince of Wales Hospital. They were either untreated or had been off antihypertensive drugs for at least two weeks. They all had SBP >160 mmHg and/or DBP >95 mmHg. Their renal function tests, fasting blood glucose and urinalysis were all normal.

Study two. As part of our studies of the metabolic syndrome (588), 85 healthy Chinese subjects aged 26-60 years were recruited at random mainly from the staff of the Prince of Wales Hospital and their friends. Their BP was all <120/80 mmHg. Their renal function tests, fasting blood glucose and lipid profiles were all normal. In addition, 47 patients with essential hypertension but no other diseases were recruited from our medical clinics. They were either untreated or had been off treatment for at least four weeks. They all had BP above 140/90 mmHg. Their renal function tests, fasting blood glucose and lipid profiles were all normal.

5.2.2 *BP measurement*

All these participants attended our Clinical Pharmacology Studies Unit at the Prince of Wales Hospital as out-patients for BP measurements.

Study one. Sitting (after 5 minutes) BP was measured on three occasions by a research nurse, using a Hawksley random-zero sphygmomanometer. [This device was originally chosen with the view to reduce the bias of multiple readings per person (589). It is now known to underestimate systolic readings by 2.0-3.7 mmHg (590).] An average of the three readings was taken as the subject's BP. Phase V DBP was used. In six of the patients with hypertension recruited in 1991, BP was measured by a semi-automatic sphygmomanometer (Dinamap), which had become the standard equipment for hypertension-related studies in our Department.

Study two. Sitting (after 5 minutes) BP was measured at one-minute interval by a Dinamap. An average of three readings was taken as the subject's BP.

5.2.3 *Urine collections and measurement of urinary cations and creatinine*

All the participants were asked to provide one 24-hour urine collection while on their usual diets, but alcohol and medications were avoided. Clear instructions on the need and the method for complete collection were given.

In study one, urine was collected into 2-litre plastic bottles containing 100 ml of 0.5 M HCl to ensure a pH of <3 and the stability of DA. In study two, urine was collected into 2.5-litre plastic bottles containing 40 ml of 2 g/L boric acid. All the bottles were returned on the same day of completion of the collection. Aliquots were taken and stored deep frozen at -20°C until analysis.

Urinary sodium and potassium were measured by indirect ion-selective electrodes and creatinine by Jaffe reaction on a Beckman Astra-8 Chemistry Analyser (Beckman Instruments, USA).

The 24-hour urinary output was calculated for sodium, potassium, creatinine and free DA. In study two, urinary kallikrein was also measured. Creatinine output was used as an index of under-or over-collection. In study one, seven subjects with values outside the reference range quoted by our laboratory (7.1-17.7 mmol/day in men and 5.3-15.5 mmol/day in women) were excluded. In study two, subjects with values outside the reference range were asked to repeat the collection.

5.2.4 *Measurement of urinary free catecholamines*

Urinary free catecholamines were measured in batches by high performance liquid chromatography (HPLC) with electrochemical detection. The methodology

used and the performance of the assays have been described in detail elsewhere (30,587,591). The principles of the methods are briefly summarised here.

The method used until 1995 (HCl used as preservative for urine collection)

With the help of Wong (587) and Ho (591), an ion-pair reversed phase HPLC method was developed.

The extraction of urinary free catecholamines was based on the method of Davidson & Fitzpatrick (592). However, after the adsorption of free catecholamines onto the alumina in 3 M Tris buffer, the alumina was washed with 0.1 M Tris buffer instead of 0.1 M barbitone buffer. The adsorbed free catecholamines were eluted with 0.1 M HCl instead of 0.5 M HCl.

The separation and quantification of free catecholamines by HPLC was based on the method of Weicker et al. (593). Due to the use of a different type of column (stationary phase) in our HPLC system, the acetate-citric buffer mobile phase was modified. Methanol 14% was used instead of methanol 5%, and the flow rate was increased from 1.0 to 2.0 ml/minute. A 3 M Tris buffer was used to maintain an optimal pH condition of 8.6 during the adsorption process. The main objective was to maintain a total elution time of 15 minutes for all catecholamines. Furthermore, the eluted catecholamines were further washed with ethyl acetate to remove electro-active species from the eluant. Electrochemical detector with the applied potential set at +0.6 V was used for the quantification of catecholamines.

The 'optimal' preservative (acid) for urine collection, storage conditions and pretreatment method before HPLC measurement were systematically studied. It has been a common practice to use acids as preservatives for urine collections to prevent catecholamine degradation. However, higher concentrations of inorganic acids may

hydrolyse conjugates of catecholamines, resulting in falsely high free catecholamine concentrations (594). To study the effects of duration of storage of acidified human urine, a final acid concentration of 0.025 M HCl was used. This concentration was selected based on the collection of 24-hour urine samples into 2-litre containers with 100 ml of 0.5 M HCl. In previous experiments by our group, 0.5 M HCl solution had not been shown to increase free catecholamines after a short period. As more urine was collected in the bottle, the acid solution will be diluted further. The lower acid concentration would not be sufficient to cause hydrolysis of the conjugated catecholamines. Urine samples acidified with 0.025 M HCl were kept at 30°C to simulate collection conditions by out-patients. There were no significant changes in the urinary free catecholamines over 48 hours. The same samples were also stored frozen at -20°C to simulate practical situations of storing batches of samples before analysis. Although small changes in free DA concentrations were detected after one month, the range of changes were within $\pm 5\%$ of the fresh samples. This magnitude of variation was within the inter-batch precision limits. However, both noradrenaline and adrenaline changed significantly by about 10% after one month. The effects of storage at -70°C need to be determined.

Therefore, for the collection of 24-hour urine, 100 ml of 0.5 M HCl solution was added to each 2-litre plastic container. The urine samples were kept at 4°C if the analyses could be done within three days. Otherwise, the aliquots were stored deep frozen at -20°C and the analyses completed within one month.

The analytical 'performance' of the assay had been studied by Wong (587) and Ho (591). The results of recovery and intra-batch and inter-batch precision studies are summarised in Table 5a and Table 5b.

Table 5a. Results of recovery and intra-batch precision study.

	Concentration of standard (nM)	Measured concentration (nM)	% Recovery	Intra-batch CV (%)
DA	0	1002	-	1.0
	500	1472	94.0	1.0
	1000	2003	100.1	3.1
	2000	3031	101.5	1.1
NA	0	304	-	3.2
	200	503	99.5	3.0
	400	715	102.8	2.2
	600	905	100.2	1.4
A	0	58	-	17.4
	100	147	89.0	3.6
	200	246	94.0	8.4
	300	346	96.0	3.2

Data from Ho (591).

Table 5b. Inter-batch precision study using 2 quality control urine samples.

Quality control sample	Free catecholamines	Mean concentration (nM)	CV (%)
A (n = 97)	DA	1011	4.8
	NA	279	2.0
	A	69	11.6
B (n = 47)	DA	612	6.2
	NA	91	9.9
	A	27	29.6

Data from Ho (591).

As can be seen in Table 5a, the average recovery of free DA was 98.5%. The intra-batch coefficient of variation ranged from 1.0 to 3.1%. The average recovery of free NA was 100.8%. The intra-batch coefficient of variation ranged from 1.4 to 3.2%.

As can be seen in Table 5b, the inter-batch coefficient of variation for free DA was 4.8-6.2%. The figure for free NA was 2.0-9.9%.

The method used since 1996 (boric acid used as preservative for urine collection)

In the past, the roles of renal DA, the SNS and the renal kallikrein-kinin system in ECF volume and BP homeostasis were studied in isolation. Attempts to study these systems simultaneously were hampered by incompatibility of the urine preservatives for catecholamines and kallikrein.

In order to measure acid-stable DA and NA and acid-labile kallikrein enzyme together, Lee & Critchley (30,595) compared the use of boric acid and HCl as the preservative for urine collection before measurements by HPLC using the method of Wong & Ho described above. As can be seen in Table 5c, the variations between the DA and NA concentrations preserved in HCl or boric acid at different time intervals (tested by 2-way analysis of variance) were not statistically significant. The aliquots could be stored at -20°C for up to three months. Hence, in our studies of renal DA since 1996, boric acid was used as the urine preservative. The advantage of using boric acid is that it would not interfere with the measurement of urinary kallikrein (Section 5.2.5). The inter-assay coefficients of variation were 4.8% at 1,011 nmol/L for DA and 2.9% at 279 nmol/L for NA. The intra-assay coefficients of variation were 1.0% at 1,472 nmol/L for DA and 3.0% at 503 nmol/L for NA. The mean recovery of urinary free DA and NA was 92% and 93 %, respectively (30,595).

Table 5c. Urinary free DA and NA concentrations (nmol/L) in 10 human urine samples containing 0.5 M HCl or 2 g/L boric acid at ambient temperature over 48 hours and -20°C over 3 months.

	Time	Temp ($^{\circ}\text{C}$)	HCl (0.5 M)	Boric acid (2 g/L)	Two-way ANOVA
DA (n = 10)	0 hour (Plain)	Ambient	1,286 \pm 163	1,286 \pm 163	Timer factor: p = 0.950
	24 hours	Ambient	1,337 \pm 178	1,289 \pm 176	Acid factor: p = 0.762
	48 hours	Ambient	1,298 \pm 176	1,211 \pm 172	Interaction of two factors: P=0.971
	2 weeks	-20	1,235 \pm 161	1,256 \pm 165	Time factor: p = 0.988
	1 month	-20	1,315 \pm 181	1,306 \pm 182	Acid factor p = 0.964
	2 months	-20	1,341 \pm 170	1,330 \pm 169	Interaction of two factors:
	3 months	-20	1,343 \pm 182	1,315 \pm 178	p = 1.000
	NA (n = 10)	0 hour (Plain)	Ambient	147 \pm 23	147 \pm 23
24 hours		Ambient	146 \pm 25	145 \pm 25	Acid factor: p = 0.954
48 hours		Ambient	148 \pm 24	145 \pm 24	Interaction of two factors: p = 0.999
2 weeks		-20	149 \pm 23	150 \pm 24	Time factor: p = 0.944
1 month		-20	159 \pm 27	195 \pm 27	Acid factor p = 0.889
2 months		-20	167 \pm 27	166 \pm 27	Interaction of two factors:
3 months		-20	161 \pm 27	162 \pm 26	p = 1.000

Data from Lee (30).

5.2.5 *Measurement of urinary kallikrein*

The methodology used and the performance of the assay have been described in detail elsewhere (30,595).

In brief, urinary kallikrein was measured by the method of Amundsen et al. (596). However, we used a 96-well ELISA plate instead of test tubes and cuvettes. This modification saves on substrate volume, increases the number of samples being assayed and simplifies the absorbance measurement using an ELISA plate reader instead of measuring absorbance in cuvettes one by one. This is an amidolytic assay in which urinary kallikrein is measured by its capacity to cleave the amido bond of a synthetic chromogenic tripeptide D-valyl-leucyl-arginine-*p*-nitronilide, S-2266 (AR KABI Diagnostica, Stockholm, Sweden), which exhibits good specificity and sensitivity for glandular kallikrein. Aliquots were centrifuged at 2000 x *g* for 10 minutes at 4°C and the supernatant removed. Later, 50 µl of 0.2 mol/L Tris-HCL buffer with pH 8.2 (Merck) was added before incubation at 37°C for 5-10 minutes using a 96-well ELISA plate. Supernatants of urine or kallikrein standards of porcine origin (Bayer, Leverkusen, GFR) (120 µl) were mixed with the buffer and incubated at 37°C for 2-5 minutes. The reaction was started by adding 40 µl of 1.2 mmol/L of S-2266 and was stopped by mixing with 30 µl of 50% acetic acid (Merck) after incubating at 37°C for 30 minutes. The absorbance at 405 nm was measured by the Dynatech MR5000 microplate spectrophotometer and compared with that of the same incubation mixture containing 50 kIU/ml aprotinin (Bayer) within 4 hours. By adding aprotinin, a potent inhibitor of glandular kallikrein, to the sample blank, protease activities not inhibited by aprotinin as well as the colour from the urine itself can be subtracted. Results were expressed as units of kallikrein activity per litre (KU/L).

The intra-assay coefficients of variation were 1.8% at 8.4 ± 0.1 KU/L, 2.7% at 13.2 ± 0.3 KU/L and 1.2% at 300.4 ± 3.4 KU/L. The inter-assay coefficients of variation were 2.4% and 5.6% at the kallikrein concentrations of 3.1 ± 0.01 KU/L and 322.4 ± 17.0 KU/L, respectively. The recoveries of urinary kallikrein at various concentrations ranged from 90 to 100%. The stability of urinary kallikrein with time was analysed by one-way repeated measures ANOVA.

Table 5d. Urinary kallikrein concentrations (KU/L) in the 10 human urine samples preserved with and without 2 g/L boric acid at ambient temperature over 48 hours and -20°C over 3 months.

Time	Temperature ($^{\circ}\text{C}$)	Urinary kallikrein concentration measured in urine preserved with 2 g/L of boric acid (KU/L)	One-way repeated ANOVA
0 h (plain)		82 ± 26	
24 hours	Ambient	82 ± 39	
48 hours	Ambient	81 ± 38	$p = 0.189$
2 week	-20	83 ± 39	
1 month	-20	82 ± 39	
2 months	-20	82 ± 39	
3 months	-20	83 ± 40	$p = 0.198$

Data from Lee (30).

If HCl was used as the urine preservative, the recovery of urinary kallikrein was only 5.7% after storage at -20°C for up to three months (30). Hence, when urine was collected for measurements of kallikrein and/or free DA and NA, boric acid was

used as the preservative. Aliquots were stored at -20°C and the analyses completed within one (for DA and NA) or three months (for kallikrein).

5.2.6 *Statistical analysis*

Throughout my thesis, data are expressed as mean \pm SEM. In this study, significance of difference for all variables was assessed by analysis of variance (ANOVA). Correlation coefficients between urinary sodium, potassium, free DA and kallikrein were determined by linear regression analysis. P values of <0.05 were regarded as statistically significant.

5.3 **Results**

5.3.1 *Study one*

Thirty of the 89 normotensive subjects with a complete urine collection had one or more first-degree relatives with hypertension requiring drug treatment. As can be seen in Table 5e, there was a female predominance across the three groups of subjects. This was due to the reluctance of the males to participate in a study like this, because they would have to take a few days off work. The two groups of normotensive subjects were otherwise comparable with respect to age, body height and weight and BP. When compared with the normotensive subjects, hypertensive patients were heavier and had a higher SBP and DBP ($p<0.05$ by ANOVA).

The 24-hour urinary excretion of sodium, potassium, creatinine and free DA did not differ between the normotensive subjects and hypertensive patients ($p>0.05$ by ANOVA).

Table 5e. Characteristics of 89 normotensive (NT) Chinese with or without a family history of hypertension (FH) and 24 hypertensive Chinese recruited in 1989-1991.

	NT with no FH (n=59)	NT with FH (n=30)	Hypertensives (n=24)	P value (ANOVA)
Males:females	21:38	5:25	8:16	
Age (years)	43.4 ± 2.0	48.3 ± 1.3	45.5 ± 1.7	NS
Height (cm)	157.3 ± 1.0	156.0 ± 1.3	157.9 ± 2.0	NS
Weight (kg)	55.4 ± 1.1	55.3 ± 1.3	63.6 ± 1.9	<0.05
SBP (mm Hg)	104.7 ± 1.5	104.4 ± 1.6	161.2 ± 2.3	<0.05
DBP (mm Hg)	69.5 ± 1.1	68.8 ± 1.4	100.7 ± 1.2	<0.05
24-hour urine				
Volume (l)	1.54 ± 0.1	1.46 ± 0.1	1.59 ± 0.1	NS
Creatinine (mmol)	9.0 ± 0.3	8.9 ± 0.4	10.0 ± 0.7	NS
Sodium (mmol)	128.5 ± 6.5	133.7 ± 6.2	147.5 ± 13.5	NS
Potassium (mmol)	37.4 ± 1.8	40.4 ± 2.7	40.2 ± 2.7	NS
Free DA (nmol)	1,461 ± 78	1,540 ± 86	1,593 ± 71	NS

NS = not significant

As can be seen in Figure 5a, there was a positive correlation between 24-hour urinary sodium and free DA outputs in normotensive subjects with no family history ($r=0.42$, $p<0.001$, x coefficient=5.02). However, such a relationship was not seen in normotensive subjects with a family history of hypertension ($r=0.22$, $p<0.5$) or hypertensive patients ($r=-0.02$, $p>0.5$).

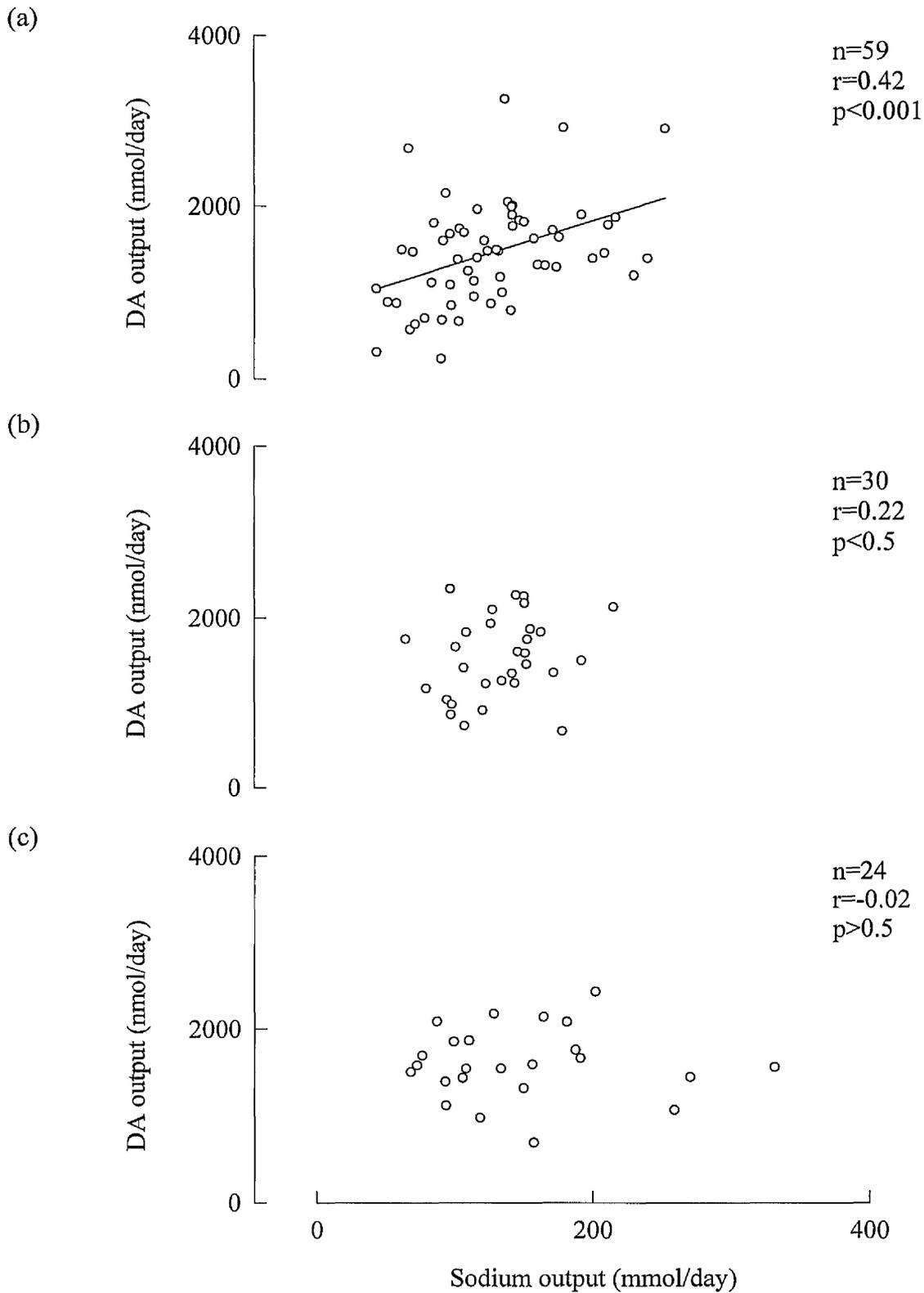


Figure 5a. Relationship between 24-hour urinary sodium and DA outputs in normotensive Chinese with (b) or without (a) a family history of hypertension and in (c) hypertensive Chinese recruited in 1989-1991.

5.3.2 *Study two*

Twenty-seven of the 85 normotensive subjects had one or more first-degree relatives with hypertension requiring treatment. The characteristics of these subjects and hypertensive patients are summarised in Table 5f.

Table 5f. Characteristics of 85 normotensive (NT) Chinese with or without a family history of hypertension (FH) and 47 hypertensive Chinese recruited in 1996-1998.

	NT with no FH (n=58)	NT with FH (n=27)	Hypertensives (n=47)	P value (ANOVA)
Males:females	23:35	14:13	25:22	
Age (years)	42.1 ± 1.2	39.1 ± 1.4*	42.3 ± 1.0	NS
Height (cm)	158.3 ± 0.9	162.7 ± 1.3	160.2 ± 1.3	<0.05
Weight (kg)	59.4 ± 10.5	63.7 ± 10.0	69.6 ± 2.0	<0.05
SBP (mm Hg)	115.9 ± 1.4	117.2 ± 2.1	155.9 ± 2.1	0.000
DBP (mm Hg)	69.3 ± 1.4	69.4 ± 2.0	97.6 ± 1.3	0.000
24-hour urine				
Volume (l)	1.7 ± 0.1	1.9 ± 0.2‡	1.8 ± 0.1*	NS
Creatinine (mmol)	9.9 ± 0.4	10.5 ± 0.8§	11.3 ± 0.6§	NS
Sodium (mmol)	163.2 ± 8.4	191.2 ± 15.0*	171.3 ± 11.4	NS
Potassium (mmol)	42.0 ± 2.5	44.9 ± 2.8†	43.8 ± 2.0	NS
Free DA (nmol)	1,618 ± 65	1,554 ± 108†	1,857 ± 93	<0.05
Kallikrein (KU)	8.1 ± 0.6‡	8.5 ± 1.0*	7.0 ± 0.6‡	NS

Data from *1, †2, ‡3 or §4 subjects were missing.

The three groups of subjects were different ($p < 0.05$ by ANOVA) with respect to the height, body weight, SBP and DBP (Table 5f). As expected, the SBP and DBP of patients with hypertension were much higher than those of normotensive subjects.

The 24-hour urinary excretion of sodium, potassium, creatinine and kallikrein did not differ between the normotensive subjects and hypertensive patients ($p > 0.05$ by ANOVA). However, the free DA output was 14.8-19.0% higher in hypertensives than in normotensive subjects ($p < 0.05$ by ANOVA).

As can be seen in Figure 5b, there was a positive correlation between 24-hour urinary sodium and free DA outputs in normotensive subjects without a family history ($r = 0.36$, $p = 0.006$, x coefficient = 2.77), normotensive subjects with a family history ($r = 0.57$, $p = 0.004$, x coefficient = 4.0) and patients with hypertension ($r = 0.44$, $p = 0.002$, x coefficient = 3.57).

As can be seen in Figure 5c, a positive correlation between urinary potassium and free DA outputs was seen in normotensives with a family history of hypertension ($n = 23$, $r = 0.53$, $p = 0.010$, x coefficient = 20.1) and patients with hypertensive ($n = 47$, $r = 0.34$, $p = 0.020$, x coefficient = 15.7). However, such a relationship was not seen in normotensives without a family history of hypertension ($n = 58$, $r = 0.21$, $p < 0.5$).

As can be seen in Figure 5d, there was no correlation between 24-hour urinary sodium and kallikrein outputs in either normotensives ($r = 0.04$, $p > 0.5$ and $r = 0.17$, $p < 0.5$) or patients with hypertension ($r = 0.26$, $p < 0.1$).

As can be seen in Figure 5e, there was a positive correlation between urinary potassium and kallikrein outputs in patients with essential hypertension ($n = 44$, $r = 0.35$, $p = 0.020$, x coefficient = 0.10). However, such a relationship was not seen in normotensive subjects ($n = 55$, $r = 0.18$, $p < 0.5$ and $n = 24$, $r = 0.18$, $p < 0.5$).

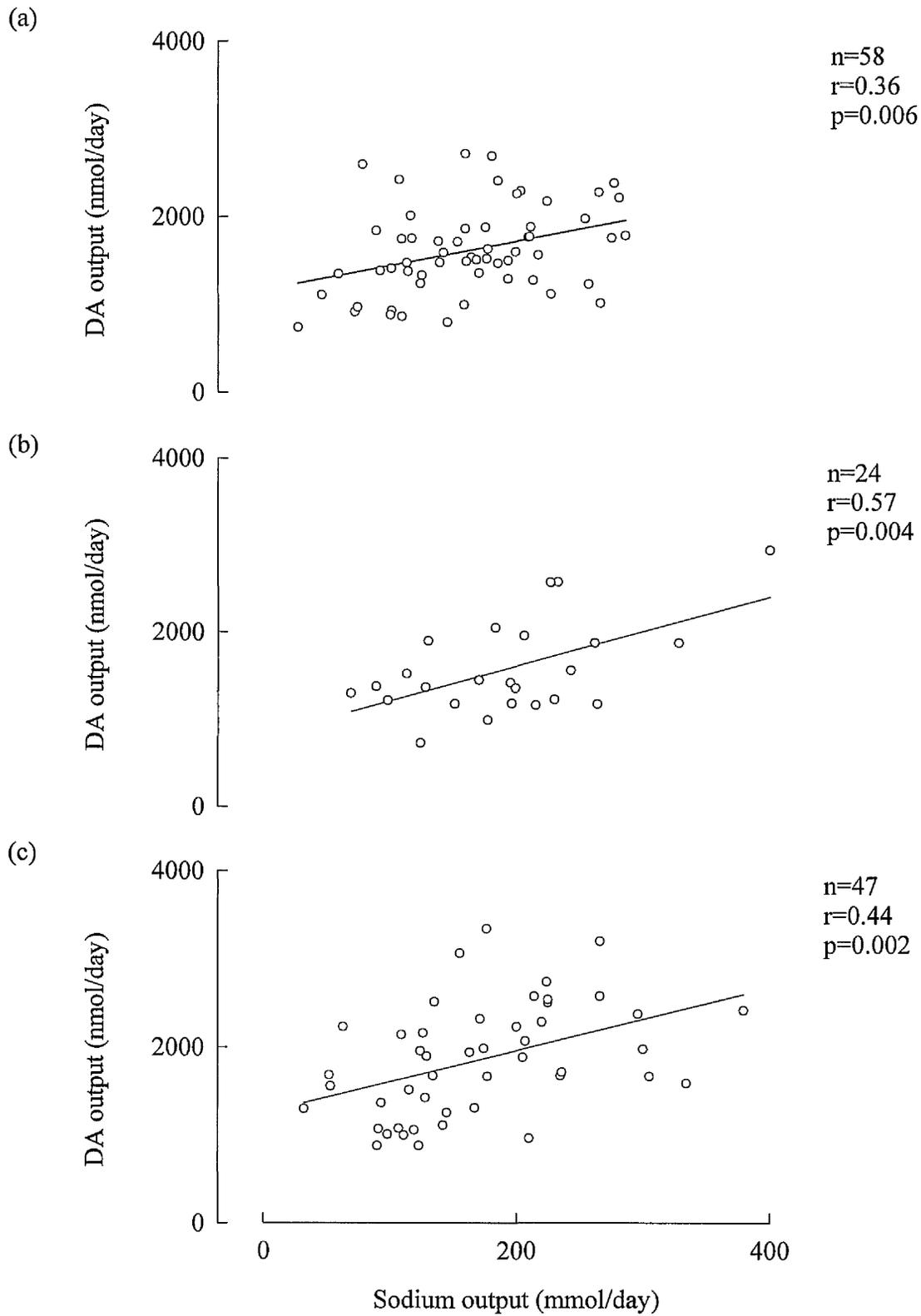


Figure 5b. Relationship between 24-hour urinary sodium and DA outputs in normotensive Chinese with (b) or without (a) a family history of hypertension and in (c) hypertensive Chinese recruited in 1996-1998.

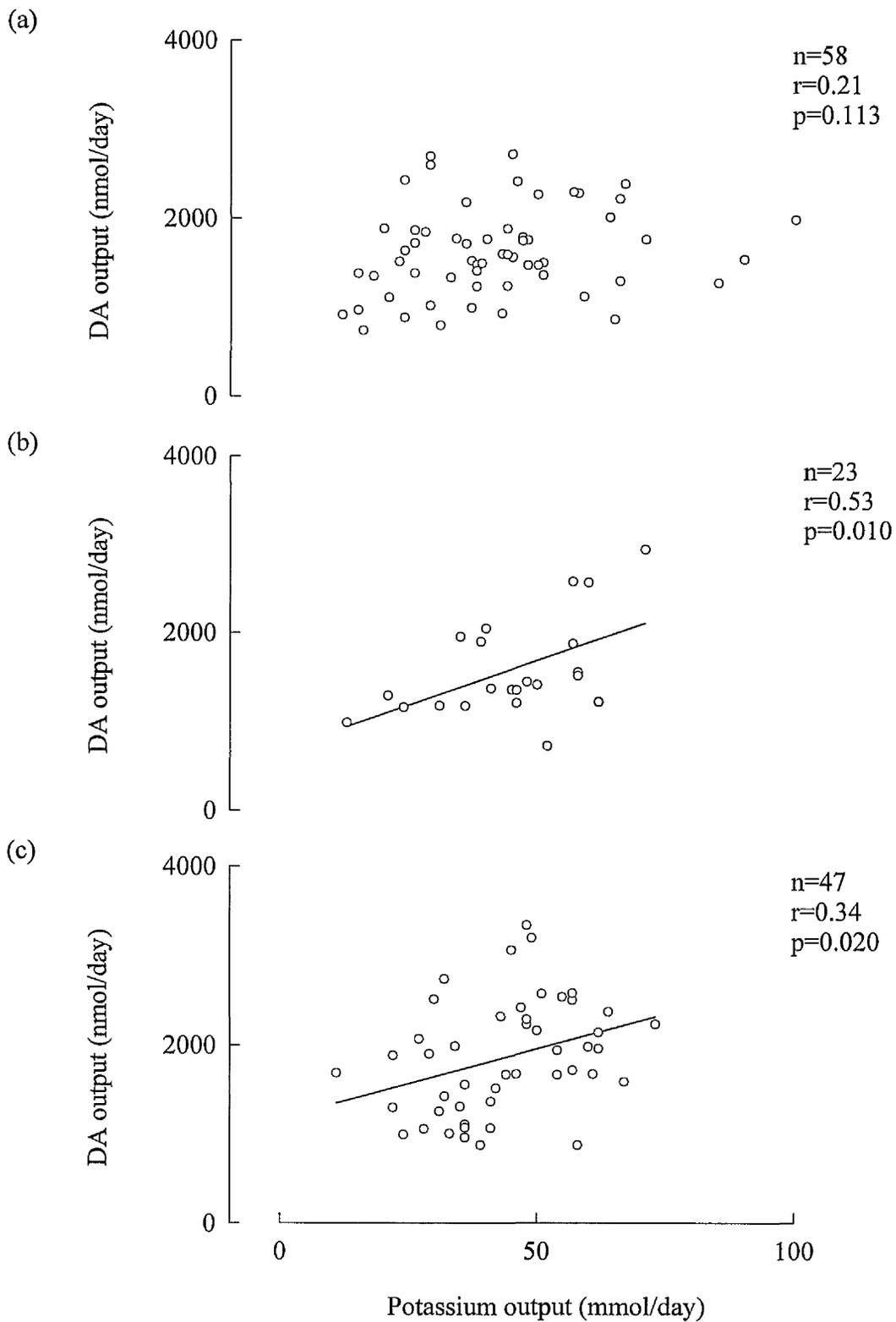


Figure 5c. Relationship between 24-hour urinary potassium and DA outputs in normotensive Chinese with (b) or without (a) a family history of hypertension and in (c) hypertensive Chinese recruited in 1996-1998.

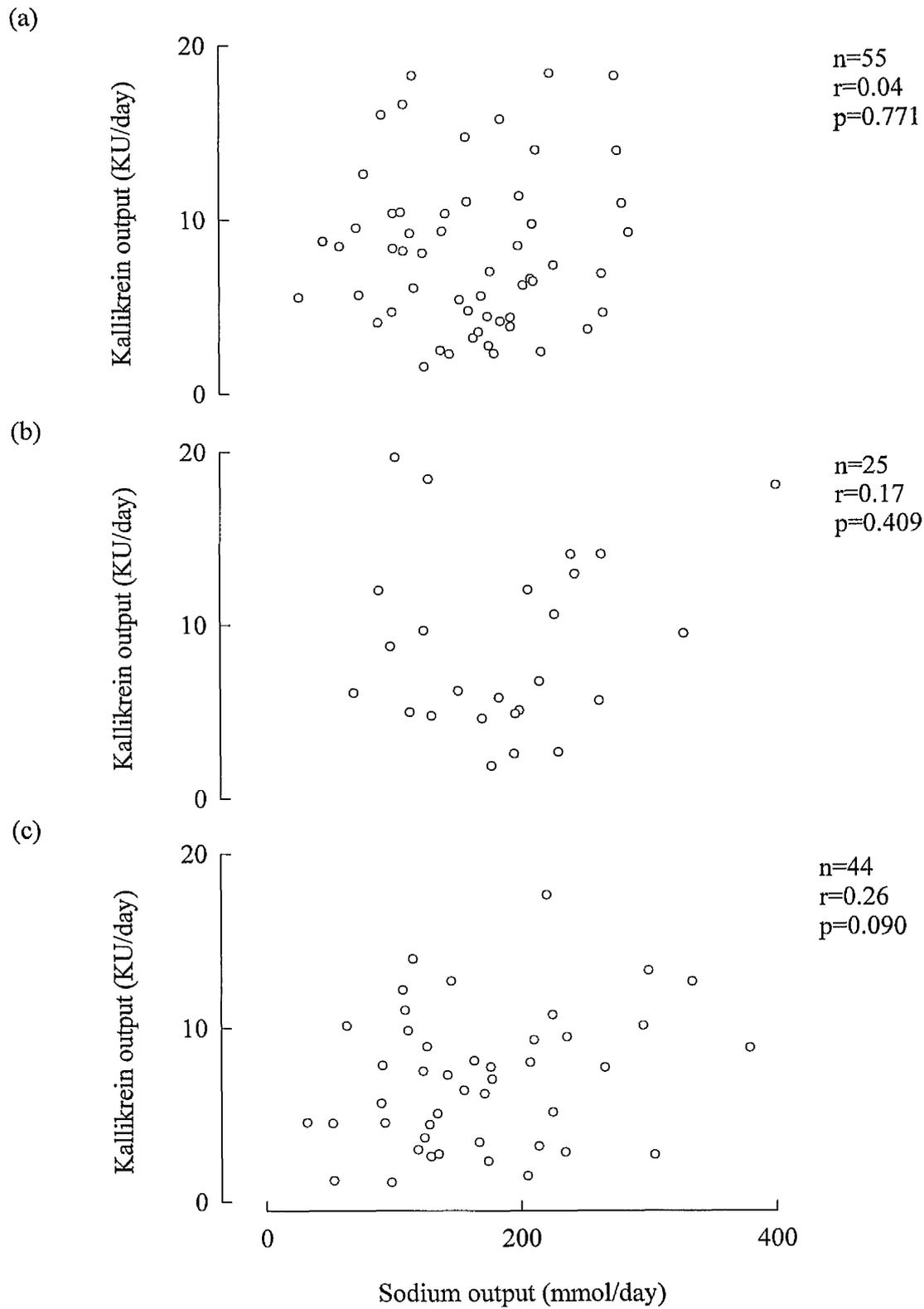


Figure 5d. Relationship between 24-hour urinary sodium and kallikrein outputs in normotensive Chinese with (b) or without (a) a family history of hypertension and in (c) hypertensive Chinese recruited in 1996-1998.

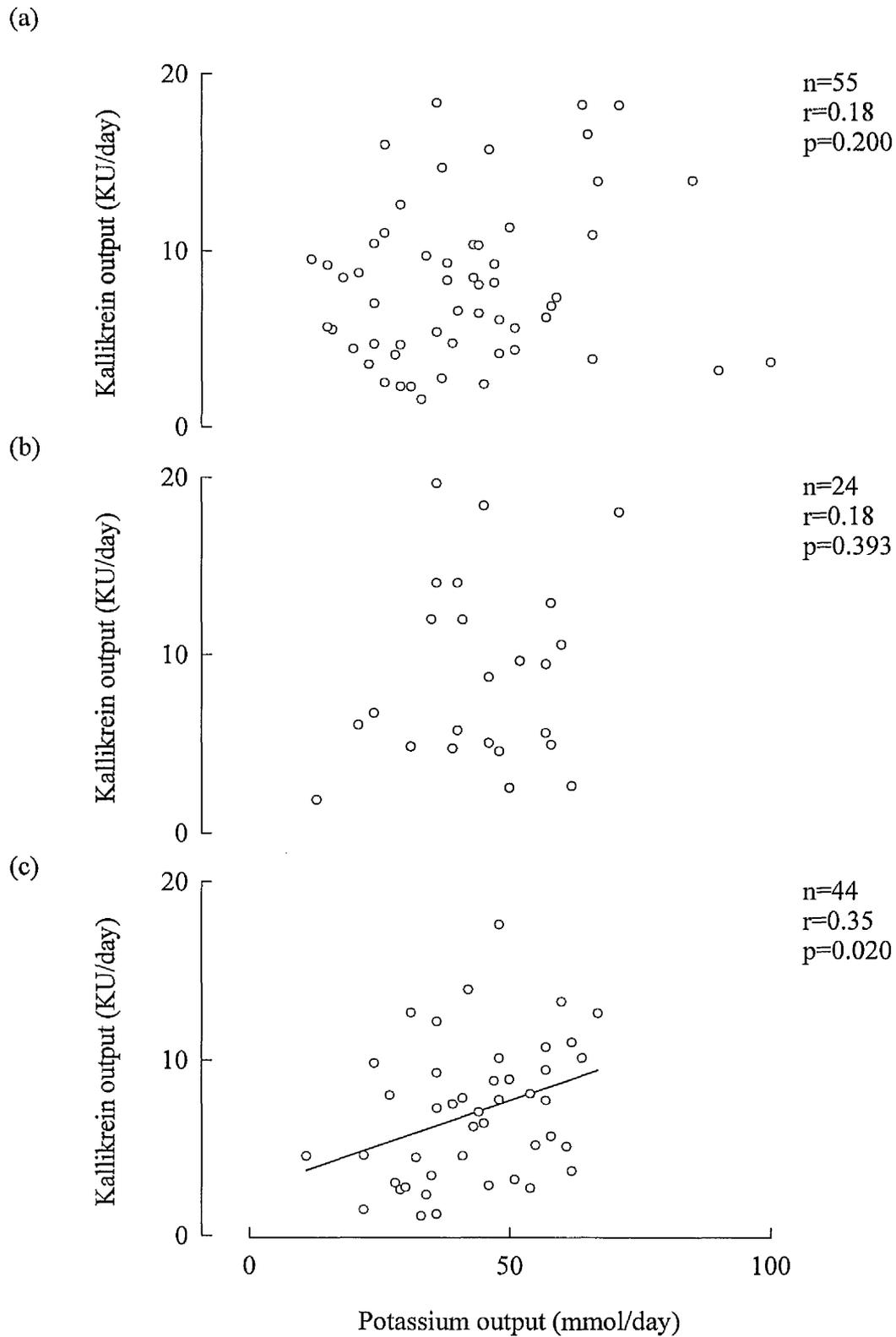


Figure 5e. Relationship between 24-hour urinary potassium and kallikrein outputs in normotensive Chinese with (b) or without (a) a family history of hypertension and in (c) hypertensive Chinese recruited in 1996-1998.

5.4 Discussion

Between 1989-1991 and 1996-1998, the salt intake among the local Chinese, as reflected by their 24-hour urinary sodium output, had increased considerably in both normotensive (27.0-43.0%) and hypertensive subjects (16.1%) (Table 5e and Table 5f). This increase in salt intake was accompanied by a trend for larger 24-hour urinary free DA output (0.9-10.7% and 16.6%).

As expected of ethnic groups living in salt-abundant regions (Table 3g), a positive correlation between the 24-hour urinary sodium and free DA outputs was seen in normotensive Chinese subjects in Hong Kong (Figure 5a and Figure 5b).

5.4.1 *Variations in findings between study one and study two*

Since these studies involved different subjects recruited at different times, direct comparison and interpretation of the findings may be difficult. Unlike in study one (Figure 5a), normotensive subjects with a family history of hypertension and patients with hypertension in study two also showed a positive correlation between urinary sodium and free DA outputs (Figure 5b). The inclusion criteria (BP and its measurement), recruitment method (community-based or not) and characteristics of the participants (age, body weight, BP levels and salt intake) were different between the two studies and these could have contributed to the variations in findings.

It is always difficult to verify the parental history of hypertension in a study like this. Parents not known to have hypertension requiring treatment might not be available for BP measurement at the study centre. Hence, the offspring of patients having undiagnosed hypertension could have been 'misplaced' in the group without a family history. However, it is unlikely that my findings regarding the renal sodium-DA relationships would be much different in view of the small (if any) number of

cases involved. Anyway, there was a positive correlation between 24-hour urinary sodium and free DA outputs in both groups of normotensives in study two (Figure 5b) and a trend for such a relationship among normotensives with a family history of hypertension in study one (Figure 5a). It is possible that genetics (Section 4.1), salt-sensitivity (Section 2.6 and Section 4.1), other determinants (Section 3.2.2) and their interactions will influence the renal sodium-DA relationships in these subjects.

Patients with essential hypertension in study two were younger (42.3 ± 1.0 vs 45.5 ± 1.7 years) but heavier (69.6 ± 2.0 vs 63.6 ± 1.9 kg) than patients in study one (Table 5f and Table 5e). Their salt intake was also higher than patients in study one (urinary sodium output 171.3 ± 11.4 vs 147.5 ± 13.5 mmol/day). They had less severe hypertension than patients in study one ($155.9 \pm 2.1 / 97.6 \pm 1.3$ vs $161.2 \pm 2.3 / 100.7 \pm 1.2$ mmHg). The group difference in BP should be larger in view of the falsely low readings due to the use of a random-zero sphygmomanometer in study one (590). Unfortunately, the duration of hypertension had not been recorded in detail in both studies. Their age and BP levels might indicate that patients in study two had less severe hypertension of shorter duration. Their sodium-DA relationships tended to resemble those of the normotensive subjects. Unlike patients in study one (Figure 5a and Table 5e), they had a positive urinary sodium-DA correlation (Figure 5b) and hyperdopaminergic response (Table 5f) (see Section 4.1.1 and Section 5.4.5).

5.4.2 *Published studies in Chinese*

My findings regarding the renal sodium-DA relationship in Chinese subjects were confirmed by two other studies in Mainland China and Hong Kong. However, in both studies, the parental history of hypertension among the participants was not stated.

Hou & Zhang (597) in Guangzhou studied the correlation between 24-hour urinary sodium and free DA outputs in 22 healthy volunteers (14 males, 8 females, aged 22-53 years, mean 31.4 years) on unrestricted diets. [Ten of these subjects also participated in an oral salt loading study and the results will be presented in Section 7.4.3.] Their 24-hour urinary free DA outputs (measured by HPLC) varied greatly between 1,500 and 6,900 nmol. The mean \pm SEM values for free DA and sodium outputs were not given. There was a strong positive correlation between the 24-hour urinary excretion of sodium and free DA ($r=0.87$, $p<0.01$).

Ho et al. (598) recruited 41 young healthy females aged 18-23 years from a study of determinants of bone mass in Hong Kong. Their 24-hour urinary sodium (132 ± 8.2 , range 54-302 mmol) and free DA ($1,463 \pm 47.5$, range 902-2,134 nmol) was significantly correlated ($r=0.55$, $p<0.001$). Stepwise multiple regression analysis with DA as the dependent variable and sodium, potassium, calcium and digoxin-like immunoreactive substance as independent variables showed that sodium was the only contributor to DA excretion.

5.4.3 *Comparison with other ethnic groups*

In cross-sectional studies of the relationship between 24-hour urinary sodium and DA outputs, the x coefficient or 'slope' of the linear regression (Section 3.1.6) provides an estimate of the factor by which free DA output is increased in proportion to an increase in sodium output. As can be seen in Table 3g and in this study, the x coefficient is higher among the Thais (5.93) and Zimbabweans (5.10) than in Hong Kong Chinese (2.77-5.02) and Caucasians (3.19).

Critchley et al. (361) pointed out that the correlations between 24-hour urinary sodium and free DA outputs are based on populations rather than individual

data. In their study, the 'r' values for Caucasians, Zimbabweans and Thais only represented variances of 16%, 41% and 28% (Table 3g). The 'r' value for Caucasian subjects in the study of Patrick et al. (362) represented variance of 26%. The 'r' value for Japanese in the study of Saito et al. (363) represented variance of 27%. The 'r' values for Hong Kong Chinese in this study and the study of Ho et al. (552) represented 13-32% and 30%, respectively.

5.4.4 *The West Africans and Iranians*

In contrast, West Africans and Iranians do not show a positive correlation between 24-hour urinary sodium and free DA (Section 3.1.6). They come from regions of high ambient temperature and, previously, a salt-scarce environment. The evolutionary benefit of diminished salt excretion is obvious (Section 1.3). Lee (19) and Critchley et al. (361) proposed that the lack of or 'uncoupling' of the renal sodium-DA relationship in these subjects may be a mechanism to help conserve salt.

5.4.5 *Patients with hypertension and their first-degree relatives*

Previously, studies on the renal sodium-DA relationship in patients with hypertension have only focused on the 24-hour urinary free DA output under basal conditions (Section 4.1.1). Whether there is a positive correlation between urinary sodium and free DA outputs was never studied.

In Caucasian and Japanese patients with 'established' hypertension, urinary free DA excretion under basal conditions is decreased compared with normotensive subjects (509-513). In Caucasian patients, a hyperdopaminergic state with increased 24-hour urinary DA output is seen in the 'early' stage of hypertension - borderline (labile) hypertension (576). Once hypertension becomes established, the decreased

urinary DA output typical of older patients with hypertension is seen. Unlike older patients (513), younger Japanese patients with hypertension have a higher 24-hour urinary free DA output than normotensive subjects (515). Taken together, these findings suggest that the increased intrarenal DA synthesis in younger patients with borderline or mild to moderate hypertension may represent an early antihypertensive compensatory mechanism (Section 4.1.1). Therefore, it is not entirely surprising that a hyperdopaminergic response under basal conditions is seen among the younger patients with less severe hypertension in study two (Table 5f) but not in the older patients with more severe hypertension in study one (Table 5e). The absence of a significant urinary sodium-DA relationship among older patients with more severe hypertension in study one (Figure 5a) also suggest a dysregulation of the renal DA system in established hypertension.

Apart from older age and more severe hypertension, salt-sensitvity may be associated with a suppressed renal DA output (Section 4.1.1). In fact, decreased urinary free DA output under basal conditions is seen in Japanese patients with salt-sensitive hypertension and low-renin hypertension (512,513).

Previously, a lower 24-hour urinary free DA excretion under basal conditions among the relatives of hypertensive patients compared with healthy subjects without a family history of hypertension was reported in a Japanese study (513). However, this finding could not be confirmed in the present study (Table 5e and Table 5f) and previous studies of Japanese (363) or Caucasian (371) subjects.

Unlike the Japanese subjects (363), the normotensive first-degree relatives of Chinese patients with hypertension did show a significant correlation between 24-hour urinary sodium and free DA outputs (Figure 5b).

Hence, our findings regarding the renal sodium-DA relationships in normotensive subjects do not provide support for the presence of a dysregulated renal DA system at the prehypertensive stage.

5.4.6 *Potassium intake and renal DA production*

Potassium supplements given by mouth or as an intravenous infusion can increase urinary free DA excretion (306,453). A positive correlation between urinary potassium and free DA outputs was seen in the present study of hypertensives and some normotensives. These observations suggest that renal DA production is also determined by the potassium intake (Section 3.2.2). This could be one reason why higher potassium intake is associated with a lower BP in population studies (599) and dietary potassium restriction exacerbates hypertension (600). On the other hand, potassium supplementation can lower BP, and this effect is more pronounced in subjects with a higher sodium intake (601).

In the present study, a positive correlation between urinary potassium and free DA outputs was seen only in normotensives with a family history and patients with hypertension (Figure 5c). This observation might suggest that potassium intake as a determinant of renal DA production was more important in hypertensives and their close relatives. As can be seen in Table 5f, both subject groups had a higher sodium intake and/or BP than normotensives with no family history of hypertension.

5.4.7 *Renal kallikrein-kinin system activity*

Like renal DA, the renal kallikrein-kinin system plays an important role in ECF volume and BP homeostasis (Section 2.3.3).

Decreased 24-hour urinary kallikrein excretion under basal conditions could indicate suppressed activity in the renal kallikrein-kinin system. This was reported among hypertensive patients in Sweden (602) and Italy (603,604) and among salt-sensitive hypertensives in Italy (87). Urinary kallikrein output was even lower in low-renin hypertensives from Italy (604) and patients with malignant hypertension from Sweden (602). However, like Simonetti et al. (605) in Italy, I could not find any evidence of impaired production of renal kallikrein among patients with essential hypertension (Table 5f).

As in Japanese subjects (494,517), urinary kallikrein output in normotensive subjects with a family history of hypertension was found to be similar to those with no family history (Table 5f). However, Rey et al. (606) reported that the boys with hypertensive parents had a lower ratio of urinary kallikrein to creatinine compared with boys with normotensive parents.

Previous studies rarely examined the environmental determinants of renal kallikrein production. Hunt et al. (607) in the U.S. studied 69 pairs of monozygous twins to investigate possible dietary, biochemical and anthropometric determinants of urinary kallikrein excretion. Urinary potassium and sodium excretion differences were significantly related to kallikrein differences ($r=0.46$, $p=0.0001$ and $r=0.30$, $p=0.01$). Urinary pH ($r=0.23$, $p=0.03$) and SBP ($r=-0.25$, $p=0.03$) differences were related to urinary kallikrein output differences independently of urinary potassium. Song et al. (86) studied the determinants of urinary kallikrein excretion in 204 normotensives from different ethnic groups living in the U.S. In a multivariate analysis, potassium excretion was found to be the strongest correlate of kallikrein excretion. Stimulation of renal kallikrein production could be the other reason why potassium intake is inversely related to BP (599-601).

In the present study, there was no correlation between urinary sodium and kallikrein outputs in either normotensives or hypertensives (Figure 5d). This finding, which needs to be confirmed by a bigger study, may suggest that under basal states, renal kallikrein production is not linked to the prevailing salt balance. To determine the relative importance of renal kallikrein-kinin system in sodium balance, salt loading studies will be required (Chapter 10). A positive correlation between 24-hour urinary potassium and kallikrein outputs was seen, only among hypertensives (Figure 5e). Thus, potassium intake appeared to be a factor affecting renal kallikrein synthesis. Potassium supplementation could lower BP among hypertensives by stimulating both renal DA and kallikrein-kinin systems.

5.4.8 *Significance and implications of my findings*

Among younger Chinese patients with less severe hypertension, but not older patients with more severe hypertension, 24-hour urinary free DA output under basal conditions is increased compared with normotensive subjects. This enhanced renal DA response may represent an early antihypertensive compensatory mechanism.

There is a positive correlation between 24-hour urinary sodium and free DA outputs in normotensive Chinese with or without a family history of hypertension and younger patients with less severe hypertension. This may suggest that among these subjects, intrarenal DA synthesis is linked to the prevailing salt status and renal DA may regulate the renal excretion of sodium.

Normotensive subjects with or without a family history of hypertension do not differ with regards the 24-hour urinary free DA output under basal conditions and the urinary sodium-DA relationship. In other words, there is no evidence for any dysregulation of the renal DA system at the prehypertensive stage.

Since these cross-sectional studies of renal sodium-DA relationship involved different subjects recruited at different times using slightly different methodology, the conclusions regarding hypertensive patients and their first-degree relatives cannot be considered to be definitive. A bigger comparative study using identical methodology is needed to confirm my findings

A positive correlation between urinary potassium and free DA outputs was seen in hypertensives and normotensives with a family history of hypertension, suggesting that renal DA production is also determined by the potassium intake of these subjects.

Renal kallikrein production under basal conditions, as indicated by 24-hour urinary kallikrein output, is not different between normotensives and hypertensives. The absence of a positive correlation between 24-hour urinary sodium and kallikrein outputs may suggest that renal kallikrein production is not linked to the prevailing salt balance among normotensives and hypertensives. However, acute salt loading studies will be more useful in determining the relative importance of renal kallikrein-kinin system in sodium balance. The presence of a correlation between 24-hour urinary potassium and kallikrein outputs may suggest that potassium intake is one factor affecting renal synthesis of kallikrein among hypertensives.

Chapter 6 A sibling-pair analysis of renal dopamine and kallikrein-kinin system activities and their relationships to sodium excretion and BP

6.1 Background of the study and main objectives

Recognition that hypertension is part genetically determined has motivated studies to identify mutations that confer susceptibility. Mutations in at least 10 genes have been shown to alter BP. These mutations alter BP through a common pathway, changing salt and water reabsorption in the kidney (608). The molecular genetics of salt-sensitivity (Section 2.6) and hypertension were recently reviewed (609).

In view of the physiological role of endogenous DA (Chapter 3) and renal kallikrein-kinin system (Section 2.3.3) in ECF volume and sodium homeostasis, it would be of great interest to study the genetic influence on the activities of these natriuretic systems under basal states and during salt loading. There could be several approaches, as already discussed in Section 2.3.3, Chapter 3, Section 4.1 and Chapter 5. For example, renal sodium-DA or sodium-kallikrein relationships and 24-hour urinary free DA and kallikrein outputs were compared between healthy subjects with or without a family history of hypertension and among different ethnic groups. The natriuretic response and renal DA response to salt loading were compared between normotensives with or without a family history of hypertension (also see Chapter 7 and Chapter 8). The association between DA receptor gene polymorphisms with hypertension was studied. Pedigree analyses were used to study gene-environment interaction and the genetic influence on urinary kallikrein excretion and the risk of hypertension. Rat models of hypertension were also used.

Siblings have approximately 50% of their genes in common. They are less likely to be subjected to environmental differences than unrelated individuals. Thus, sibling-pair studies reduce the variability inherent in population-based studies of multifactorial disorders, such as hypertension (610). This study was conducted to examine the renal DA and kallikrein-kinin system activities and their relationships to BP and sodium and potassium excretion in Chinese by studying siblings discordant for hypertension.

6.2 Subjects and methods

This study had been approved by the Clinical Research Ethics Committee of the Chinese University of Hong Kong. All subjects gave informed consent to the study.

As part of our studies on the genetics and clinical characteristics of metabolic syndrome in Hong Kong (611), Chinese patients with essential hypertension, aged 25-55 years, with at least one available normotensive sibling, were recruited from our Hypertension Clinic at the Prince of Wales Hospital. Those with diabetes, renal disease, secondary hypertension or hyperlipidaemia were not included. Hypertensive probands were either untreated or their antihypertensive drugs were withheld, under supervision, for at least four weeks. Sitting (after 10 minutes) BP was measured by a Dinamap. The mean of three readings taken at 1-minute intervals on two occasions was used for analysis. The hypertensive probands all had BP >140/90 mmHg, while their siblings all had BP <120/80 mmHg.

All the participants were asked to provide one 24-hour urine collection while on their usual diets, but alcohol and medications were avoided. Clear instructions on

the need and the method for complete collection were given. Creatinine output was used as an index of completeness of urine collection. Subjects with values outside the reference range were asked to repeat the collection.

Urine was collected into 2.5-litre plastic bottles containing 40 ml of 2 g/L boric acid as preservative (Section 5.2.4). Bottles were returned on the same day of completion of collection. Aliquots were taken and stored at -20°C until analysis.

Urinary sodium and potassium were measured by indirect ion-selective electrodes. Urinary creatinine was measured by Jaffe reaction on a Beckman Astra-8 Chemistry Analyser (Beckman Instruments, USA). Urinary free DA and kallikrein were measured as in study two in Chapter 5 (Section 5.2.4 and Section 5.2.5). The 24-hour urinary output was calculated for sodium, potassium, creatinine, free DA and kallikrein.

Significance of difference between groups was tested by Student's unpaired t test. Correlation coefficients between urinary sodium, potassium, free DA and kallikrein and between BP and urinary free DA and kallikrein were determined by linear regression analysis. P values of <0.05 were regarded as statistically significant.

6.3 Results

The characteristics of these 43 hypertensive and normotensive sibling-pairs are summarised in Table 6a. The hypertensives were heavier (69.1 ± 1.7 vs 62.7 ± 1.6 kg, $p < 0.01$) and had a larger urinary creatinine output. Their BP was also higher (147.0 ± 2.5 / 91.4 ± 1.5 vs 120.0 ± 1.3 / 71.8 ± 1.2 mmHg, $p < 0.01$) than their normotensive siblings.

Table 6a. Characteristics of 43 normotensive and hypertensive sibling-pairs.

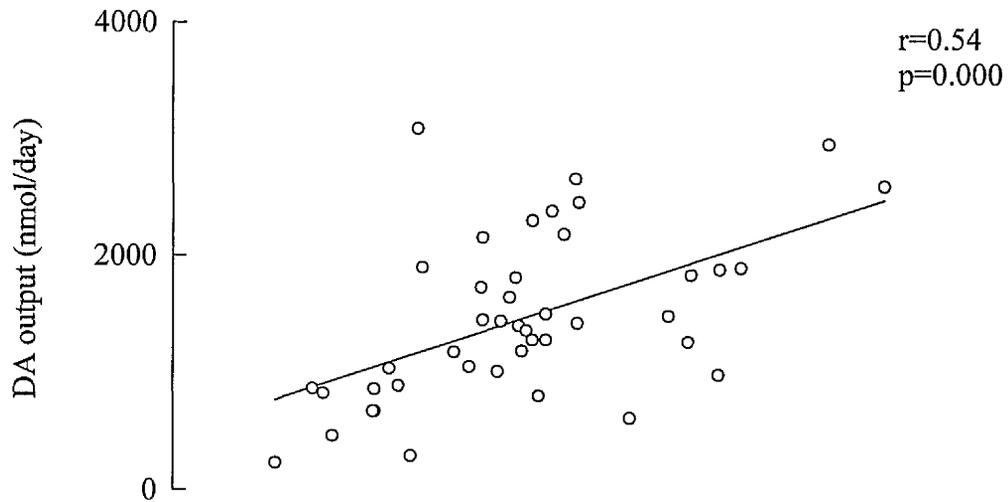
	Normotensives	Hypertensives	P value
Males:females	14:29	21:22	
Age (years)	39.0 ± 1.0	40.0 ± 0.9	NS
Height (cm)	160.0 ± 1.2	160.5 ± 1.2	NS
Weight (kg)	62.7 ± 1.6	69.1 ± 1.7	<0.01
SBP (mm Hg)	120.0 ± 1.3	147.0 ± 2.5	<0.01
DBP (mm Hg)	71.8 ± 1.2	91.4 ± 1.5	<0.01
24-hour urine			
Volume (l)	1.72 ± 0.1	1.90 ± 0.1	NS
Creatinine (mmol)	9.4 ± 0.6	11.2 ± 0.6	<0.05
Sodium (mmol)	196.7 ± 13.8	188.2 ± 14.9	NS
Potassium (mmol)	47.0 ± 2.4	47.6 ± 2.4	NS
Free DA (nmol)	1,457 ± 107	1,765 ± 108	<0.05
Kallikrein (KU)*	7.5 ± 0.7	7.0 ± 0.5	NS

NS = not significant. *Data from 11 subjects were missing.

The sibling-pairs did not differ with respect to the 24-hour urinary sodium, potassium and kallikrein outputs. However, the 24-hour urinary free DA output was 21.1% higher among the hypertensives ($1,765 \pm 108$ vs $1,457 \pm 107$ nmol, $p < 0.05$).

The correlations between urinary sodium, potassium, free DA and kallikrein outputs are summarised in Figure 6a, Figure 6b, Figure 6c and Figure 6d.

(a)



(b)

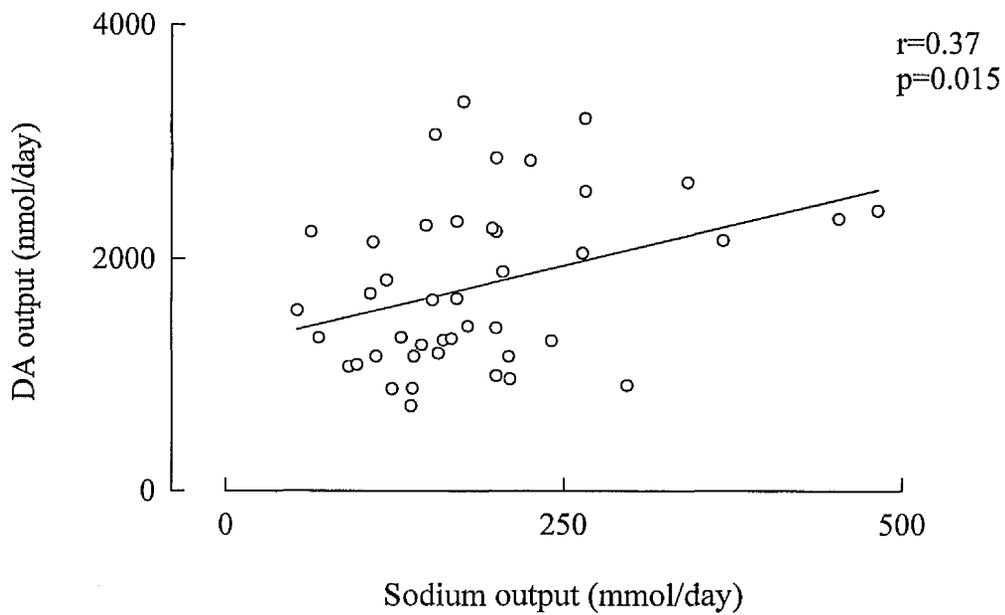
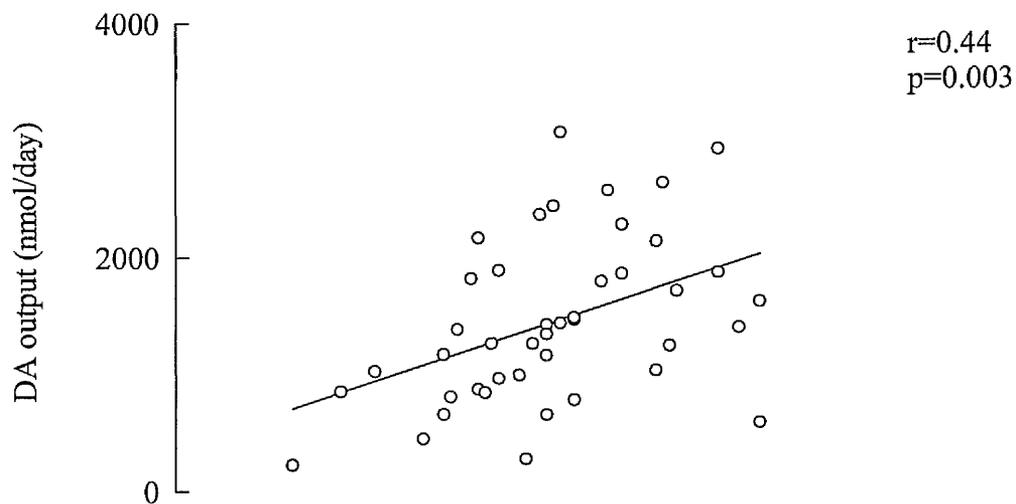


Figure 6a. Relationship between 24-hour urinary sodium and DA outputs in 43 (a) normotensive and (b) hypertensive sibling-pairs.

(a)



(b)

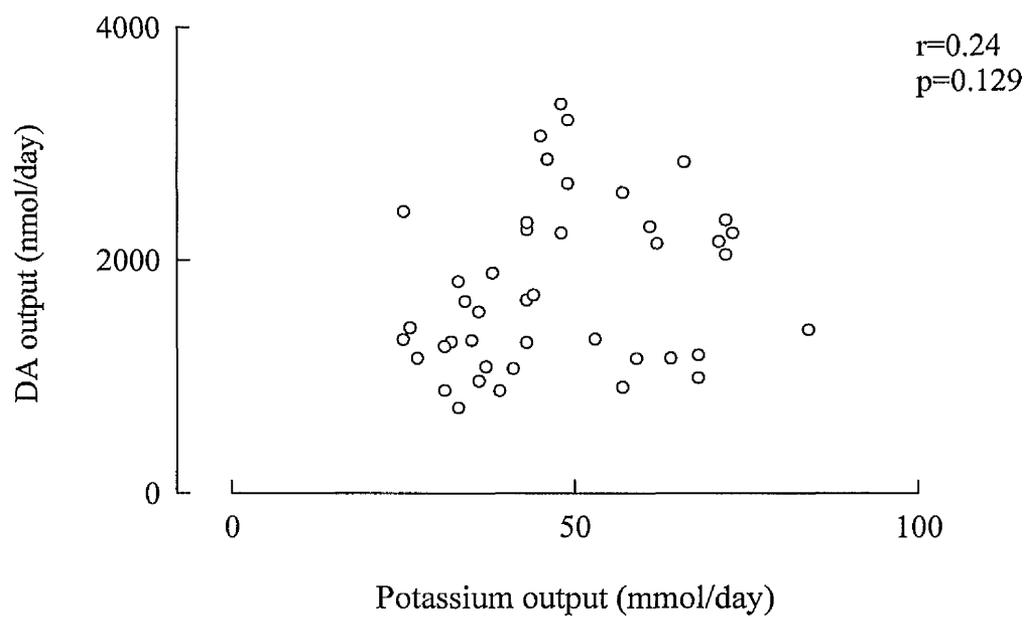


Figure 6b. Relationship between 24-hour urinary potassium and DA outputs in 43 (a) normotensive and (b) hypertensive sibling-pairs.

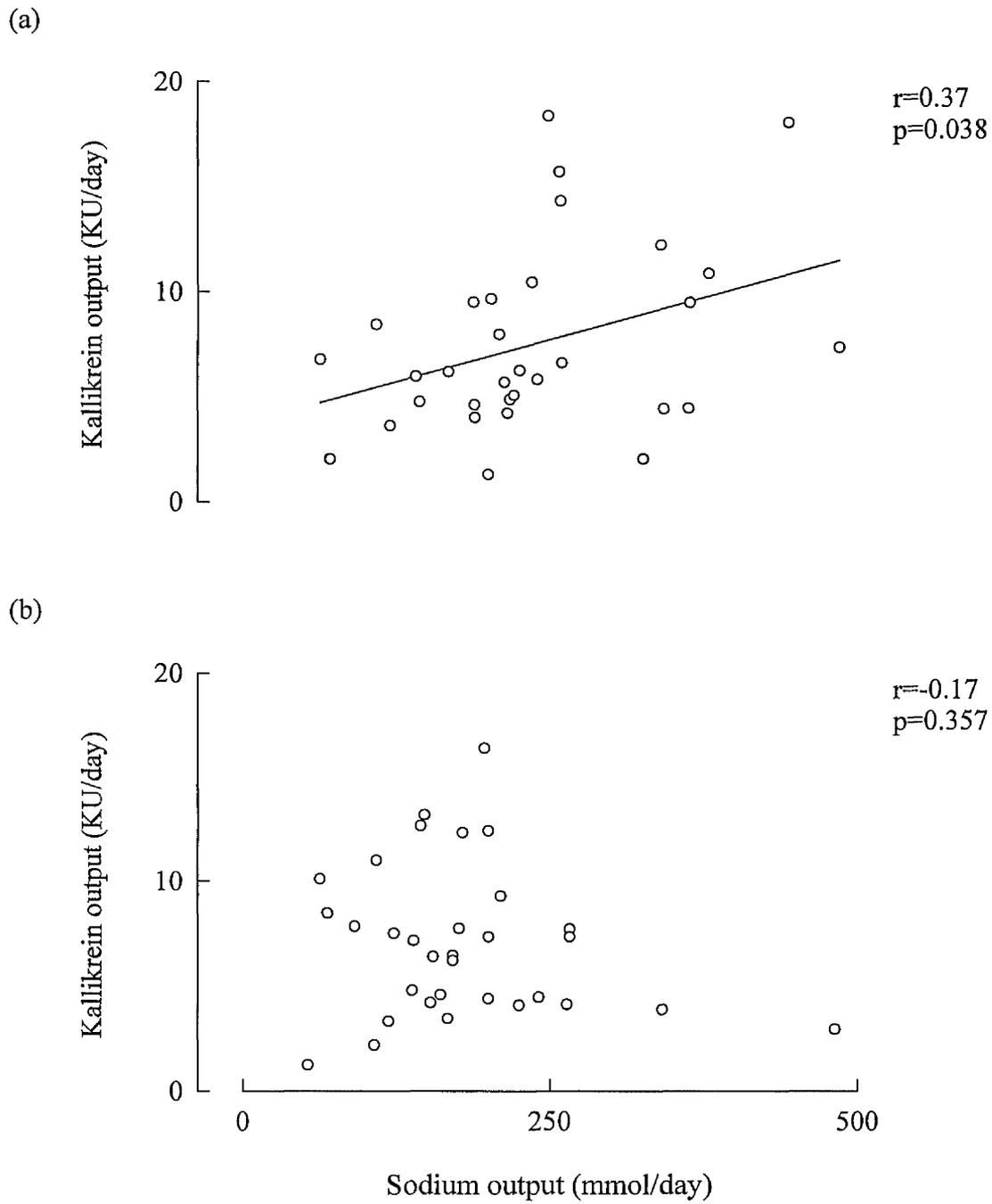


Figure 6c. Relationship between 24-hour urinary sodium and kallikrein outputs in 32 (a) normotensive and (b) hypertensive sibling-pairs.

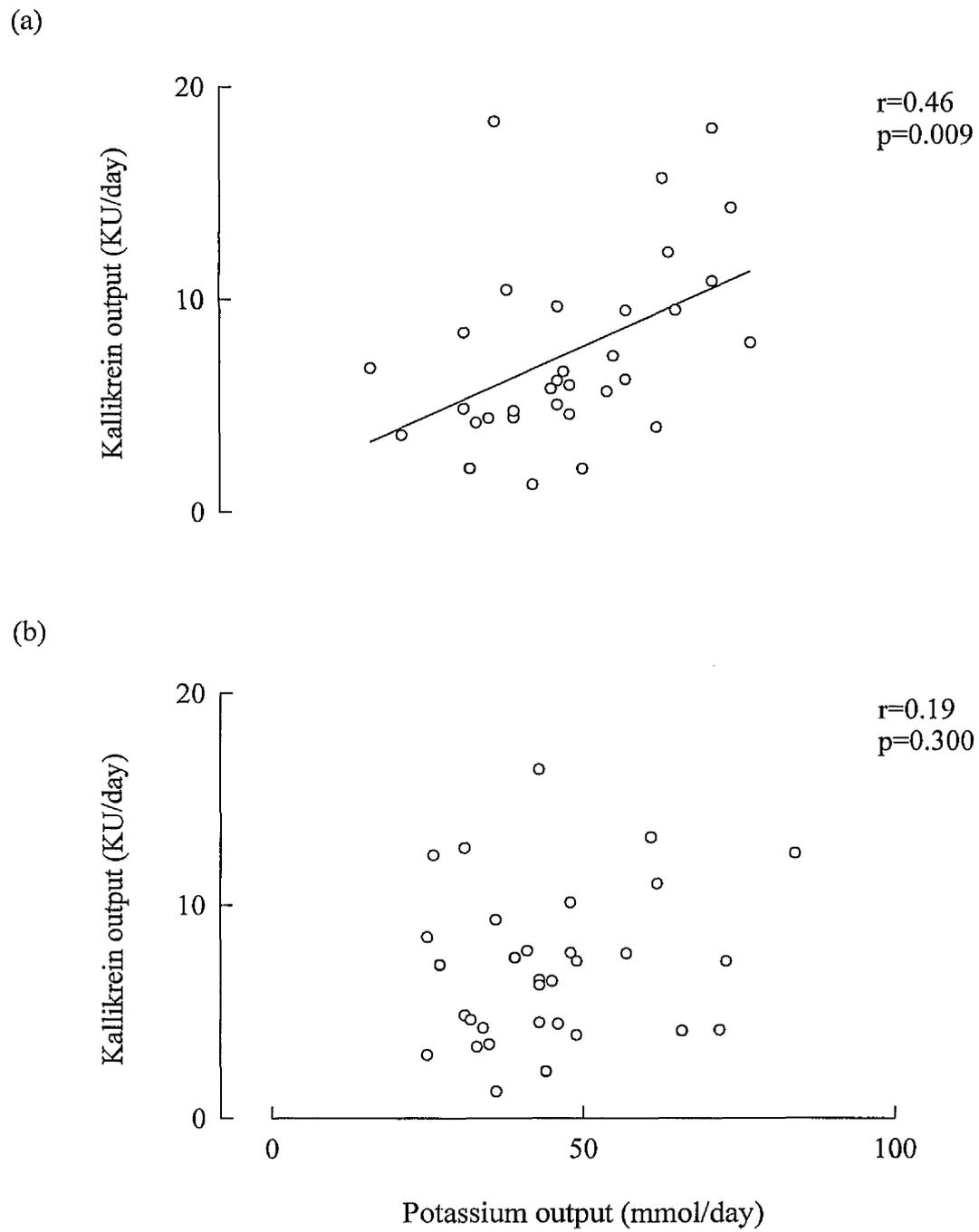


Figure 6d. Relationship between 24-hour urinary potassium and kallikrein outputs in 32 (a) normotensive and (b) hypertensive sibling-pairs.

As can be seen in Figure 6a, there was a positive correlation between 24-hour urinary sodium and free DA outputs in normotensive ($r=0.54$, $p=0.000$, x coefficient =5.02) and hypertensive ($r=0.37$, $p=0.015$, x coefficient=3.23).

As can be seen in Figure 6b, free DA excretion was significantly related to urinary potassium in normotensives ($r=0.44$, $p=0.003$, x coefficient=2.28) but not in hypertensive ($r=0.235$, $p=0.129$) siblings.

As can be seen in Figure 6c, kallikrein excretion was related to urinary sodium in normotensives ($r=0.37$, $p=0.038$, x coefficient=5.32) but not in their hypertensive siblings ($r=0.17$, $p=0.357$).

As can be seen in Figure 6d, kallikrein excretion was related to urinary potassium in normotensives ($r=0.46$, $p=0.009$, x coefficient=8.28) but not in their hypertensive counterparts ($r=0.19$, $p=0.300$).

Among normotensive siblings, SBP was not related to 24-hour urinary free DA ($r=0.133$, $p=0.39$) or kallikrein ($r=0.04$, $p=0.843$) output. DBP was not related to 24-hour urinary free DA ($r=0.01$, $p=0.973$) or kallikrein ($r=-0.28$, $p=0.190$) output.

Among hypertensive siblings, there was a trend for an inverse relationship between SBP and urinary free DA output ($r=-0.30$, $p=0.052$) and between DBP and urinary kallikrein output ($r=-0.34$, $p=0.054$). However, there was no correlation between DBP and 24-hour urinary free DA ($r=0.01$, $p=0.931$) or between SBP and urinary kallikrein ($r=-0.22$, $p=0.230$).

6.4 Discussion

These sibling-pairs discordant for hypertension were recruited during 1996-1998. Their sodium intake, as reflected by their 24-hour urinary sodium output (see

Table 6a), was much higher than those of normotensives (47.1-53.1%) and hypertensives (27.6%) recruited during 1989-1991 (see Table 5e). There was a corresponding 10.8% increase in 24-hour urinary free DA output among the hypertensive siblings, whereas the free DA output among the normotensive siblings was similar to that of normotensives studied in 1989-1991. It was also obvious from Table 6a and Table 5e that normotensive siblings were also heavier and had a higher BP than the normotensive subjects in 1989-1991.

6.4.1 *Renal sodium-DA relationships*

In this sibling-pair study, I confirmed the finding from study two of Chapter 5 that there was a positive correlation between 24-hour urinary sodium and free DA outputs in both normotensives and hypertensives (Figure 6a). This may suggest that renal DA is an important natriuretic factor and dietary sodium is a major determinant of renal DA synthesis (Section 3.1.6) in Chinese normotensives and hypertensives. Of the variation in free DA excretion in normotensives and hypertensives in this study, 29% and 14% could be accounted by variation in sodium intake. These were comparable to the figures (13-32% and 19%) in study two of Chapter 5.

In this study, I also confirmed the presence of a hyperdopaminergic response among younger patients with less severe hypertension (Table 6a). This could represent an early antihypertensive compensatory mechanism in these siblings (Section 4.1.1 and Section 5.4.5).

6.4.2 *Potassium intake and renal DA production*

Potassium intake can only affect renal DA production (Section 5.4.6), but the intrarenal mechanism involved is not known. In study two of Chapter 5, a positive

correlation was seen between 24-hour urinary potassium and free DA outputs among hypertensives and normotensives with a family history of hypertension (Figure 5c). It is possible that potassium intake has a greater effect on renal DA synthesis when the salt intake or the BP of the subjects is higher (Section 5.4.6). In the present study, there was a positive correlation between 24-hour urinary potassium and free DA outputs among normotensives but not in hypertensives (Figure 6b). Although salt intake was higher among normotensive siblings (Table 6a), the group difference was only 8.5 mmol. In the study of Ho et al. (598), there was no correlation between 24-hour urinary potassium and free DA output among young healthy females.

Therefore, the findings so far seemed to suggest that potassium intake could affect renal DA synthesis in some subjects. Both the underlying mechanisms and the modulatory effects of salt intake, BP and other factors are not known.

6.4.3 *Renal kallikrein-kinin system activity*

I confirmed that renal kallikrein-kinin system activity under basal conditions was not different between hypertensives and normotensives (Section 5.4.7).

There were considerably variations in urinary cation-kallikrein relationships seen in this sibling-study and study two of Chapter 5. Among the normotensives, 24-hour urinary kallikrein output was related to the urinary excretion of sodium (Figure 6c) and potassium (Figure 6d). Such a correlation was not seen in study two of Chapter 5 (Figure 5d and Figure 5e). Among the hypertensive siblings, urinary kallikrein output was not related to either urinary sodium (Figure 6c) or potassium (Figure 6d). A positive correlation between 24-hour urinary potassium and kallikrein outputs was seen in hypertensives in study two of Chapter 5 (Figure 5e). Due to the variations in diet and the lack of consistency in findings regarding cation-kallikrein

relationships, cross-sectional studies are not very useful in determining the role of renal kallikrein-kinin system in ECF volume and BP homeostasis. Therefore, there is a need for salt-loading studies (Chapter 10).

6.4.4 *BP in relation to urinary DA and kallikrein outputs*

Hyperdopaminergic response may be seen in younger hypertensives (Section 4.1.1, Section 5.4.5 and Section 6.4.1). SBP is inversely related to urinary kallikrein in monozygous twins (607). It will be of interest to find out if BP of normotensives and hypertensives is related to urinary free DA and kallikrein excretion.

Among normotensive siblings, neither SBP nor DBP was related to urinary free DA or kallikrein outputs. In contrast, a trend for an inverse relationship between SBP and urinary free DA excretion ($r=-3.0$, $p=0.052$) was seen in hypertensive siblings. Like the hyperdopaminergic response (Section 5.4.5 and Section 6.4.1), this may indicate the presence of an antihypertensive compensatory mechanism in these hypertensives. The inverse relationship between DBP and 24-hour urinary kallikrein output may suggest that hypertensives with suppressed renal kallikrein synthesis will be at risk of more severe hypertension (Section 2.3.3).

6.4.5 *Significance and implications of my findings*

In this sibling-pair study, I have confirmed the presence of enhanced renal DA production hypertensive subjects. This may represent an early antihypertensive compensatory mechanism. In accord with this hypothesis is the finding of a trend for an inverse relationship between SBP and 24-hour urinary free DA output among hypertensive subjects.

I have also shown that there is a positive correlation between 24-hour urinary sodium and free DA outputs in both normotensive and hypertensive siblings. This may suggest that in these subjects, intrarenal DA synthesis is linked to the prevailing salt status and endogenous DA may regulate the renal excretion of sodium.

A positive correlation between urinary potassium and free DA outputs was seen in normotensive siblings, suggesting that renal DA production is also determined by the potassium intake of these subjects.

I have confirmed that urinary kallikrein output under basal conditions was not different between hypertensives and normotensives. In hypertensive siblings, the presence of an inverse relationship between DBP and 24-hour urinary kallikrein output may suggest that subjects with suppressed renal kallikrein synthesis will be at risk of more severe hypertension. Cross-sectional studies of normotensives and hypertensives so far have yielded inconsistent results regarding urinary cation-kallikrein relationships.

Salt-loading studies would be required in determining the relative importance of renal DA, renal kallikrein-kinin system and other neurohormones in ECF volume and BP homeostasis in Chinese with or without a genetic risk of hypertension.

Chapter 7 Urinary free DA and NA outputs during acute changes in oral salt intake in healthy Chinese subjects

7.1 Background of the study and main objectives

I have shown that normotensive Chinese subjects with or without a family history of hypertension show a positive correlation between 24-hour urinary sodium and free DA outputs (Chapter 5 and Chapter 6). This may suggest that DA has an important intrarenal natriuretic role in Chinese as well as normotensive Caucasians and Japanese and dietary salt is a major determinant of its intrarenal synthesis (Section 3.1.6). It was, thus, anticipated that they would respond to oral salt loading with an increase in urinary free DA output similar to that reported in Caucasians and Thais (Section 3.1.7).

In my preliminary investigation into the role of renal DA in the natriuretic response to oral salt loading (612) and the tolerability and feasibility of such studies in healthy volunteers, five normotensive Chinese subjects (4 males and 1 female, aged 23-35 years) without a family history of hypertension received a 70 mmol sodium diet for three days (days 1 to 3) followed by their usual diets with unrestricted salt intake for six days (days 4 to 9). On days 4 to 6, they also received 10 'Slow sodium' tablets (Ciba-Geigy) at 9 a.m. and 1 p.m. equivalent to 200 mmol of sodium per day. There was a 5- to 8-fold increase in mean daily sodium excretion (40 mmol on day 3 vs 216-389 mmol on days 4-6 and 183-317 mmol on days 7-9, $p < 0.05$). As a group, the mean daily urinary free DA output did not change (1,284 nmol on day 3 vs 1,298-1,408 nmol on days 4-6 and 1,481-1,670 nmol on days 7-9, $p > 0.05$). None of these subjects had any discomforts during the study. I then realised that my results could have been affected by other confounding factors in the

diet such as variations in protein, potassium, calcium and phosphate intakes (Section 3.2.2). Furthermore, the basal sodium intake was only reduced to 70 mmol/day, compared with a daily intake of 20 mmol or lower in most published studies (Table 3h). It was not known if my results would have been different if a basal sodium intake of 20 mmol/day had had been used. The use of a similar protocol would also enable me to establish if there is indeed an ethnic difference in renal DA response to oral salt loading.

Another mechanism which appears to contribute to the natriuresis following salt loading is a reduction in SNS activity, which can be demonstrated by a reduction in plasma concentration and/or urinary excretion of free NA (Section 2.4.2).

In this study, my main objective was to determine the roles of renal DA and suppression of SNS activity in the natriuretic response to oral salt loading in healthy Chinese subjects taking a constant diet with two different sodium intakes.

7.2 Subjects and methods

This study had been approved by the Clinical Research Ethics Committee of the Faculty of Medicine, the Chinese University of Hong Kong. After the nature and purpose of the study were explained, consent was obtained from each participant.

Eight healthy Chinese males (aged 22-24 years) were studied in July 1992 after a routine physical and laboratory examination to rule out hypertension, diabetes mellitus, renal disease or other significant illness. These medics were living in the air-conditioned student hostel next to the Prince of Wales Hospital, where they had their clinical attachments. Their parents all had a BP <140/90 mmHg, as indicated by the recent readings recorded by the medics themselves or family doctors.

The study was performed in the out-patient setting. During the 10-day study period, they had deliberately avoided outdoor activities so as to minimise sodium loss from sweating. All the meals they consumed during the study were prepared in the hospital kitchen. From days -4 to 0 and days 1 to 5, subjects were given the same basic diet containing 1,900 calories, 75 g protein, 20 mmol sodium and 45 mmol potassium. In addition, from days 1 to 5, subjects were given 20 'Slow sodium' tablets (Ciba-Geigy) equivalent to 200 mmol of sodium per day. These were taken in two divided doses with breakfast and lunch. After salt restriction (10 mmol/day), the amount of sodium excreted in urine on each day approaches the new intake within four days (613). Subsequent salt loading (350 mmol/day) results in a greatly increased urinary sodium output that reaches the new steady state within three days.

Body weight and BP after lying supine for 10 minutes were measured in the morning before breakfast at the end of each 5-day period. The mean of three consecutive readings measured by a Dinamap at intervals of one minute was taken as the subject's BP. MAP was calculated by adding one-third of the pulse pressure to the diastolic pressure (phase V).

Urine was collected for 24 hours from 8:00 a.m. to 8:00 a.m. in plastic bottles containing 100 ml of 0.5M HCl on day -4 and from days 0 to 5. The bottles were kept in the air-conditioned hospital compound until urine collection was complete. The bottles were then returned immediately. Aliquots were stored at -20°C until assayed for sodium, potassium, creatinine, free DA and free NA.

Urinary sodium and potassium were measured by indirect ion-selective electrodes and creatinine by Jaffe reaction on a Beckman Astra-8 Chemistry Analyser (Beckman Instruments, USA). Urinary free DA and NA were measured by HPLC using electrochemical detection, as in study one of Chapter 5 (Section 5.2.4).

The 24-hour urinary output was calculated for sodium, potassium, creatinine, free DA and free NA.

Differences from the baseline and between each study period for all variables were tested using the Wilcoxon sign-rank test. P values <0.05 were regarded as statistically significant.

7.3 Results

Table 7a summarises the data on MAP, pulse rate and body weight observed at entry and on the last day of each of the study periods. There were no significant changes in MAP; the eight subjects were therefore regarded as 'salt-resistant' (369,371). As expected, sodium restriction resulted in a decrease in body weight and sodium loading resulted in subsequent increase in body weight.

Table 7a. MAP, pulse rate and body weight in eight healthy Chinese subjects during ingestion of 20 or 220 mmol of sodium per day.

	At entry (day -4)	20 mmol sodium (day 0)	220 mmol sodium (day 5)
MAP (mmHg)	78.2 ± 2.4	76.8 ± 1.8	75.0 ± 2.8
Pulse (beats/min)	65.9 ± 3.3	63.5 ± 2.4	61.6 ± 4.0
Body weight (kg)	58.3 ± 1.9	57.3 ± 1.9*	57.9 ± 1.9†

*p<0.05 when compared with 'at entry'.

†p<0.05 when compared with '20 mmol sodium'.

The daily excretion of sodium, potassium, creatinine, free DA and free NA and the urine volume in response to changes in sodium intake is shown in Table 7b and Figure 7a. As a group, there was an 8- to 9-fold increase in sodium excretion during salt loading ($p < 0.01$). The rate of increase in urine sodium was the greatest in the first two days of salt loading during which an 8-17% increase in urinary DA excretion was seen ($p < 0.05$). However, by day 4, urinary free DA output did not differ significantly from the baseline value on day 0. On day 5, there was a 22% reduction in urinary free NA output ($p < 0.05$).

Table 7b. 24-hour urine volume and urinary excretion of sodium, potassium, and creatinine in eight healthy Chinese subjects during low (days -4 to 0) and high (days 1 to 5) sodium intakes.

Days	Sodium intake (mmol/day)	Volume (l)	Sodium (mmol)	Potassium (mmol)	Creatinine (mmol)
-4	20	1.8 ± 0.2	$85 \pm 8^\dagger$	32.1 ± 2.2	13.4 ± 0.6
0	20	1.7 ± 0.1	22 ± 4	38.9 ± 3.1	13.3 ± 0.6
1	220	1.8 ± 0.2	$86 \pm 17^\dagger$	$50.0 \pm 1.2^\dagger$	14.8 ± 0.9
2	220	$2.8 \pm 0.3^\dagger$	$199 \pm 12^\dagger$	$49.4 \pm 2.5^\dagger$	14.3 ± 0.8
3	220	2.2 ± 0.2	$216 \pm 19^\dagger$	42.1 ± 1.7	14.3 ± 0.9
4	220	$2.2 \pm 0.2^\dagger$	$186 \pm 18^\dagger$	40.9 ± 2.3	14.4 ± 0.9
5	220	$2.1 \pm 0.1^*$	$260 \pm 14^\dagger$	44.1 ± 3.5	$19.0 \pm 1.3^\dagger$

* $p < 0.05$, $^\dagger p < 0.01$ when compared with day 0.

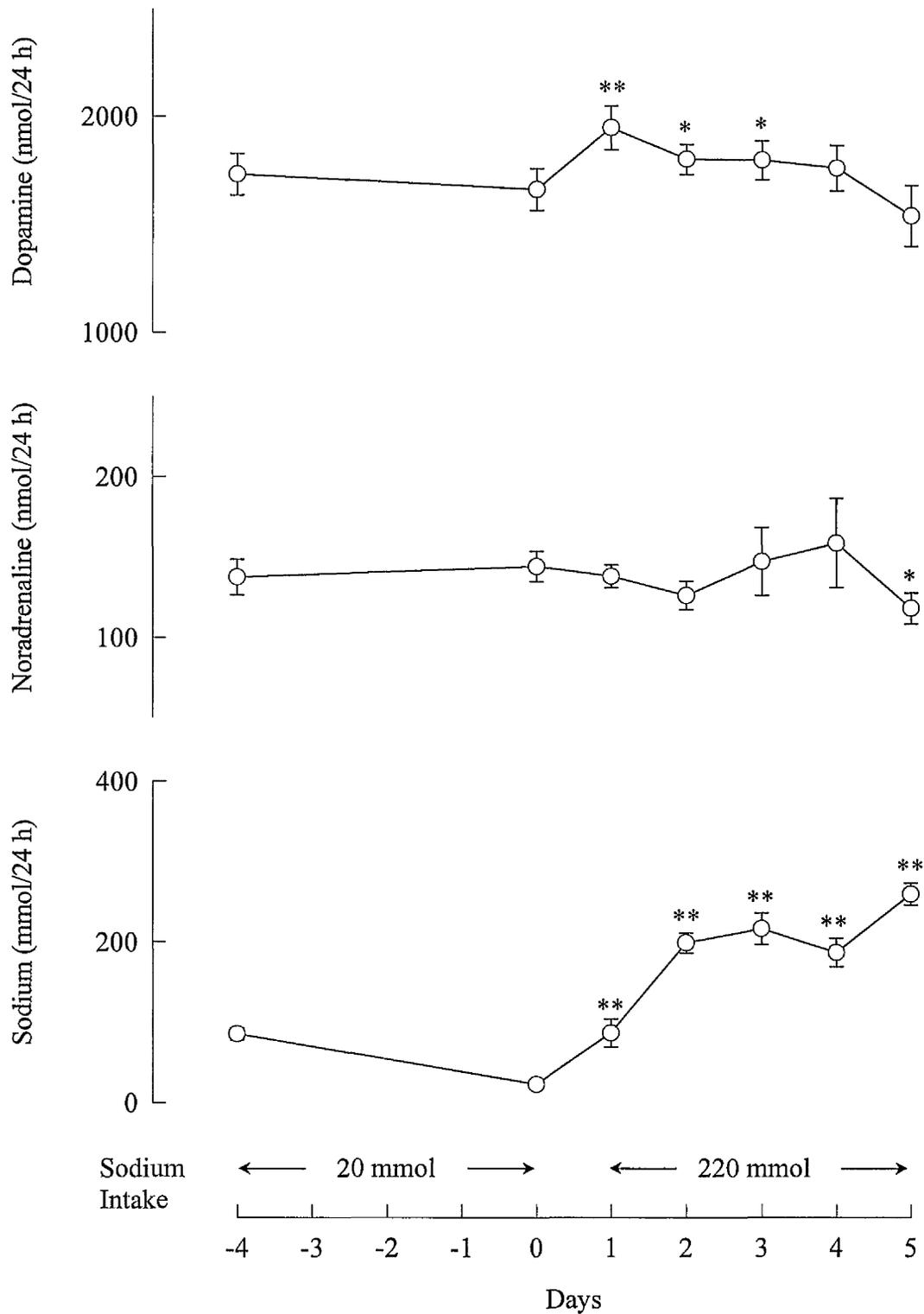


Figure 7a. Mean \pm SEM of 24-hour urinary sodium, DA and NA outputs in eight healthy Chinese subjects when their sodium intake was increased from 20 mmol/day (days -4 to 0) to 220 mmol/day. * $p < 0.05$, ** $p < 0.01$ when compared with day 0.

7.4 Discussion

Dietary salt loading (Section 3.17) and the acute infusion of isotonic saline (Section 3.19) have been widely used to determine factors controlling natriuresis in health and disease. Activation of the renal DA (Chapter 3) and other natriuretic systems (Section 2.3) and suppression of the SNS activity and other antinatriuretic systems (Section 2.4) are expected to facilitate sodium excretion. In the present study, I have investigated the role of two of these factors in modulating urinary sodium output in healthy Chinese subjects during periods of different sodium intake.

7.4.1 *Effects of salt loading on MAP*

As in Caucasians (367-369,373), a high sodium intake of around 250 mmol/day for about one week was not associated with an increase in MAP in healthy Chinese subjects. This is in marked contrast to normotensive American blacks in whom a modest increase in sodium intake from 40 to 180 mmol/day is associated with a 7% increase in MAP after 14 days (375).

Sodium kinetics differ considerably between normotensive whites and blacks when they are given 10, 200 and 400 mmol/day sodium for seven days in random order (614). With increasing salt intake, the half-life of sodium increases in both whites and blacks. Mean figures for whites are 1.08, 1.57 and 2.88 days compared with 1.65, 2.81 and 5.81 days for blacks. At the 400 mmol/day intake, whites on average accumulate 385 mmol of sodium compared with 909 mmol for blacks. It was previously observed that normotensive blacks excrete an intravenous salt load over 24 hours to a lesser extent than do normotensive white subjects (22). It has been suggested that a decreased renal production of DA may be an important

pathogenic factor in the development and maintenance of hypertension in American blacks (370,375).

7.4.2 *Effects of salt loading on the renal DA response*

A positive correlation between 24-hour urinary sodium and free DA outputs was seen in normotensive Chinese subjects with or without a family history of hypertension (Chapter 5 and Chapter 6). However, my preliminary study (Section 7.1) on the effects of oral sodium loading yielded conflicting results with regarding to the renal DA response; the expected increase in urinary free DA output was not seen. Apart from variations in protein, potassium, calcium and phosphate intakes before and after salt loading, differences in the basal levels of sodium intake (70 mmol instead of 20-40 mmol/day used in the studies listed in Table 3h) before sodium loading might be of importance. For example, in healthy Caucasian subjects given intravenous saline infusion (520), those with a family history of hypertension differed from those without such a history in having lower urinary sodium and free DA outputs during the low (20 mmol/day) or medium (170 mmol/day) sodium intake. However, such differences in urinary sodium and DA responses were not seen during the high (340 mmol/day) sodium intake.

In this study, I have therefore used a constant diet containing either 20 or 220 mmol sodium/day for five days each. I have shown that the renal DA response to oral salt loading in healthy Chinese subjects was relatively small and not sustained (see Figure 7a). The maximum increase in urinary free DA output after salt loading was only 17% in Chinese subjects. The increase in free DA output was attenuated by days 2 and 3 and the output was not different from the baseline by days 4 and 5.

Moreover, a temporal relationship between urinary excretion of sodium and free DA was not seen in Chinese subjects (Figure 7a).

When changing from a low (9-40 mmol/day) to high (220-340 mmol/day) sodium intake (12,13,367-369,372-374), Caucasian subjects generally showed a much greater increase in renal DA of 26-59% (Table 3h). Studies (12,369,374) that measured 24-hour urinary free DA excretion only towards the end of the salt loading could have missed an early peak. In other words, the maximum increase in renal DA response among Caucasian subjects might even be greater than the range indicated above if daily urinary free DA output was measured in every study. In previous studies that included a daily 24-hour urine collection during the high sodium intake period (13,367,368,372,373), urinary free DA output remained elevated throughout the high salt intake period in Caucasian subjects (Table 3h). Moreover, a temporal relationship between urinary excretion of sodium and free DA was seen in Caucasian subjects (13).

Thus, healthy Chinese subjects show a relatively small renal DA response for three days after acute changes in salt intake, suggesting that renal DA contributes only partly to the early natriuretic response to salt loading. Ideally, to establish if there is an ethnic difference in renal DA response to salt loading, larger number of Chinese and Caucasian subjects living in the same environment should be studied using identical protocol.

7.4.3 *Published studies on the renal DA response to salt loading in Chinese*

Hou & Zhang (597) in Guangzhou studied the renal DA response to salt loading in 10 healthy Chinese volunteers. They were put on a constant diet with two different sodium intakes (34 and 170 mmol/day) each given for four days. On the last

day of each salt intake, 24-hour urine and blood samples were collected. HCl was added to the aliquots to maintain a pH of 3.5-4.0. Urinary free DA was measured by HPLC with electrochemical detection. Following salt loading, an increase in mean urinary sodium (50.8 to 168 mmol/day) and free DA (1,020 to 1,550 nmol/day) outputs was seen. There was a 92-93% reduction in PRA and plasma aldosterone concentration. Thus, the increase in free DA excretion (52%) appears much larger than that of my subjects (17%). Basal BP (Section 4.1.1) and salt intake (Section 7.4.2) in these subjects and exact constituents of the diet (Section 3.2.2) were not known but could have influenced the results. Moreover, the subjects in Guangzhou could have a genetic background that is different from my subjects.

7.4.4 *Effects of salt loading on the SNS activity*

Another mechanism which may contribute to the natriuresis following salt loading is a reduction in SNS activity, which may be reflected in a reduction in urine free NA output (Section 2.4.2). Impaired suppression of SNS activity during sodium loading, which is seen in salt-sensitive hypertensives but not in normal subjects or salt-resistant hypertensives, is associated with greater sodium retention and an increase in MAP.

In this study, a 22% reduction in urinary NA was seen on day 5 when the subjects were in sodium balance (Figure 7a). The lack of a temporal relationship between urine sodium and urine NA during the first four days of oral salt loading suggests that a reduction in the SNS activity does not contribute to salt-induced natriuresis in healthy Chinese subjects. However, any tendency to hypervolaemia-related increases in BP might have been partly offset by an appropriate reduction in SNS activity towards the end of the high salt intake period (Section 2.4.2).

Previous studies on the relationship between SNS activity and natriuresis in healthy subjects never reported the daily urinary NA excretion during the high salt intake. In the study of Alexander et al. (12), an average of the last three collections on a low sodium intake (9 mmol/day for eight days) and last four collections on a high sodium intake (209 or 259 mmol/day for 10 days) were used; a 44% reduction in urinary free NA output was seen. In the study of Luft et al. (201), the 24-hour urinary free NA on the last day of each level of sodium intake (each given for at least three days) was used. A 28% fall in urine free NA was seen after the daily sodium intake was increased from 10 to 300 mmol. In the study of Turner & Reilly (203), subjects were given one week of low sodium (10 mmol/day) diet and one week of high sodium (200 mmol/day) diet. A 28% fall in the 24-hour urinary free NA output on the 6th day was seen. In the study of Gill et al. (369), the response of five subjects to sodium intakes of 9 and 249 mmol/day each given for seven days was studied by comparing the plasma NA and the urinary free NA output on the last day of each intake. Plasma NA concentration fell by 6%, but mean urinary NA output did not change (124 to 89 nmol/day, $p>0.1$). Since 24-hour urinary free NA output was not measured throughout the salt loading period, the possibility of an earlier reduction in SNS activity could not be excluded.

7.4.5 *Significance and implications of my findings*

Findings from this study suggest that in healthy Chinese subjects, renal DA contributes only partly to the natriuretic response to oral salt loading. The lack of a temporal relationship between the natriuretic response and urinary free NA output in the first four days of salt loading suggests that a reduction in SNS activity plays no significant role. However, any tendency to hypervolaemia-related increases in BP

may be partly offset by an appropriate reduction in SNS activity towards the end of the high salt intake period.

Since their MAP remains unchanged and the natriuretic response to a high sodium diet for five days is similar to those reported previously, Chinese subjects must have other mechanism(s) for dealing with a salt load.

Chapter 8 Urinary free DA and NA outputs during acute changes in oral salt intake in healthy Chinese subjects with a family history of hypertension

8.1 Background of the study and main objectives

There is a strong association between genetic factors and hypertension (608,609) and between salt intake and hypertension (Section 1.1). Among young normotensive subjects, BP is higher in the offspring of hypertensive parents than in the offspring of normotensive parents (615). The normotensive first-degree relatives of patients with hypertension have a blunted natriuretic response to an intravenous salt load (204) and are more likely to be salt-sensitive (Section 2.6). A prevailing hypothesis to explain the familial predisposition to hypertension is that inherited factors have effects that impair the kidney's ability to excrete sodium (616), and this primary renal 'defect' may be related to the dysregulation of one or more of the natriuretic or antinatriuretic systems (Chapter 2 and Chapter 3).

I have already established that the renal DA response to oral salt loading in healthy Chinese subjects without a family history of hypertension is 'unusual' in that: (a) the increase in renal DA output is small; (b) the increase becomes attenuated from day 2 (Chapter 7). However, any tendency to hypervolaemia-related increases in BP may be partly offset by a reduction in SNS activity towards the end of the high salt intake period. Subjects with a family history of hypertension also have a significant renal sodium-DA relationship (Chapter 5) and should show a renal DA response to oral salt loading. Previous oral salt loading studies by others had only focused on normotensives or hypertensives (Section 4.1.1).

In this study, my main objective was to determine if the effects of oral salt loading on BP and the natriuretic response are different in normotensives without a family history of hypertension. If this is the case, I wanted to know if these are related to a defect in intrarenal DA synthesis or an abnormal SNS response.

8.2 Subjects and methods

This study had been approved by the Clinical Research Ethics Committee of the Faculty of Medicine, the Chinese University of Hong Kong. The study setting and design were as described in Section 7.2.

Seven healthy Chinese male medical students (aged 21-31 years) were studied in February 1995. One (n=3) or both (n=4) of their parents were known to have hypertension requiring regular drug treatment.

During the 10-day study period (days -4 to 0 and days 1 to 5), subjects were given the same diet containing 1,900 calories, 75 g protein, 20 mmol sodium and 45 mmol potassium. From days 1 to 5, they also took 20 'Slow sodium' tablets (Ciba-Geigy) equivalent to 200 mmol of sodium per day. Body weight and supine BP were measured in the morning before breakfast at the end of each 5-day period.

Urine was collected for 24 hours in bottles containing 100 ml of 0.5M HCl on day -4 and from days 0 to 5. Aliquots were refrigerated until assayed for sodium, potassium, creatinine (as in Section 5.2.3), free DA and free NA (as in study one in Chapter 5, see Section 5.2.4).

To assess the 'synergistic' effect of activation of renal DA and suppression of the SNS activity on urinary sodium excretion (617), changes in the 'natriuretic index' (the ratio of 24-hour urinary free DA to NA output) were also examined.

The significance of differences between variables was tested using the Wilcoxon sign-rank test. The data were compared with those from subjects without a family history of hypertension (Chapter 7). Significance of differences between the two groups was tested using the Mann-Whitney U test.

8.3 Results

The seven subjects with a family history of hypertension were heavier (Table 8a). After salt loading, their MAP slightly increased and their pulse rates decreased. These changes were not seen in the eight subjects without a family history.

Table 8a. MAP, pulse rate and body weight in seven healthy Chinese subjects with a family history of hypertension during ingestion of 20 or 220 mmol of sodium per day. [The corresponding figures from the eight subjects without a family history (Chapter 7) are given in brackets.]

	At entry (day -4)	20 mmol sodium (day 0)	220 mmol sodium (day 5)
MAP (mmHg)	81.0 ± 2.0 (78.2 ± 2.4)	80.1 ± 2.5* (76.8 ± 1.8)	83.1 ± 2.1† (75.0 ± 2.8)§
Pulse (beats/min)	73.4 ± 4.6 (65.9 ± 3.3)	69.4 ± 4.4 (63.5 ± 2.4)	64.7 ± 4.5‡ (61.6 ± 4.0)
Body weight (kg)	75.5 ± 3.4 (58.3 ± 1.9)§	73.9 ± 3.2 (57.3 ± 1.9)	73.6 ± 3.2 (57.9 ± 1.9)

*p<0.02 when compared with at entry.

†p<0.05, ‡p<0.02 when compared with day 0.

§p<0.001 when comparing the two groups.

||p<0.05 when comparing the two groups with regards the changes from baseline.

The urinary outputs of sodium, potassium, creatinine, free DA, free NA and DA/NA ratio and urine volume in response to changes in salt intake are shown in Table 8b and Figure 8a. Following salt loading, there was a 7- to 10-fold increase in sodium excretion ($p < 0.02$). The natriuretic response was not different from that seen in subjects without a family history of hypertension. There was also a tendency for urine volume and the urinary excretion of potassium and creatinine to increase.

There was a 23% increase in urinary free DA output ($p < 0.02$) on the first day of high sodium intake (Table 8b and Figure 8a). From days 2 to 5, the increase in urinary DA output also became attenuated (12-15% higher than the baseline) but still reached statistical significance compared with baseline on days 2 and 5. Throughout the high sodium intake period, urinary free DA output appeared to be consistently higher among those with a family history of hypertension (Table 8b). However, the difference between the two groups was not statistically significant ($p > 0.05$).

Unlike the subjects without a family history of hypertension who showed a 22% reduction in free NA output on day 5, those with a family history showed no significant changes in urinary NA output throughout the high sodium intake period. Subjects with a family history tended to show a higher urinary free NA output throughout the study period (Table 8b) and the difference was statistically significant on the second day of salt loading.

There was a 20% increase in the DA/NA ratio ($p < 0.02$) only on day 2 of high sodium intake (Table 8b). Subjects without a family history of hypertension showed an increase in the DA/NA ratio on day 1 (22.4%), day 2 (25.9%) and day 5 (13.8%). The DA/NA ratio appeared to be consistently lower in those with a family history of hypertension, but this difference reached statistical significance only on day 1.

Table 8b. 24-hour urine volume and urinary outputs of sodium, potassium, creatinine, free DA and free NA in seven healthy Chinese subjects with a family history of hypertension during 20 mmol (days -4 to 0) and 220 mmol (days 1 to 5) sodium intakes. [The corresponding figures from the eight subjects without a family history of hypertension (Chapter 7) are given in brackets.]

Days	Volume (l)	Sodium (mmol)	Potassium (mmol)	Creatinine (mmol)	DA (mmol)	NA (nmol)	DA/NA ratio
-4	2.5 ± 0.2	111 ± 22† (85 ± 8)	40.1 ± 2.8	15.2 ± 0.9	2015 ± 134 (1730 ± 96)	194 ± 15 (138 ± 11)	10.9 ± 1.3 (13.2 ± 1.4)
0	2.1 ± 0.2	22 ± 4 (22 ± 4)	39.1 ± 5.2	14.8 ± 1.1	1794 ± 106 (1659 ± 96)	194 ± 11 (145 ± 9)	9.4 ± 0.7 (11.6 ± 0.5)
1	2.2 ± 0.2	60 ± 9† (86 ± 17)	48.9 ± 4.7†	16.8 ± 0.8*	2197 ± 172† (1947 ± 102)	230 ± 16 (138 ± 7)‡	9.9 ± 1.1 (14.2 ± 0.7)§
2	2.5 ± 0.3	170 ± 25† (199 ± 12)	54.7 ± 5.7	16.1 ± 1.1	2070 ± 140* (1799 ± 70)	189 ± 21 (126 ± 9)	11.9 ± 1.7† (14.6 ± 0.8)
3	2.8 ± 0.3	201 ± 19† (216 ± 19)	46.0 ± 3.8	15.9 ± 1.0	2047 ± 145 (1795 ± 91)	194 ± 16 (148 ± 21)	11.4 ± 1.8 (13.7 ± 1.8)
4	2.4 ± 0.4	169 ± 13† (186 ± 18)	37.1 ± 5.0	15.3 ± 1.3	2049 ± 185 (1758 ± 105)	206 ± 19 (159 ± 28)	10.2 ± 0.9 (12.9 ± 1.7)
5	2.2 ± 0.3	252 ± 22† (260 ± 14)	49.1 ± 6.4	19.1 ± 1.5*	2011 ± 132* (1537 ± 141)	192 ± 17 (118 ± 10)	10.9 ± 1.1 (13.2 ± 1.0)

*p<0.05, †p<0.02 when compared with day 0.

‡p<0.05, §p<0.02 when comparing the two groups with regards the changes from baseline.

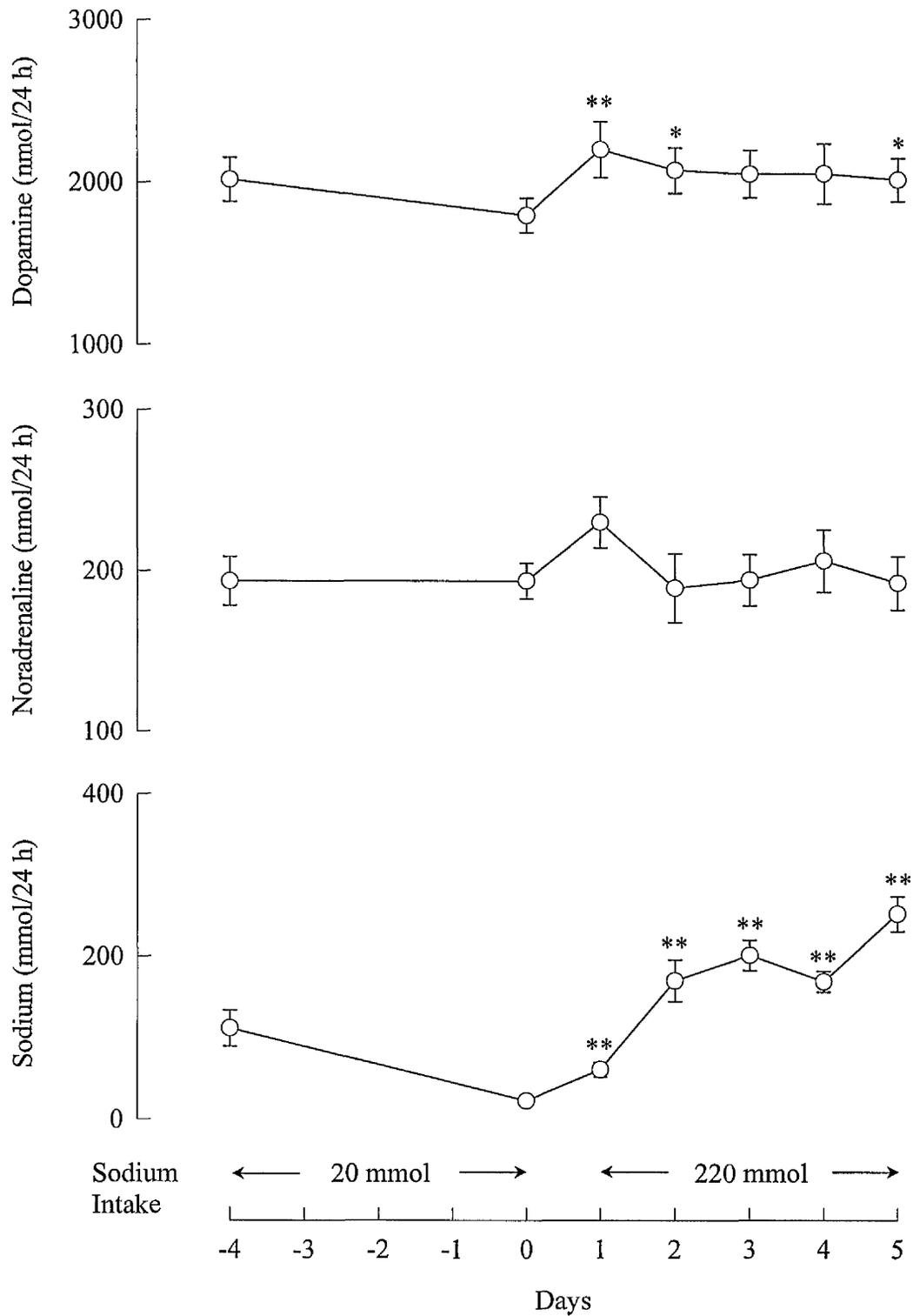


Figure 8a. Mean \pm SEM of 24-hour urinary sodium, DA and NA outputs in seven healthy Chinese subjects with a family history of hypertension when their sodium intake was increased from 20 mmol/day to 220 mmol/day. * $p < 0.05$, ** $p < 0.02$ when compared with day 0.

8.4 Discussion

Both clinic and ambulatory BP is higher in the normotensive offspring of hypertensive parents than in the offspring of normotensive parents even before sustained hypertension occurs (618). Normotensive offspring of hypertensive parents also differ from offspring of normotensive parents in having an exaggerated rise in BP following oral (619) or intravenous (204) sodium loading and a blunted renal sodium excretion after saline infusion (204,620). This enhanced effect of sodium on BP and an impaired natriuretic response to a salt load may be related to abnormalities in some regulating factors of BP and sodium balance (Chapter 2 and Chapter 3). Surprisingly, the role of renal DA has rarely been evaluated (520), despite its known natriuretic and vasodilatory actions in humans (Chapter 3).

8.4.1 *Effects of salt loading on MAP*

Unlike Chinese subjects without a family history of hypertension, those with a positive family history showed an increase (average 3.7%) in MAP on changing from a 20 to 220 mmol sodium diet (Table 8a).

Similar findings have been reported by previous studies. Pusterla et al. (619) in Switzerland studied the BP responses to a low (15-18 mmol/day) or high (>200 mmol/day) sodium diet for one week each in 23 healthy males (aged 20-32 years, 13 with a positive family history of hypertension). Following salt loading, BP increased only in subjects with a positive family history ($119/76 \pm 3/2$ vs $126/80 \pm 4/2$ mmHg). Myers & Morgan (621) in Australia studied the BP responses to a low (70 mmol/day) or high (200 mmol/day) sodium diet for two weeks each in 154 healthy volunteers. In those aged 20-49 years with a positive family history of hypertension, BP was higher at entry [actual figures not given in their publication]. These

volunteers also showed a greater increase in MAP after salt loading than the subjects with a negative family history (males 3.0 ± 1.0 vs -0.2 ± 1.7 mmHg, $p < 0.05$; females 3.0 ± 2.8 vs 2.3 ± 1.3 mmHg, $p = \text{NS}$). Overlack et al. (622) in Germany studied the BP responses to a low (20 mmol/day) or high (300 mmol/day) salt diet for one week each in 163 Caucasian, non-obese healthy subjects (98 men and 65 women). Compared with subjects with a negative family history of hypertension, subjects with a positive family history were younger (33.0 ± 1.6 vs 40.7 ± 1.7 years) and had a higher MAP at baseline (89.1 ± 1.1 vs 85.9 ± 0.8 mmHg). Only those with a positive family history showed a rise in MAP (mean 0.9%) after salt loading. Salt sensitivity (an increase in MAP of ≥ 5 mmHg) was seen in 25.6% of subjects with a positive family history and 14.4% of those with a negative history.

Other investigators reported that the offspring of hypertensive parents do not differ in their responses to salt loading from those whose parents are normotensive. Weissberg et al. (623) in the U.K. recruited 20 male medical students who reduced their sodium intake to 40 mmol/day for one week by adhering to a diet sheet. Then, they returned to their normal diets supplemented by sodium 200 mmol/day (20 Ciba-Geigy 'Slow sodium' tablets) for one week. The change in diet was associated with a rise in supine SBP of 6.0 ± 1.1 mmHg and standing SBP of 4.8 ± 1.3 mmHg. The 12 subjects with a family history of hypertension did not differ in their responses from subjects without a family history. Turner & Reilly (203) in the U.S. studied the changes in MAP after one week of a low (10 mmol/day) and high (200 mmol/day) sodium diet in 11 normotensive sons (aged 46.9 ± 1.6 years) of two hypertensive parents and 11 normotensive sons (aged 41.4 ± 1.2 years) of two normotensive parents. MAP remained 7 mmHg higher in sons of hypertensive parents (low salt diet 100 ± 2 vs 93 ± 2 mmHg, high salt diet 97 ± 3 vs 90 ± 2 mmHg).

8.4.2 *Effects of salt loading on natriuresis*

In my study, daily excretion of sodium did not differ between subjects with or without a family history of hypertension on any day of the high salt diet (Table 8a). Hence, there is no evidence that the renal excretion of sodium after salt loading is impaired in healthy Chinese subjects with a family history of hypertension.

Turner & Reilly (203) in the U.S. have determined whether renal sodium excretion is impaired and whether tubular reabsorption of sodium is increased in normotensive sons of hypertensive parents. Urinary sodium excretion after one week of low (10 mmol/day) or high (200 mmol/day) salt diet did not differ between normotensive sons of normotensive or hypertensive parents. On day 7 of each diet, filtered load of sodium, absolute excretion of sodium, fractional excretion of sodium (an index of total tubular sodium reabsorption) and fractional excretion of lithium (an inverse index of proximal tubular sodium reabsorption) also did not differ between the two groups. They suggested that higher BP (see Section 8.4.1) in the offspring of hypertensive parents may have compensatory renal effects that restore renal sodium handling to normal (see Section 2.2). Compensatory changes in the natriuretic and antinatriuretic systems (Section 8.4.3 and Section 8.4.4) may also help restore renal sodium handling to normal.

Städler et al. (624) in Switzerland compared the renal tubular handling of sodium in normotensive subjects with or without a family history of hypertension. After changing from a low (15-18 mmol/day) to high (>200 mmol/day) sodium diet, GFR, as estimated by the clearance of inulin, is similar in the two groups. The renal plasma flow, as estimated by the clearance of para-aminohippurate (PAH), tended to be slightly, but not significantly, higher in those with a positive family history. The tubular reabsorption of sodium from the proximal tubule, as estimated by the

difference between the clearances of inulin and lithium, or from the distal tubule, as estimated by the difference between the clearances of lithium and sodium, were comparable in the two groups. These findings do not support the concept of a familial predisposition to hypertension being associated with an enhanced proximal reabsorption of sodium.

8.4.3 *Effects of salt loading on the renal DA response*

If DA is an important intrarenal natriuretic hormone and dietary sodium is the major determinant of its intrarenal synthesis, there should be a positive correlation between urinary sodium and free DA (Section 3.1.6). Like healthy Chinese subjects without a family history of hypertension, those with a family history show a positive correlation between 24-hour urinary sodium and free DA outputs (Chapter 5). It is expected that healthy Chinese subjects with a family history of hypertension will show a urinary DA response to oral sodium loading.

In the present study of healthy subjects with a family history of hypertension, an increase in urinary free DA output (15-23%) was seen during the first two days of sodium loading (Figure 8a). This increase in urinary DA excretion preceded the major component of the natriuretic response by one day, suggesting that renal DA leads, rather than follows, sodium excretion (13). Like the normotensive Chinese subjects without a family history of hypertension (Table 7b and Figure 7a), the renal DA response was relatively small (15-23% and 8-17%) and tended to be attenuated after 2-3 days (Table 8b and Figure 8a). Thus, renal DA response to oral salt loading differs between Chinese and Caucasian subjects both quantitatively and qualitatively. Moreover, the increase in urinary DA in both groups of Chinese subjects was obviously small compared to the 7- to 10-fold increase in sodium excretion. These

results suggest that renally produced DA only contributes partly to the acute natriuretic response to oral sodium loading in Chinese and the renal DA response to salt loading does not differ significantly between the offspring of normotensive and hypertensive parents (Table 8a).

Compared with normotensive subjects, urinary free DA output under basal conditions is higher in some normotensive Japanese subjects with a family history of hypertension (363), young Japanese patients with essential hypertension (515) and Caucasian patients with borderline hypertension (516). It has been suggested that the increased intrarenal DA synthesis in these subject groups may represent an early antihypertensive defense response (Section 4.1.1, Section 5.4.5 and Section 6.4.1).

Compared with subjects without a family history of hypertension, there was a trend ($p=NS$) for a higher urinary free DA outputs after salt loading among subjects with a family history (Table 8b). This 'enhanced' renal DA response may represent an early antihypertensive defense response (Section 4.1.1, Section 5.4.5 and Section 6.4.1) or a compensatory response to an overactive SNS activity that persists during oral salt loading (see Section 8.4.4).

Krzesinski et al. (520) in Belgium compared the renal DA response to salt loading in 20 young normotensive subjects with ($n=12$) or without ($n=8$) a family history of hypertension. While these subjects were on three different sodium diets (20, 170 and 340 mmol/day) each for one week, one liter of 0.9% saline was infused over 30 minutes with urine being collected for three hours for measurements of sodium and free DA. During the low and medium sodium diets, but not during the high sodium diet, the natriuresis and DA excretion were lower in the subjects with a positive family history. Thus, a genetic predisposition to hypertension seems to be characterised by a lower urinary sodium excretion and an impaired renal DA

synthesis during acute salt loading while on a normal or low sodium diet. It is conceivable that subjects with a family history of hypertension might be able to show an apparently normal renal DA response during large acute changes in salt intake.

Gerlo et al. (625) in Belgium and Young et al. (626) in the U.S. reported an inverse correlation between age and 24-hour urinary free DA excretion among free living subjects. In my cross-sectional study (Chapter 5), normotensive subjects with a family history of hypertension were considerably older (39.1 ± 1.4 and 48.3 ± 7.2 years) than subjects in salt loading study (21-31 years). It will be of great interest to find out if there is an age-related difference in the renal DA response to salt loading.

8.4.4 *Effects of salt loading on the SNS activity*

During salt loading, a reduction in SNS activity will facilitate renal sodium excretion (Section 2.4.2) and help offset any tendency to hypervolaemia-related rises in BP (Section 7.4.4). This has been shown in healthy subjects as well as patients with salt-resistant hypertension (Section 2.4.2).

In the present study, urinary free NA output remained unchanged throughout the high salt intake period (Table 8b and Figure 8a). Thus, there was no evidence that a reduction in SNS activity plays a role in the natriuretic response to oral salt loading in Chinese subjects with a family history of hypertension. This may be one reason why they showed an increase in MAP after salt loading (Table 8a). In contrast, subjects without a family history of hypertension showed a reduction in SNS activity towards the end of high sodium intake and their MAP was unchanged (Chapter 7).

In the study of Turner & Reilly (203), urinary free NA output on day 6 of a low (10 mmol/day) and high (200 mmol/day) sodium diet each given for seven days was compared between normotensive subjects with or without a family history of hypertension. On a low sodium diet, mean NA excretion was 139 nmol/day greater in the sons of hypertensive parents (466 ± 171 vs 327 ± 60 nmol/day, $p=0.20$). On a high sodium diet, it was only 53 nmol/day greater in the sons of hypertensive parents (286 ± 66 vs 236 ± 79 nmol/day, $p=0.10$). With the shift from a low to high sodium diet, there was a greater reduction in mean NA excretion among the sons of hypertensive parents (177 vs 91 nmol/day). The authors suggested that increased SNS activity may mediate the increase in vascular resistance and higher BP in the sons of hypertensive parents.

8.4.5 *Effects of salt loading on the urinary free DA/NA ratio*

Weinberger et al. (617) reported a close correlation between the ratio of urine free DA to urine free NA and the state of sodium balance in normal white subjects receiving a low (10 mmol/day) or high (800 mmol/day) sodium diet. The natriuretic response to salt loading was accompanied by an increase in this 'natriuretic index' (due to activation of renal DA synthesis and suppression of SNS activity).

In the present study, the expected increase in DA/NA ratio after salt loading was seen in subjects with a family history of hypertension on day 2 and in subjects without a family history from day 1 to day 3 (Table 8b). Despite the difference in the 'natriuretic index', subjects with a family history of hypertension had a natriuretic response similar to those with a family history (Figure 8a and Figure 7a). Higher BP in the offspring of hypertensive parents (Section 8.4.1) may have compensatory renal

effects that help restore renal sodium excretion to normal (Section 2.2). There could also be compensatory changes in other neurohormonal systems (see Section 8.4.6).

8.4.6 *Effects of salt loading on other neurohormonal systems*

To identify factors that might account for differences in sodium handling and BP responses to salt loading between healthy subjects with or without a family history of hypertension, several investigators also assessed indexes of SNS activity and circulating natriuretic and antinatriuretic substances after salt loading. Turner & Reilly (203) reported a similar reduction in urinary aldosterone but no changes in urinary PGE₂ or plasma endothelin. Pusterla et al. (619) reported similar reductions in plasma levels of NA, renin and aldosterone but no changes in urinary PGE₂ or PGF_{2α} output. Weissberg et al. (623) reported similar reductions in PAC, plasma aldosterone and sodium pump activity.

Ferrari et al. (627) in Switzerland measured the plasma ANP in 51 offspring of normotensive parents and 45 offspring of hypertensive parents during liberal salt intake. Groups were subdivided according to their 24-hour sodium excretion. In the offspring of hypertensive parents, plasma ANP was slightly lower on a modest sodium intake (5.3 ± 0.7 vs 7.7 ± 0.7 fmol/L, $p < 0.05$) and markedly reduced when on a high salt intake (8.0 ± 1.3 vs 15.0 ± 1.3 fmol/L, $p < 0.001$). Moreover, the slope of the relationship between plasma ANP and 24-hour urinary sodium was flatter among the subjects with hypertensive parents (z test=2.4). Twenty men were studied after four days of low (70 mmol/day) and high (350 mmol/day) salt intake. After salt restriction, plasma ANP did not differ between subjects with or without a family history. On salt loading, plasma ANP increased only in the subjects with normotensive parents. After four days of a 130 mmol sodium diet, 16 men were

given two litres of 0.9% saline over 90 minutes. The acute plasma ANP response did not differ between the subjects with or without a family history of hypertension. Thus, an altered ANP response in hypertension-prone subjects becomes apparent during chronic, rather than acute, stimulation of ANP release by salt intake.

8.4.7 *Effects of salt loading on urinary creatinine output*

In this study and in my previous study (Chapter 7), there were 15-43% and 8-43% increases respectively in urinary creatinine output following salt loading. There could be two possible explanations. Firstly, creatinine clearance tends to increase when changing from a low to high salt intake (628,629). Secondly, when plasma creatinine is measured using the Jaffe reaction or other methods, interference by DA is now recognised (630). To what extent urinary DA may interfere with the measurement of urinary creatinine has not yet been determined.

8.4.8 *Implications and significance of my findings*

As in healthy Chinese subjects without a family history of hypertension, those with a family history show an early but unsustained rise in urinary free DA during oral sodium loading. Such an increase is relatively small compared to the 7- to 10-fold increase in urinary sodium excretion. These data suggest that renal DA only contributes partly to the natriuretic responses to sodium loading. Unlike those with normotensive parents, subjects with a family history of hypertension show an increase in MAP after oral sodium loading, possibly because of the inappropriately high SNS activity. Subjects with a family history tend to have a higher urinary free DA output during sodium loading. This might represent an early antihypertensive defense response or a compensatory response to increased SNS activity.

Chapter 9 Urinary free DA and NA outputs in response to gradually increasing oral sodium intake over eight days in healthy Chinese subjects

9.1 Background of the study and main objectives

I have so far described that renal DA appears to contribute only partly to the acute natriuretic response to oral salt loading in healthy Chinese subjects (Chapter 7 and Chapter 8). This conclusion is based on the observations that following acute changes in oral sodium intake from 20 to 200 mmol/day: (a) the increase in free DA is relatively small, compared to the 7- to 10-fold increase in urine sodium and the DA response reported in Caucasian subjects; (b) the increase in urine DA becomes attenuated after two to three days.

All previous studies on the effects of oral salt loading on the renal DA response and hence the role of renal DA in sodium homeostasis (Section 3.1.7) have used sudden and large changes in salt intake (e.g. from 20 to 220 mmol sodium/day). Such an approach has a major limitation as it does not test the sensitivity of the renal DA and other natriuretic or antinatriuretic systems to small and 'physiological' changes in salt intake. If renal DA system plays an important role in sodium and ECF volume homeostasis in Chinese, it should be sensitive to small and gradual increases in sodium intake. Similarly, a prompt response in SNS activity might also be expected.

Therefore, in this study, I have focused on the natriuretic response and the sensitivity of renal DA and SNS activity to small and gradual increases in sodium intake in healthy Chinese subjects.

9.2 Subjects and methods

This study had been approved by the Clinical Research Ethics Committee of the Faculty of Medicine, the Chinese University of Hong Kong. After the nature and purpose of the study were explained, consent was obtained from each participant.

Seven healthy Chinese males (aged 23-25 years) were studied in September-October 1995 after a physical and laboratory examination to rule out any significant illness. They were medical students living in the air-conditioned student hostel next to the Prince of Wales Hospital. The BP of their parents was said to be <140/90 mmHg, as indicated by the recent measurements by the students or family doctors.

The study was performed in the out-patient setting. During the 12-day study period, they had deliberately avoided outdoor activities so as to minimise sodium loss from sweating. From days -3 to 0 and days 1 to 8, they were given the same diet prepared by the Prince of Wales Hospital's kitchen containing 1,900 calories, 75 g protein, 20 mmol sodium and 45 mmol potassium. From days 1 to 8, the subjects also received 'Slow sodium' tablets (Ciba-Geigy) equivalent to 50 mmol on day 1, 100 mmol on day 2, 150 mmol on day 3, 200 mmol on day 4, 250 mmol on day 5, and 300 mmol on days 6 to 8. These were taken in three divided doses with the main meals.

Body weight and 10-minute supine BP was recorded in the morning before breakfast on entry and at the end of the low and high sodium intake periods. The mean of three consecutive readings measured by a Dinamap at intervals of one minute was taken as the subject's BP. MAP was calculated.

Urine was collected for 24 hours from 8 a.m. to 8 a.m. in bottles containing 100 ml of 0.5M HCl on day -3, day 0 and days 1 to 8. Aliquots were stored at -20°C until assayed for sodium, potassium, creatinine, free DA and free NA.

Urinary sodium and potassium were measured by ion-selective electrodes and creatinine by the Jaffe reaction on a BM/Hitachi 911 automatic analyzer (Boehringer Mannheim, Germany). Urinary free DA and NA were measured as in study one in Chapter 5 (Section 5.2.4).

Differences from baseline and between the study periods for all variables were tested using the Wilcoxon sign-rank test. P values of less than 0.05 were regarded as statistically significant.

9.3 Results

After four days of salt restriction, the subjects showed a mean reduction in their MAP and body weight of 4.3% and 1.3%, respectively (Table 9a). Following salt loading, their average heart rate fell by 10.5%, but their body weight and MAP did not change.

Table 9a. MAP, pulse rate and body weight in seven healthy Chinese subjects at entry and during the ingestion of 20 or 70-320 mmol of sodium/day.

	At entry (day -3)	20 mmol sodium (day 0)	70-320 mmol sodium (day 8)
MAP (mmHg)	83.0 ± 1.3	79.4 ± 0.5*	81.6 ± 2.1
Pulse (beats/min)	63.1 ± 2.4	64.1 ± 2.8	57.4 ± 2.6‡
Body weight (kg)	70.2 ± 3.1	68.3 ± 3.0‡	68.5 ± 2.9

*p<0.05, †p<0.02 when compared with at entry.

‡p<0.02 when compared with day 0.

The mean daily outputs of sodium, potassium, creatinine, free DA and NA and the urine volume in response to changes in sodium intake are shown in Table 9b and Figure 9a. By the last day of the low salt intake period (Day 0), urinary sodium excretion was 29 ± 3 mmol/day. As the oral sodium intake was gradually increased, there were corresponding increases in urinary sodium excretion. By the last day of salt loading period, sodium excretion had increased 13-fold ($p < 0.02$). There were no significant changes in urine volume or urinary potassium and creatinine outputs.

Table 9b. 24-hour urine volume and urinary excretion of sodium, potassium and creatinine in seven healthy Chinese subjects during low (days -3 to 0) and high (days 1 to 8) sodium intakes.

Days	Sodium intake (mmol/day)	Volume (l)	Sodium (mmol)	Potassium (mmol)	Creatinine (mmol)
-3	20	2.4 ± 0.3	116 ± 27	46.1 ± 3.6	14.7 ± 0.6
0	20	2.1 ± 0.2	29 ± 3	50.9 ± 3.3	16.1 ± 0.9
1	70	1.9 ± 0.2	41 ± 9	51.7 ± 4.0	16.2 ± 0.8
2	120	1.7 ± 0.3	$64 \pm 8^*$	56.1 ± 4.9	16.9 ± 0.8
3	170	1.9 ± 0.2	$152 \pm 24^\dagger$	55.6 ± 2.1	16.2 ± 1.0
4	220	1.9 ± 0.2	$186 \pm 26^\dagger$	47.3 ± 3.7	17.2 ± 0.7
5	270	2.1 ± 0.2	$283 \pm 12^\dagger$	44.7 ± 2.8	16.6 ± 0.9
6	320	2.1 ± 0.1	$362 \pm 17^\dagger$	45.1 ± 3.2	15.5 ± 0.6
7	320	2.3 ± 0.3	$337 \pm 22^\dagger$	44.7 ± 5.5	15.9 ± 0.5
8	320	2.3 ± 0.2	$403 \pm 12^\dagger$	51.1 ± 5.3	16.3 ± 0.7

* $p < 0.05$, $^\dagger p < 0.02$ compared with day 0.

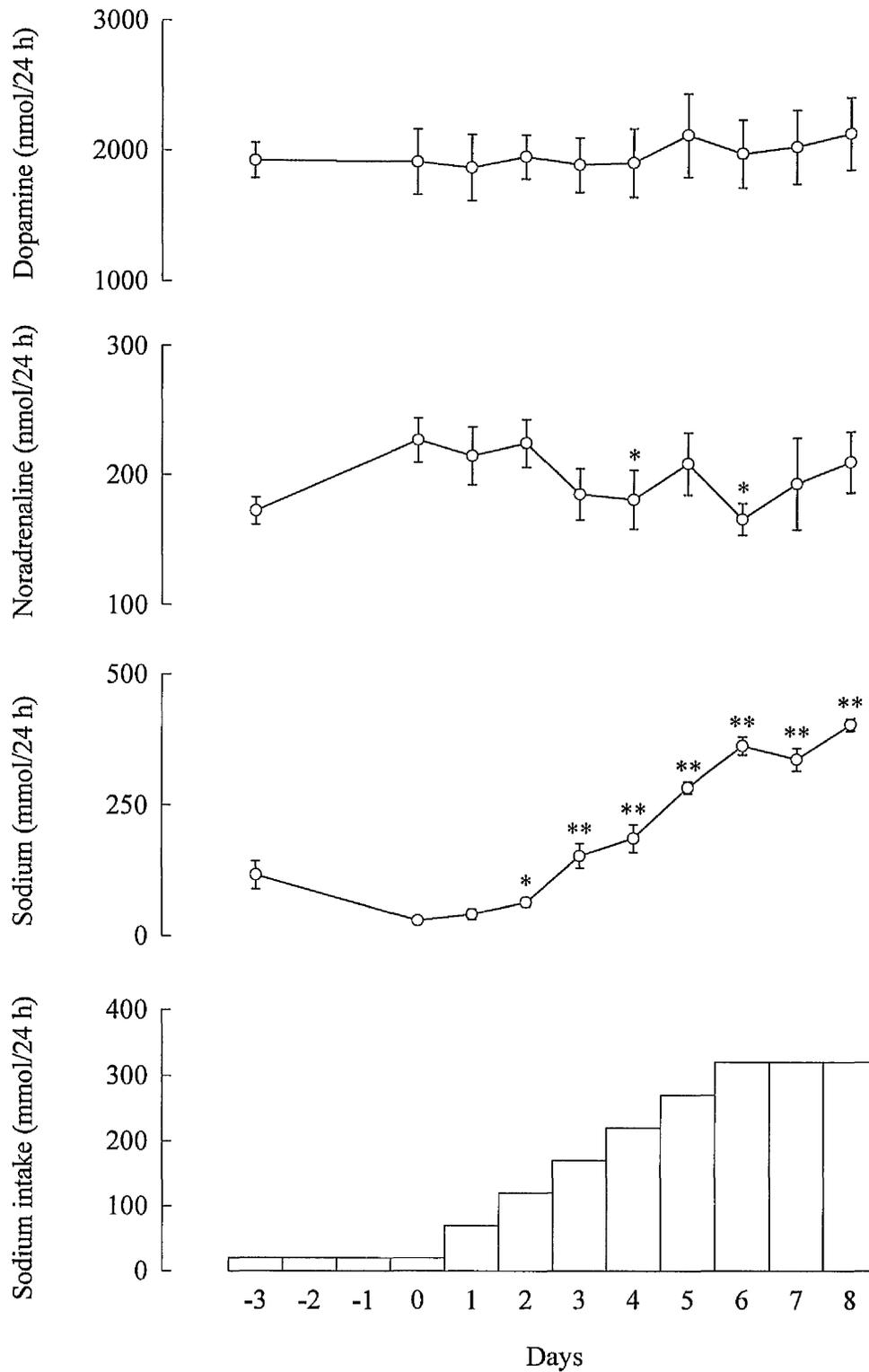


Figure 9a. Mean \pm SEM 24-hour urinary sodium, DA and NA outputs in seven healthy Chinese subjects when their sodium intake was increased gradually from 20 to 320 mmol/day. *p<0.03, **p<0.02 when compared with day 0.

There were no significant changes in urinary free DA output throughout the high sodium intake period.

In contrast, there was a 19.9-26.5% reduction ($p < 0.03$) in urinary free NA output four and six days after the start of the increase in sodium intake.

9.4 Discussion

The results from this study demonstrated that small, gradual increases in oral sodium intake in healthy Chinese subjects resulted in corresponding increases in urinary sodium excretion. This was not, however, accompanied by any increase in urinary free DA excretion. Instead, a reduction in SNS activity towards the end of the high salt intake period was seen.

To have a better appreciation of how sensitive the neurohormonal systems to changes in ECF volume can be, the only other published study of the effects of gradual changes in oral sodium intake (631) is reviewed below.

9.4.1 *Effects of gradual increases in salt intake on plasma ANP and the RAS*

Sagnella et al. (631) in the U.K. studied the sensitivity of plasma ANP and the RAS to gradual changes in dietary salt intake in six normal subjects (19-21 years, 4 men, 2 women). After two days on their normal sodium intakes, they were put on a low salt intake diet of 10 mmol/day for four days (days -3 to 0). Sodium intake was then gradually increased in increments of 50 mmol/day (day 1) up to 350 mmol/day (day 7). Subjects were maintained on this level for the subsequent two days (days 8 to 9). Blood was taken for the measurements of ANP, PAC and

aldosterone, and 24-hour urine samples were collected for the measurements of sodium, potassium and creatinine outputs.

By the fourth day on the low salt diet (day 0, Table 9c), mean urinary sodium excretion was 12.2 mmol, which was similar to the dietary intake. As the sodium intake was gradually increased by 50 mmol/day, there were corresponding increases in urinary sodium excretion. Throughout the study, there were no significant changes in BP, urinary potassium excretion and creatinine clearance.

Table 9c. Urinary sodium, plasma ANP, aldosterone and PRA in six healthy Caucasian subjects during gradual increases in dietary salt intake.

Days	Sodium intake (mmol/day)	Urinary sodium (mmol/day)	Plasma ANP (pg/ml)	Plasma aldosterone (pmol/ml)	PRA (ng/ml/h)
0	10	12.2 ± 4.2	9.9 ± 1.1	2,520 ± 147.4	7.1 ± 1.3
1	50	13.3 ± 2.8	11.4 ± 1.6	1,393 ± 125.4*	7.5 ± 1.9
2	100	27.2 ± 7.4*	14.4 ± 1.8*	785.8 ± 75.6*	3.6 ± 1.0*
3	150	89.6 ± 21.8*	16.9 ± 2.1*	360.5 ± 60.3*	2.0 ± 0.4*
4	200	169.8 ± 17.8*	20.9 ± 2.9*	291.1 ± 43.3*	1.8 ± 0.5*
5	250	268.5 ± 36.5*	20.9 ± 3.7*	496.8 ± 118.8*	1.9 ± 0.5*
6	350	295.8 ± 15.9*	23.3 ± 2.2*	204.8 ± 26.5*	1.1 ± 0.3*
7	350	314.8 ± 31.4*	23.1 ± 2.2*	251.6 ± 78.7*	1.6 ± 0.4*

Data from Sagnella et al. (631). *p<0.05 vs basal levels (day 0).

It can be seen from the study of Sagnella et al. (631) that with gradual increases in sodium intake (50 mmol/day), there were corresponding increases in plasma ANP (Table 9c). There was a 15.2% increase in mean plasma ANP even on day 1, but this did not reach statistical significance. There were also marked reductions in plasma aldosterone from days 1 to 7 (Table 9c). Changes in PRA paralleled those in plasma aldosterone concentration with the exception that there was no significant change in PRA on the first day of the raised salt intake (Table 9c). These results indicate that plasma ANP and the RAS are extremely sensitive to relatively small increases in sodium intake.

9.4.2 *Effects of gradual increases in salt intake on the renal DA response*

In my study, I have clearly shown that healthy Chinese subjects did not show any renal DA response at all during gradual increases in oral salt intake (Figure 9a). In particular, there were still no changes in urinary free DA output, despite a sodium intake that was much larger (320 vs 220 mmol/day) than that used in the preceding studies (Chapter 7 and Chapter 8) and resulted in a larger (13- vs 9-10-fold) increase in urinary sodium excretion. This finding is surprising since sudden, large changes in oral salt intake (20 to 220 mmol/day) produced an early, although unsustained, increase in free DA output (Chapter 7 and Chapter 8).

9.4.3 *Effects of gradual increases in salt intake on the SNS activity*

Another mechanism which contributes to the natriuretic response to sodium loading is a reduction in SNS activity (Section 2.4.2). Impaired suppression of SNS activity after salt loading, which is seen in patients with salt-sensitive hypertension

(Section 2.4.2) and healthy Chinese subjects with a family history of hypertension (Chapter 8), is associated with greater sodium retention and an increase in MAP.

In my study, the temporal profile of urinary NA output did not resemble that of urinary sodium output (Figure 9a), again suggesting that a reduction in SNS activity is not the mediator of the natriuretic response to salt loading in healthy Chinese subjects. This is not surprising since I had already found that sudden, large changes in sodium intake (20 to 220 mmol/day) did not produce an early reduction in urinary free NA in normotensive Chinese subjects (Chapter 7 and Chapter 8). However, as in the offspring of normotensive parents given sudden, large changes in oral salt intake (Chapter 7), a late reduction in SNS activity (Figure 9a) may counteract any tendency to hypervolaemia-related rises in BP towards the end of the high salt intake period.

9.4.4 *Significance and implications of my findings*

My findings described so far indicate that the renal DA system in Chinese is insensitive to gradual and small increases in oral salt intake. A relatively small and unsustained renal DA response is seen only after sudden and large changes in oral salt intake. Furthermore, when Chinese subjects are subjected to both sudden, large increases and gradual, small increases in dietary salt intake, a reduction in SNS activity is not seen until towards the end of the high salt intake period. This finding suggests that increased activity of the SNS does not contribute to the early natriuretic response to salt loading. However, the late reduction in SNS activity seen in the offspring of normotensive parents may offset a tendency to hypervolaemia-related increases in BP.

Chapter 10 Urinary free DA and NA outputs in response to intravenous saline infusion in healthy Chinese subjects

10.1 Background of the study and main objectives

Sodium intake is considered to be the most important factor regulating the synthesis of free DA by the proximal tubules in the kidney (Section 3.2).

I have so far demonstrated that the renal DA response to oral salt loading in normotensive Chinese subjects is less than that reported in Caucasian subjects (Chapter 7 and Chapter 8) or absent (Chapters 9), depending how the salt load is given. It was thus of great interest to find out if normotensive Chinese would exhibit a renal DA response to intravenous saline infusion in a manner similar to that described in Caucasians (Section 3.1.9). The renal DA responses to salt loading might be different when the oral or intravenous administration route is used. Few investigators have used both methods of salt loading to evaluate the renal DA response in their subjects (Section 3.1.7 and Section 3.1.9). There are marked ethnic differences in renal sodium-DA relationships both under basal conditions (Section 3.1.6) and after salt loading (Section 3.1.7 and Section 3.1.9). Since their MAP is unchanged and the natriuretic response to a high salt diet is similar to those reported previously (Chapter 7 and Chapter 9), Chinese subjects without a family history of hypertension must have other mechanism(s) for dealing with a salt load. In view of the role of the renal kallikrein-kinin system in the control of ECF volume, BP and sodium and water excretion (Section 2.3.3) and its aetiological role in salt-sensitivity (Section 2.5.8), our group has developed in 1996 an assay for the simultaneous measurement of acid-labile urinary kallikrein and acid-stable free catecholamines using boric acid as a preservative (Section 5.2.4 and Section 5.2.5).

In the present study, my main objective was to determine the relative roles of the renal DA and kallikrein-kinin systems and suppression of SNS activity in the natriuretic response to an intravenous salt load in Chinese subjects.

10.2 Subjects and methods

This study had been approved by the Clinical Research Ethics Committee of the Faculty of Medicine, the Chinese University of Hong Kong. Informed consent was obtained from each participant after the nature and purpose of the study were explained.

Eight healthy, male Chinese medical students (aged 23-25 years) were studied in April 1996 after a routine physical and laboratory examination to rule out hypertension, diabetes mellitus, renal disease or other significant illnesses. None of their parents had hypertension (BP >140/90 mmHg), which was verified during recent BP measurements by family physicians or the medical students themselves.

All subjects continued to take their usual diets. In order to quantify their usual sodium intake, they collected their urine for the 24-hour period preceding the study.

All subjects were fasted overnight. On arrival in our Clinical Pharmacology Studies Unit, they drank 400 ml of distilled water. At 08:30 (time 0), they emptied their bladder and discarded this urine. An intravenous Venflon cannula was inserted into a vein in each forearm. After an equilibration period of one hour (hour 0), they received an intravenous infusion of 0.9% saline (1,000 ml) over two hours (hours 1 and 2), followed by a 4-hour recovery period (hours 3 to 6). From hours 0 to 4, the subjects remained in the supine position, except to void urine which was collected

hourly. To ensure an adequate diuresis, about 200 ml/h of distilled water was given orally at hourly intervals from hours 1 to 6. From hours 0 to 4, supine BP and pulse measurements and blood sampling (for renal function tests and plasma protein) were followed by urine voiding. The means of three consecutive SBP and DBP readings, measured by a Dinamap at intervals of one minute, were taken. MAP was then calculated. Body weight was measured after urine voiding from hours 0 to 4. I was present throughout the study to ensure that the study run smoothly and the volunteers did not have any discomfort.

Urine was collected into plain bottles. Aliquots were then taken and stored in boric acid at -20°C . Free DA and NA were measured by an alumina extraction procedure followed by quantitation by HPLC with electrochemical detection, as in study two of Chapter 5 (Section 5.2.4). Urinary kallikrein was measured as in study two of Chapter 5 (Section 5.2.5).

Urinary sodium and potassium were measured by ion-selective electrodes. Urinary creatinine was measured by Jaffe reaction on a BM/Hitachi 911 automatic analyzer (Boehringer Mannheim, Germany). Renal function tests and total protein were measured on a DuPont Dimension AR chemistry analyser using standard routine laboratory methods.

Differences from baseline (hour 0) for all variables were tested using the Wilcoxon Sign-Ranks test. P values of less than 0.05 were regarded as statistically significant.

10.3 Results

The 24-hour urinary sodium excretion in the eight subjects prior to the study day ranged from 125 to 339 mmol (mean 232 mmol).

Following intravenous saline infusion, MAP and pulse rate in the eight subjects did not change (Table 10a). There was a small increase in body weight of about 1% ($p < 0.05$).

Table 10a. MAP, pulse rate and body weight in eight healthy Chinese subjects before (hour 0), during (hours 1 to 2) and after (hours 3 to 4) the intravenous infusion of 0.9% saline (1,000 ml over 2 hours).

	Time (hours)				
	0	1	2	3	4
MAP (mmHg)	80.4 ± 2.5	79.6 ± 3.2	81.8 ± 3.6	79.2 ± 2.7	80.7 ± 1.8
Pulse (beats/min)	64.3 ± 2.9	64.1 ± 3.6	66.3 ± 3.7	65.7 ± 2.6	66.6 ± 4.2
Body Weight (kg)	64.9 ± 1.9	65.0 ± 1.8	65.4 ± 1.8*	65.5 ± 1.8*	65.5 ± 1.8

* $p < 0.05$ when compared with the baseline (hour 0).

The plasma electrolytes and total protein of these subjects during the saline infusion are summarised in Table 10b. As a result of the haemodilution, their plasma urea, creatinine and total protein concentrations all decreased.

Table 10b. Plasma electrolytes and total protein in eight healthy Chinese subjects before (hour 0), during (hours 1 to 2) and after (hours 3 to 4) the intravenous infusion of 0.9% saline (1,000 ml over 2 hours).

	Time (hours)				
	0	1	2	3	4
Sodium (mmol/L)	140.6 ± 0.9	140.9 ± 0.7	140.8 ± 0.8	140.9 ± 0.6	140.8 ± 0.7
Potassium (mmol/L)	4.2 ± 0.1	4.1 ± 0.1	4.0 ± 0.1	4.1 ± 0.1	4.1 ± 0.1
Urea (mmol/L)	5.3 ± 0.5	5.1 ± 0.5*	4.8 ± 0.4†	4.5 ± 0.5†	4.4 ± 0.4†
Creatinine (μmol/L)	86.3 ± 2.8	84.9 ± 2.6	82.4 ± 3.5*	83.4 ± 2.9	79.5 ± 1.7*
Protein (g/L)	76.6 ± 1.6	73.3 ± 1.4†	70.8 ± 1.6†	71.6 ± 1.5†	72.5 ± 1.3†

* $p < 0.05$, † $p < 0.02$ when compared with the baseline (hour 0).

The hourly urinary outputs of sodium, potassium, creatinine, free DA, free NA and kallikrein and urine volume in response to the saline infusion are shown in Table 9c and Figure 9a. There was a 31-39% increase in sodium excretion ($p < 0.05$) during the second hour of infusion (hour 2) and first hour of recovery period (hour 3). There were no significant changes in urinary free DA throughout the study. Urinary free NA did not change while the subjects remained supine. However, an increase in free NA output of 91-105% ($p < 0.02$) was seen following ambulation (hours 5 to 6). Urinary kallikrein excretion showed a sustained increase during the saline infusion (103.0-140.4%) and for the first hour of the recovery period (74.1%).

Table 10c. Urine volume and urinary outputs of sodium, potassium and creatinine in eight healthy Chinese subjects before (hour 0), during (hours 1 to 2) and after (hours 3 to 6) the intravenous infusion of 0.9% saline (1,000 ml over 2 hours).

	Time (hours)						
	0	1	2	3	4	5	6
Volume (l)	0.46 ± 0.05	0.38 ± 0.05	0.47 ± 0.09	0.36 ± 0.05	0.37 ± 0.10	0.17 ± 0.05*	0.14 ± 0.05†
Sodium (mmol)	18.4 ± 2.3	19.8 ± 3.0	25.5 ± 4.1*	24.1 ± 3.1*	23.5 ± 2.0	15.5 ± 3.7	12.8 ± 3.8
Cumulative sodium balance (mmol)‡	-	55.2	104.7	80.6	57.1	41.6	28.8
Potassium (mmol)	3.9 ± 0.5	3.5 ± 0.5	4.0 ± 0.7	3.5 ± 0.4	3.1 ± 0.3	3.0 ± 0.5	2.9 ± 0.5
Creatinine (mmol)	0.60 ± 0.04	0.62 ± 0.03	0.65 ± 0.03	0.57 ± 0.03	0.59 ± 0.04	0.71 ± 0.12	0.74 ± 0.11

*p<0.05, †p<0.02 when compared with the baseline (hour 0).

‡Input (1 litre of 0.9% saline = 150 mmol sodium) minus output.

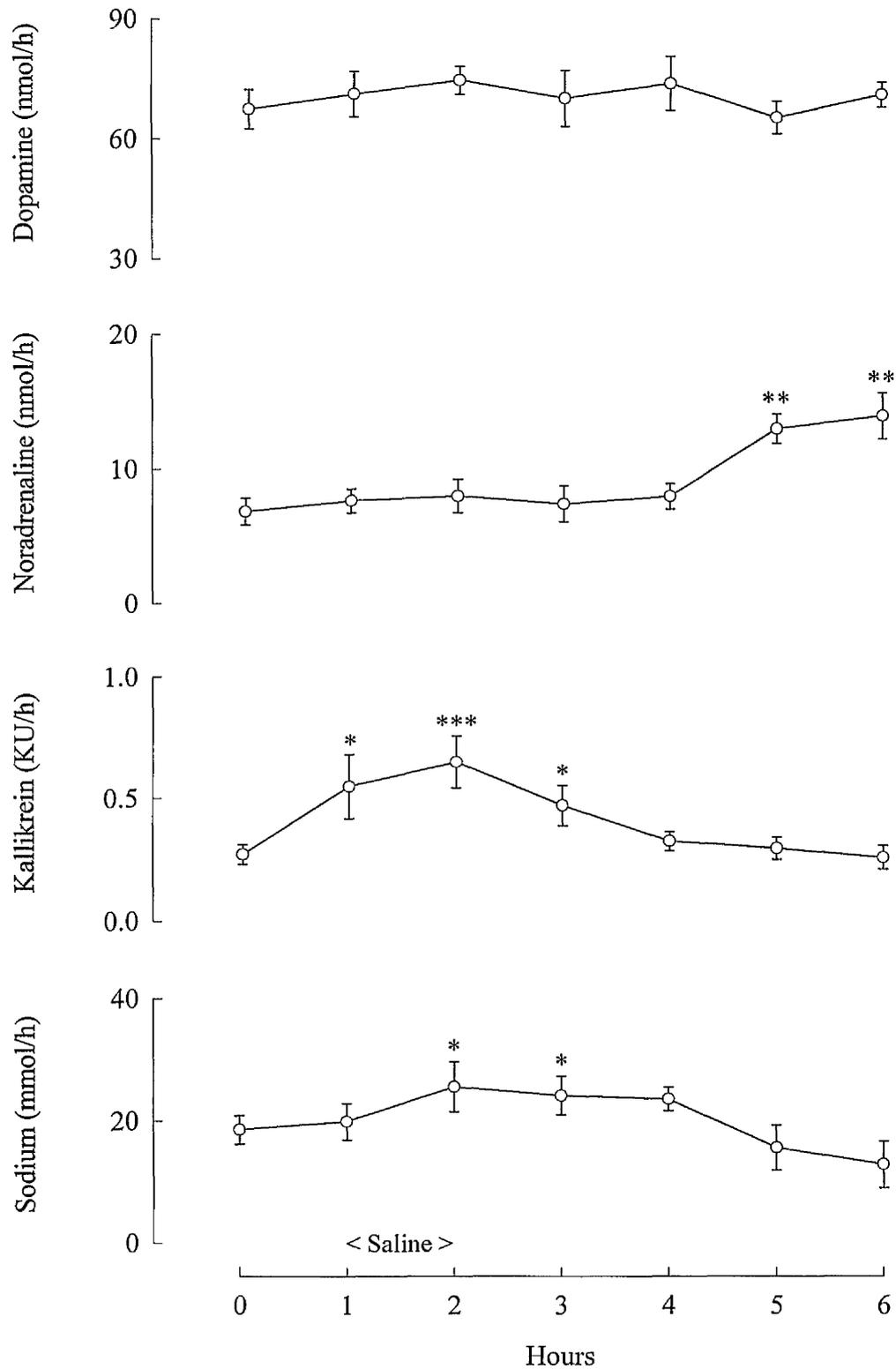


Figure 10a. The effects of intravenous saline infusion on the mean \pm SEM hourly urinary sodium, DA, NA and kallikrein outputs in the healthy Chinese subjects. * $p < 0.05$, ** $p < 0.02$, *** $p < 0.01$ when compared with hour 0.

10.4 Discussion

In this study, I have demonstrated that both urinary free DA and NA outputs in healthy Chinese subjects were not altered by the acute ECF volume expansion induced by an intravenous saline infusion. In contrast, this manoeuvre significantly increased the urinary excretion of kallikrein. These findings suggest that only the renal kallikrein-kinin system contributes to the acute natriuretic response to saline infusion in Chinese subjects.

10.4.1 *Effects of salt loading on the renal DA response in Chinese subjects*

Subjects in this study did not show an increase in renal DA output following acute saline infusion despite a 31-39% increase in urinary sodium excretion (Figure 10a). This finding suggests that renal DA is not an important natriuretic system in Hong Kong Chinese subjects. However, before reaching this conclusion, I must consider other possible explanations.

First, the intravenous salt load used in my study (1,000 ml over 2 hours or 15.4 ml/kg/2 hours) was small compared to that used in the studies done by other groups and discussed in Section 10.4.2. However, this was unlikely to be the reason for the absence of a renal DA response in my subjects. Even this modest salt load was enough to elicit a 31-39% increase in urinary sodium output and a 103.0-140.4% increase in urinary kallikrein output (Figure 10a). As already discussed in Chapter 9, if renal DA were an important natriuretic system in Chinese, this system should be sensitive to even small changes in ECF volume and body sodium status. In the published studies using Caucasian subjects (Section 10.4.2), a clear-cut, dose-response relationship between the infusion of 0.9% saline and the urinary free DA response was not seen. In other words, a mild to moderate expansion of the ECF

volume may still elicit a renal DA response. In fact, both Chen & Lokhandwala (408) and Bass & Murphy (409) have shown in rats that renal DA system appears to be relatively more important in promoting natriuresis at the lower (physiological) range of hypervolaemia (see Section 3.1.9).

The preceding level of dietary sodium intake could have a profound influence on the natriuretic response to acute saline infusion in normotensive Caucasian subjects with or without a family history of hypertension (203). Prior dietary salt restriction resulting in activated antinatriuretic systems may alter the natriuretic response to acute saline infusion and the associated changes in neurohormone systems. This was demonstrated in the study by Krzesinski et al. (520). Compared with healthy subjects without a family history of hypertension, Caucasian subjects with a family history had reduced natriuretic and renal DA responses to saline infusion after a low (20 mmol/day) or medium (170 mmol/day) sodium diet for one week. However, there were no differences between the two groups after a high (340 mmol/day) sodium diet for one week. Hence, variations in the dietary intake of sodium, and other nutrients, prior to the saline infusion could have been a confounding factor in my study.

Studies in Caucasian subjects have indicated that oral salt is a more potent stimulus to renal DA formation than intravenous saline (see Section 10.4.2). Since Hong Kong Chinese subjects have a smaller and less sustained renal DA response to oral salt loading compared with Caucasian subjects (Chapter 7 and Chapter 8), it is not entirely surprising that subjects in the present study did not show a renal DA response to intravenous saline infusion.

10.4.2 *Effects of salt loading on the renal DA response in Caucasian subjects*

Healthy Caucasian subjects generally show a renal DA response to saline infusion (Section 3.1.9). Alexander et al. (12) in the U.S. studied seven healthy women (aged 19-23 years) who were receiving a 59 mmol/day sodium diet for three days. An intravenous infusion of 5% dextrose (240 ml over 80 minutes) was followed by 0.9% saline (2,400 ml over 160 minutes). The data they published were the means of the three 20-minute intervals immediately preceding saline infusion and the means of the last four 20-minute periods of saline infusion. On changing to saline infusion, a 236% increase in sodium excretion (6.4 ± 0.8 vs 21.5 ± 2.8 mmol/hour, $p < 0.01$) and a 28% increase in free DA excretion (42.7 ± 4.3 vs 54.7 ± 3.7 nmol/hour, $p < 0.01$) were seen.

Castellano et al. (391) in Italy studied eight normal subjects (four males and four females, aged 19-57 years) without a family history of hypertension. They were given a 120 mmol/day sodium and 80 mmol/day potassium diet for five days. Urine was collected for 24 hours on the first day under basal conditions and on the second day after intravenous 0.9% saline infusion (2,000 ml over two hours). The average urinary DA excretion increased by 18% following the infusion (128 to 150 nmol/mol creatinine, $p < 0.01$).

Jeffrey et al. (392) in the U.K. studied nine healthy males (aged 20-38 years). Dietary advice was given to encourage a moderate salt intake over the preceding 48 hours. Tap water was given at intervals to ensure an adequate urine output. After a 2-hour baseline period (hours 0 to 2), the subjects were administered an intravenous infusion of 0.9% saline (20 ml/kg/hour) for three hours (hours 2 to 4) followed by a 2-hour recovery period (hours 5 to 7). The subjects were reclining during this 5-hour period but, thereafter, they were allowed to rise, and their urine was collected for a

further four hours (hours 7 to 11). Sodium excretion increased steadily from 9.0 ± 1.8 mmol/hour during the baseline period to 43.8 ± 7.2 mmol/hour in the hour after the infusion ($p < 0.01$), representing a 5-fold increase. Levels declined after this point but remained well above control during hours 7 to 11. During the entire study, only 26% of the administered salt load was excreted. A modest but significant increase in the free DA excretion of an average of 26% was detected during the infusion (79.8 ± 7.2 to 100.2 ± 7.8 nmol/hour, $p < 0.01$). This increase in urine DA was maintained until the end of the study. In other words, the increase in urinary free DA output persisted during and after the saline infusion.

Stenvinkel et al. (393) in Sweden studied nine healthy men (mean age 33, range 31-40 years). The subjects continued their usual diets with ad libitum sodium and protein intakes. On the morning of study, 500 ml of tap water was given orally, followed by 300 ml each hour. After a baseline period of one hour, an infusion of 0.9% saline was given for two hours at a rate of 25 ml/kg. The subjects were observed for a further two hours. The subjects remained in a supine position except to void urine, which was collected hourly. During the infusion, sodium excretion increased by an average of 46% (11.5 ± 1.5 to 16.2 ± 2.4 and 16.2 ± 1.8 mmol/hour, $p < 0.01$). One and two hours after termination of the saline infusion, urinary sodium excretion was still well above the basal level (19.1 ± 1.9 and 22.1 ± 2.3 mmol/hour, $p < 0.01$). A 12.8% increase in urinary free DA occurred during the second hour of the saline infusion (63.1 ± 9.1 to 71.2 ± 8.0 nmol/hour, $p < 0.01$). The urinary free DA excretion remained 16.2% and 9.5% higher than the baseline output even one and two hours after the completion of the saline infusion (73.3 ± 9.0 nmol/hour, $p < 0.01$ and 69.1 ± 9.8 nmol/hour, $p < 0.05$).

Stenvinkel et al. (394) repeated their study in 14 healthy men (mean age 34, range 25-47 years) using an identical protocol (393). During the second hour of the saline infusion, the urinary free DA excretion increased by 16.0% (77.4 ± 5.4 to 89.8 ± 5.8 nmol/hour, $p < 0.025$) and remained 16.5% higher than the basal value even one hour after the completion of the infusion (90.2 ± 6.8 nmol/hour, $p < 0.025$).

Stenvinkel et al. (396) extended their study to 15 healthy men (mean age 34, range 20-51 years) using an infusion rate of 0.9% saline 12.5 ml/kg for two hours. Urinary sodium excretion increased by 19.8% and 28.2% during the infusion (13.1 ± 1.3 to 15.7 ± 1.7 and 16.8 ± 1.5 mmol/hour, $p < 0.05$). The urinary sodium output was even higher (45.8 and 69.5%) one and two hours after the completion of the infusion (19.1 ± 1.4 and 22.2 ± 1.7 mmol/hour, $p < 0.01$). There was a 14.5% increase in urinary free DA excretion during the second hour of the saline infusion (81 ± 6 to 93 ± 7 nmol/hour, $p < 0.01$). The urinary free DA excretion remained 16.0% and 14.8% elevated one and two hours, respectively, after the completion of the saline infusion (94 ± 7 and 93 ± 9 nmol/hour, $p < 0.01$).

Stokes et al. (395) in Australia studied 20 healthy volunteers (14 men, 6 women, mean age 28 years). The subjects received a 100 mmol/day sodium, 60-80 mmol/day potassium diet for six days. On the morning of the study, 500 ml of water was given by mouth, followed by 200 ml hourly. After a baseline period of 90 minutes, two litres of 0.9% saline were given over three hours. Urine was collected every 90 minutes. The natriuretic response to the intravenous saline infusion was accompanied by an increase in the urinary free DA/NA ratio, i.e. an increase in free DA but a decrease in free NA excretion. [Data were presented in this abstract as graphs but absolute figures were not given.]

Stokes et al. (397) repeated their study in 10 healthy volunteers without a family history of hypertension (mean age 27, range 18-64 years; mean body weight 74, range 61-90 kg) using the same regimen of saline infusion (395). The natriuretic response was accompanied by a renal DA response, with the increase in urine free DA being greater in the post-infusion period. The increase in urinary free DA 1.5-3.0 hours after the completion of the saline infusion was $57 \pm 23\%$. However; a figure of $225 \pm 97\%$ was obtained if the urine had been acidified to a pH of 1-1.5 instead of 2.0-2.5 before storage (see Section 3.1.9).

A greater increase in urinary free DA output is generally seen after oral salt loading (Section 3.1.7) compared with intravenous saline infusions (Section 3.1.9), suggesting that oral salt is the more potent stimulus to renal DA formation. Lee (632) suggested that an increased oral sodium intake over several days is more effective in expanding ECF volume than an equivalent amount of sodium given intravenously. However, it is difficult to explain why an increase in urinary free DA is seen in Caucasian and Japanese patients with essential hypertension after an intravenous saline load but not an oral salt load (see Section 4.1.1).

10.4.3 *Effects of salt loading on the SNS activity*

A reduction in SNS activity might be expected to facilitate the renal excretion of a salt load (Section 2.4.2). However, in the eight healthy Chinese subjects I studied, urinary free NA output remained unchanged even after the completion of the saline infusion (Figure 10a). This observation suggests that the natriuretic response to an intravenous salt load in Hong Kong Chinese does not depend on a reduction in SNS activity.

The role of SNS activity in modulating the natriuretic response to intravenous saline infusion was studied less often. In the study of Alexander et al. (12), the combined urinary outputs of free NA and free adrenaline were used as a marker SNS activity and were unchanged after the saline infusion. In the study of Castellano et al. (391), a 17.3% reduction in mean free NA output was seen (17.0 to 14.0 $\mu\text{mol/mol}$ creatinine, $p < 0.01$). In the studies of Stokes et al. (395,397), the increase in urinary free DA/NA ratio was consistent with an activation of the renal DA system and/or a suppression of SNS activity during and after the saline infusion.

10.4.4 *Effects of salt loading on renal kallikrein-kinin activity*

The renal kallikrein-kinin system may modulate the renal excretion of sodium and water (Section 2.3.3). Furthermore, an association between defects in the renal handling of salt and water by the kallikrein-kinin system and hypertension has been suspected. Kallikrein is synthesised in the kidney and excreted into the urine. Most of the kallikrein in the urine is in the active form and predominantly reflects kallikrein secretion by the kidney (633). Thus, urinary kallikrein output has been used an index of renal kallikrein-kinin activity (see below).

In my study, the eight subjects showed a 103.0-140.4% increase in their urinary kallikrein output during the saline infusion (Figure 10a). Urinary kallikrein excretion was still much higher (74.1%) than at baseline one hour after completion of the infusion. The brisk urinary kallikrein response despite a relatively small salt load suggests that the renal kallikrein-renin system might play an important role in ECF volume and sodium homeostasis in Hong Kong Chinese.

Watson et al. (634) in the U.S. administered three litres of 0.9% saline over 60 minutes to 10 healthy men (aged 30 ± 6 years). The urinary sodium output

increased by 100% during the infusion. The urinary sodium output remained elevated 30 minutes (3-fold increase) and two hours (2-fold increase) after the completion of the infusion. The urinary kallikrein excretion increased rapidly (within 20 minutes of the start of the saline infusion) to a peak, 2-fold increase, at 40 minutes. A persistent elevation in the urinary kallikrein output was seen up to 30 minutes after the completion of the saline infusion.

Öhman & Karlberg (83) in Sweden studied the effects of the intravenous infusion of 0.9% saline (2,000 ml over four hours) on 15 healthy subjects (mean age 43, range 28-58 years) and 14 age-matched patients with essential hypertension. Urinary kallikrein excretion increased only in the healthy subjects, during the third and fourth hour of saline infusion (44%).

Nishimiya et al. (635) in Japan studied nine normotensive subjects, who were receiving a 200 mmol/day sodium diet. The subjects were given an intravenous infusion of 0.9% saline (1,000 ml over two hours). Urine volume, urinary sodium excretion and the fractional excretion of sodium were all increased. There was also an increase in urinary kallikrein excretion during the saline infusion, but the basal values and % increases were not stated in their publication.

However, two other studies failed to confirm an increase in urinary kallikrein excretion after intravenous saline infusion. Lieberthal et al. (636) in the U.S. studied six Caucasian males, who were on a diet containing 180 mmol sodium and 100 mmol potassium per day. The subjects were given 3 ml/kg water orally. Two litres of 0.85% saline were given over one hour. Both in the 3-hour period after the cessation of the saline infusion and in the subsequent 20-hour period, there were no changes in kallikrein excretion. Lewis et al. (637) in the U.K. studied seven healthy females (aged 20-29 years) on two days (a control and a saline infusion day). The

subjects were given an oral water load (20 ml of tap water/kg, followed by 300 ml every 30 minutes for six hours). Two litres of 0.9% saline were given over one hour. Urinary sodium excretion increased by 167% 60-90 minutes after the start of the infusion and then remained higher than that on the control day. However, the urinary kallikrein output did not differ significantly from the time-matched measurements on the control day.

10.4.5 *Effects of salt loading on other neurohormones in Chinese subjects*

In the present study, since 30% of the sodium load was excreted during the infusion and the MAP remained normal, it is conceivable that Hong Kong Chinese subjects might be dependent on other mechanisms for handling an intravenous salt load. These include the pressure-natriuresis mechanism and other neurohormones (Chapter 2).

Indeed, investigators in Taiwan reported that other neurohormones may also play a role in ECF volume and BP homeostasis in Chinese subjects. In the study of Chen et al. (638), nine healthy adults were given an intravenous infusion of 0.9% saline (2,000 ml over four hours). The natriuretic response was accompanied by a suppression of the PRC and plasma aldosterone concentration. In another study by Chen's group (639), nine healthy subjects taking their usual diet without salt restriction were given an intravenous infusion of 0.9% saline (2,000 ml) over four hours. Hourly urine collections revealed a 9-fold increases in urinary sodium excretion, from 10.2 ± 2.6 mmol at the baseline to 103.8 ± 13.5 mmol during the infusion. Their MAP remained unchanged throughout the study but plasma ANP increased by 24-33% during the third and fourth hour of infusion. A progressive fall in PRC of 58-87% occurred during the infusion. In the study of Hsieh et al. (640),

nine healthy subjects received two litres of 0.9% saline intravenously over four hours. A rise in plasma ANP was observed which became statistically significant by the fourth hour of the infusion. These three Taiwanese studies suggest that increased ANP release and suppression of the RAS may play a role in the regulation of ECF volume and BP homeostasis in Chinese.

10.4.6 *Significance and implications of my findings*

This was the first study to measure the simultaneous changes in renal sodium, DA, NA and kallikrein outputs in response to an intravenous salt load among healthy subjects. The absence of any changes in urinary free DA output despite a significant increase in sodium excretion after the intravenous saline infusion suggests that the renal DA system does not contribute significantly to the natriuretic response in Chinese subjects. Since the urinary free NA outputs also remain unchanged during and after the saline infusion, SNS activity also does not seem to be involved in their natriuretic response. However, the increase in urinary kallikrein output is much greater than that in sodium excretion, suggesting that the renal kallikrein-kinin system plays an important role in ECF volume and sodium homeostasis in these subjects.

Chapter 11 Conclusions

Over the past 30 years, the pioneer work of several investigators strongly suggests the importance of endogenous DA and renal DA₁ receptors in the regulation of sodium and ECF volume homeostasis (Chapter 3). There is substantial evidence that a defective renal DA system contributes to the development and maintenance of hypertension (Section 4.1). For example, some individuals may be less efficient in excreting a salt load as a result of defective renal DA production (Section 1.3). Such individuals may develop hypertension if they are frequently exposed to high salt diet.

The Chinese subjects in Hong Kong have a higher dietary sodium intake than before (Section 1.5). Hypertension and related complications such as intracerebral haemorrhage and heart failure are (increasingly) common. In my work, my aim is to establish whether Chinese subjects in Hong Kong have an efficient renal DA system that enables them to handle dietary salt loads effectively without developing an increase in BP (Section 1.6).

11.1 Are Hong Kong Chinese particularly 'salt-sensitive'?

With a substantial increase in dietary salt intake among the local population (Section 1.5), it is most important to find out whether Hong Kong Chinese are particularly 'salt-sensitive'. To address this question, I have studied the effects of oral and intravenous salt loading on BP in Chinese subjects recruited from our local population.

I have shown that the MAP in healthy Chinese subjects without a family history of hypertension remained unchanged despite large increases in sodium intake

from 20 to 220 mmol/day for five days (Chapter 7). Similar findings have been reported for Caucasian subjects (367-369,373). I have shown that the MAP in these subjects remained unchanged after gradual increases in sodium intake (50 mmol/day) over eight days (Chapter 9). In particular, there were no changes in their MAP despite a sodium intake of 320 mmol/day at the end of the salt loading period. I have also shown that an intravenous salt load (1,000 ml of 0.9% saline over two hours) was not associated with any changes in MAP in these subjects (Chapter 10). Therefore, sudden, large changes in dietary salt intake to an extent likely to be encountered in daily life do not alter the BP in healthy Chinese subjects without a family history of hypertension.

In contrast, healthy Chinese subjects with a family history of hypertension showed an increase (3.7%) in MAP after acute changes in dietary sodium intake from 20 to 220 mmol/day for five days (Chapter 8). Similar findings have been described for Caucasian subjects in Switzerland (619), Australia (621) and Germany (622). Others (248) have reported that subjects with a family history of hypertension are more likely than those without such a history to show a fall in BP after salt restriction.

11.2 Does the renal DA system have an important role in the natriuretic response to salt loading in Hong Kong Chinese?

To establish if the renal DA system has an important role in the natriuretic response to salt loading in Hong Kong Chinese, I have studied the relationship between 24-hour urinary sodium and free DA outputs in healthy subjects under basal conditions and their renal DA response to oral and intravenous salt loading.

11.2.1 *Positive correlation between 24-hour urinary sodium and free DA outputs*

If DA is an important intrarenal natriuretic substance in Chinese and dietary sodium is the major determinant of its intrarenal synthesis, there should be a positive correlation between 24-hour urinary sodium and free DA outputs (Section 3.1.6).

In my cross-sectional studies of Chinese subjects living in Hong Kong, a positive correlation between 24-hour urinary sodium and free DA outputs was seen in normotensive subjects with or without a family history of hypertension (Chapter 5), younger patients with less severe hypertension (Chapter 5 and Chapter 6), and normotensive and hypertensive sibling-pairs (Chapter 6). In contrast, patients with more severe hypertension did not show a positive correlation between 24-hour urinary sodium and free DA outputs (Chapter 5). A bigger comparative study using identical methodology is needed to confirm my findings (Section 5.4.8).

Two other studies in Guangzhou (597) and Hong Kong (598) also confirmed that there was a positive correlation between 24-hour urinary sodium and free DA outputs among healthy Chinese subjects. Similar findings have been reported in ethnic groups originating from salt-abundant regions (Table 3g).

11.2.2 *Hyperdopaminergic response in patients with hypertension*

Under basal conditions, Chinese patients with hypertension had a higher 24-hour urinary free DA output than normotensive subjects (Chapter 5 and Chapter 6). In view of the known cardiovascular and renal effects of DA in the body (Chapter 3), this increased intrarenal DA synthesis in patients with hypertension may represent an early antihypertensive compensatory mechanism.

Such a hyperdopaminergic response has also been reported in Caucasian and Japanese patients during the early phases of hypertension (Section 4.1.1).

11.2.3 *Small, unsustained renal DA response to large, acute increases in oral salt intake*

If DA is an important intrarenal natriuretic substance in Chinese and dietary sodium is the major determinant of its intrarenal synthesis, there should be a marked increase in urinary free DA output during the natriuretic response to oral salt loading (Section 3.1.7). However, when healthy Chinese subjects without a family history of hypertension were given a diet containing either 20 or 220 mmol sodium/day for five days each (Chapter 7), they only showed a 8-17% increase urinary free DA output and this increase was only sustained for three days. A similar renal DA response was seen in healthy Chinese subjects with a family history of hypertension (Chapter 8). On changing from a 20 to 220 mmol/day sodium diet, subjects with a family history showed a 23% increase in free DA output excretion on the first day of high salt intake. From day 2 to day 5, the increase in urinary free DA output became attenuated (12-15% higher than the baseline) but still reached statistical significance on days 2 and 5.

In contrast, when changing from a low (90-40 mmol/day) to high (220-340 mmol/day) sodium intake, Caucasian subjects showed a much greater increase in urinary free DA output of 26-59% and the urinary free DA output remained elevated throughout the high salt intake period (Table 3h).

Since the renal DA was small (compared to the 7- to 10-fold increase in urine sodium and the DA response reported in Caucasians) and the increase in urinary free DA output became attenuated after two to three days of salt loading, the renal DA system contributes only partly to the early natriuretic response to dietary salt loading in Chinese.

11.2.4 *Absence of a renal DA response to small, gradual increases in oral salt intake*

If renal DA plays an important role in acute sodium and ECF volume homeostasis in Chinese, it should be sensitive to small, gradual increases in dietary salt intake (Section 9.1). However, during gradual increases in salt intake (50 mmol/day) over eight days, urinary free DA output did not change in healthy Chinese subjects without a family history of hypertension (Chapter 9). In particular, despite a sodium intake of 320 mmol/day on the last three days of the high salt intake period, daily urinary free DA output remained unchanged. These findings suggest that the renal DA system in Chinese is insensitive to gradual, small increases in oral salt intake.

11.2.5 *Absence of a renal DA response to intravenous saline infusion*

If DA has an important natriuretic role in Hong Kong Chinese, there should be a concomitant increase in urinary free DA output during the natriuretic response to an intravenous salt load (Section 3.1.9). However, when healthy Chinese subjects without a family of hypertension were given an intravenous 0.9% saline infusion (1,000 ml over two hours), their hourly urinary free DA output remained unchanged (Chapter 10).

11.2.6 *Renal DA is not an important natriuretic factor in Hong Kong Chinese*

My findings so far indicate that in Hong Kong Chinese, the renal DA system is insensitive to gradual, small increases in oral salt intake and a modest intravenous salt load. Even sudden, large increases in oral salt intake are associated with a

relatively small and unsustained renal DA response. Thus, renal DA does not appear to be an important natriuretic system in Hong Kong Chinese.

11.3 Do Chinese have another mechanism(s) to deal with a salt load?

I have shown that the MAP in healthy Chinese subjects without a family history of hypertension remained unchanged despite large increases in sodium intake (Chapter 7 and Chapter 9). Their natriuretic response to oral or intravenous salt loading was brisk and comparable to that reported in other ethnic groups (Chapter 7, Chapter 9 and Chapter 10). Therefore, it is reasonable to investigate what other mechanism(s) are involved in the natriuretic response to a salt load.

11.3.1 The role of a reduction in the SNS activity

A reduction in the SNS activity may contribute to the natriuretic response to salt loading, as a concomitant fall in plasma NA concentration or urinary free NA output has been demonstrated in Caucasian subjects (Section 2.4.2). However, on changing from a low to high sodium diet, healthy Chinese subjects without a family history of hypertension showed no changes in urinary free NA output in the first four days of the high salt intake period (Chapter 7). During gradual increases in dietary salt intake, their urinary free NA output also remained constant during the first three days of increasing salt loading (Chapter 9). In both situations, a reduction (about 20%) in urinary free NA was seen towards the end of the high salt intake period, which might help offset any tendency to hypervolaemia-related increases in BP. The urinary free NA output also remained unchanged in healthy Chinese subjects during the natriuretic response to an intravenous sodium load (Chapter 10). These

observations suggest that a reduction in the SNS activity does not contribute to the natriuretic response to salt loading in Hong Kong Chinese.

11.3.2 *The role of the renal kallikrein-kinin system*

The renal kallikrein-kinin system also plays an important role in ECF volume and BP homeostasis (Section 2.3.3). Renal kallikrein production, as indicated by 24-hour urinary kallikrein output, was not different between Chinese normotensives and patients with hypertension (Chapter 5) or between normotensive and hypertensive sibling-pairs (Chapter 6). Cross-sectional studies of the interrelationships between 24-hour urinary sodium, potassium and kallikrein outputs among normotensives and hypertensives failed to show consistent results (Chapter 5 and Chapter 6). Hence, salt-loading studies would be the better means to determine the importance of renal kallikrein-kinin system in ECF volume and sodium homeostasis in Chinese.

In my study, healthy Hong Kong Chinese subjects without a family history of hypertension showed a 103-140% increase in urinary kallikrein excretion during an intravenous 0.9% saline (one litre over two hours) infusion (Chapter 10). Urinary kallikrein output was still 74% higher than the basal level one hour after completion of the infusion. This brisk urinary kallikrein response to a modest intravenous salt load suggests that the renal kallikrein-kinin system might play an important and substantial role in ECF volume and sodium homeostasis in Hong Kong Chinese.

11.4 Do the offspring of hypertensive parents respond to a salt load differently?

BP is higher in the offspring of hypertensive parents than in the offspring of normotensive parents (Section 8.1). Subjects with a family history of hypertension also tend to have a blunted natriuretic response to an intravenous salt load and are more likely to be salt-sensitive. I have therefore compared the effects of oral salt loading on BP (Section 11.1), sodium excretion, renal DA response and SNS activity in healthy Chinese subjects with or without a family history of hypertension (Chapter 8). After the sodium intake was increased from 20 to 220 mmol/day, both the natriuretic (Section 8.4.2) and renal DA (Section 8.4.3) responses were similar in the two groups. As in subjects without a family history of hypertension, subjects with a family history showed no changes in urinary NA output (Section 8.4.4) over the first four days of the high salt intake period. These findings suggest that the natriuretic response in both groups is not mediated by a reduction in SNS activity. However, unlike subjects without a family history, the offspring of hypertensive parents showed no changes in urinary free NA output even by the fifth day (Section 8.4.4). This lack of a fall in SNS activity might contribute to the increase in their MAP during high salt intake (Section 8.4.1).

11.5 Implications of my findings and the need for future studies

In healthy Chinese subjects, the renal DA response to salt loading is either attenuated (Section 11.2.3) or absent (Section 11.2.4 and Section 11.2.5). The nature of the defect in renal DA mobilisation is not known at present. In particular, it is not known if there is impaired intrarenal conversion of L-dopa to DA after, for example,

salt loading and protein meals (Section 3.2.2). Since healthy Chinese subjects in Hong Kong do not have an efficient renal DA response to salt loading, they might be more prone to salt-induced hypertension unless they have other mechanism(s) to deal with a salt load. Although my subjects without a family history of hypertension showed no changes in their BP after modest salt loading, the effects of a higher salt intake (e.g. 300 mmol/day) are not known. In those with a family history of hypertension, an increase in BP was seen when their sodium intake was increased to 220 mmol/day. The effects of a higher sodium intake in Chinese subjects with other risk factors for salt sensitivity (e.g. diabetes and obesity) are also not known. These questions and the relative importance of the renal kallikrein-kinin system and other natriuretic (Section 2.3) and antinatriuretic (Section 2.4) systems in ECF volume and BP homeostasis need to be addressed in future studies. To confirm if there is indeed an ethnic difference in the renal DA system, subjects of different ethnic background living in the same environment should be studied in future.

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