GLUCOCORTICOID RECEPTOR BINDING CHARACTERISTICS IN RAT GENETIC MODELS OF HYPERTENSION AND IN NORMAL SUBJECTS OF KNOWN GLUCOCORTICOID RECEPTOR GENOTYPE

by

Maurizio PANARELLI M.D. (Turin, Italy)

A thesis submitted for the degree of Doctor of Philosophy

Faculty of Medicine

University of Glasgow

June 1995

"We trained very hard, but it seemed that every time we were beginning to form in to teams we would be re-organised. I was to learn later in life that we tend to meet any new situation by re-organising, and a wonderful method it can be for creating the illusion of progress, while producing confusion, inefficiency, and demoralisation."

Gaius Petronius a.d. 66

AKNOWLEDGEMENTS

I wish to thank my supervisors Dr R Fraser and Dr CJ Kenyon for their advice and encouragement during the work carried out at the MRC Blood Pressure Unit.

Many collegues helped me during this work. I am grateful to Mrs CD Holloway for her technical support during the development and application of *in vitro* and *in vivo* tests for glucocorticoid receptor responsiveness, to Ms M Ingram for measuring the urinary glucocorticoid metabolites, to Dr G Inglis who introduced to me the Southern blotting technique and gave me advice when any problem arose during its application, to Professor JMC Connell for his supervision in relation to the clinical aspects of this work and to Professor AF Lever for making available the facilities in the MRC Blood Pressure Unit.

I also wish to record my appreciation to all the people at the MRC Blood Pressure Unit for their friendly support.

I also wish to thank the Society for Endocrinology (Marjorie Robinson bequest) and the British Hypertension Society for their financial support during my residence in the MRC Blood Pressure Unit.

Finally, I would like to express my thanks to Pauline M^{c}Ewan for her tremendous support over the last few years.

SUMMARY

The evidence of a familial component in the development of hypertension has been discussed in its socio-economical, genetical and hormonal aspects. Of the endocrine influences, the activity of the adrenal cortex and the way in which both mineralocorticoids and glucocorticoids interact with their target tissues has been evaluated. It has been suggested that this interaction may vary in a genetically determined manner. Recent studies suggested that the restriction fragment length polymorphism (RFLP) of the glucocorticoid receptor (GR) is associated with differences of blood pressure. Subjects from the Ladywell district of Edinburgh who were homozygous for one GR genotype had higher blood pressure than those homozygous for the other. The hypothesis that the receptor genotype might be associated with phenotypic differences in glucocorticoid responsiveness, which in turn could contribute to the development of hypertension, was drawn. This hypothesis has been investigated in strains of rat prone to develop hypertension and in a group of normotensive male subjects characterised for GR RFLP genotype.

The investigation first required the adaptation, validation and use of a number of biochemical and cell biology techniques. A new method of measuring glucocorticoid potency has been developed, based on the ability of glucocorticoids to inhibit the translation of the lysozyme gene. The lysozyme release from various types of white cells and the specificity of the inhibitory response to glucocorticoids and other steroid hormones has been examined. The results showed that the inhibition of lysozyme release from human mononuclear cells is a convenient and specific method of measuring glucocorticoid responsiveness in human tissues.

Glucocorticoid receptor binding characteristics were investigated in rat models of genetic hypertension. Preliminary results showed that white blood cells of spontaneously hypertensive rats (SHR) incubated at 24^0 bind dexamethasone with greater affinity than those from Wistar-

white blood cells of spontaneously hypertensive rats (SHR) incubated at 24^0 bind dexamethasone with greater affinity than those from Wistar-Kyoto (WKY) rat. This suggested that increased glucocorticoid activity might be a genetic component of blood pressure control. To confirm and extend these data, a method of studying glucocorticoid receptor binding, thermolability and thermostability was developed using rat liver cytosol. GR affinities and binding capacities were identical in SHR and WKY rats when tested in homologous competition studies of ^{3}H-dexamethasone after 16 h at 5^0. However, further experiments at 37^0 showed a time-dependent decrease in affinity in both SHR and WKY rats but the affinity in SHR was always higher compared to WKY rats. This suggested that an altered GR-heat shock protein function may be important in the development of hypertension.

The Milan hypertensive strain (MHS) of rat, an established model of human essential hypertension, was investigated. These rats appear to have excess mineralocorticoid activity (raised plasma volume, exchangeable sodium and plasma atrial natriuretic peptide and suppressed plasma renin) and have been shown to have higher plasma corticosterone concentration than the normotensive genetic control strain (MNS). The hypothesis of an impaired GR which leads to raised plasma corticosterone was investigated. Corticosterone, by acting via mineralocorticoid rather than glucocorticoid receptor, may cause antinatriuresis. The results showed a weaker binding affinity of endogenous steroid to GR in MHS which is in favour of the hypothesis.

In a group of normotensive male volunteers, links between polymorphism of the gene for GR and differences in GR characteristics and glucocorticoid responsiveness were tested. GR genotype was characterised by RFLP analysis. Blood pressure, plasma and urinary steroid concentrations, GR binding characteristics, responsiveness to glucocorticoids (*in vivo* and *in vitro*) and other routine biochemical variables have been compared. In a group of 64 volunteers (age range 18-

biochemical variables were observed between groups. AA subjects showed higher levels of plasma and urinary cortisol, lower apparent GR affinity for dexamethasone in white cells and higher IC_{50} values for the *in vitro* effects of dexamethasone on lysozyme activity compared to aa subjects, although the differences were not statistically significant. *In vivo* glucocorticoid responsiveness, measured as skin vasoconstriction test, showed that AA subjects were more sensitive to budesonide than those with aa ($p=0.02$). This greater sensitivity to topical steroid in subjects homozygous for the A allele may be a phenotypical expression of the glucocorticoid receptor function. However, given that other glucocorticoid indices were not different, it could be that human GR polymorphism is a marker of another cardiovascular variable which may not be directly related to receptor binding of steroid hormone.

In addition to this study, a large body of information on variables concerned with electrolyte homeostasis and the control of intermediary metabolism were collected. Many of the variables correlated independently of the receptor polymorphism. The results suggested a complex interaction of mineralocorticoid and glucocorticoid functions in the pathogenesis of hypertension.

In conclusion, studies in rats with genetic hypertension and in patients strongly indicate that glucocorticoid sensitivity may be a contributory factor in essential hypertension. If this is the case, then hypertension - or predisposition to hypertension - should be associated with a constellation of glucocorticoid sensitivity-related changes. If sensitivity is impaired, mineralocorticoid-like changes may result. If sensitivity is increased, glucocorticoid-dependant variables should change. Although neither hypothesis has been absolutely established by the experiments described in this thesis, they have provided some valuable guidelines for future studies.

CONTENTS

INDEX OF FIGURES AND TABLES

Chapter 1 INTRODUCTION

1.1 ESSENTIAL HYPERTENSION

Elevated arterial blood pressure is probably the most common public health problem in developed countries. Its prevalence depends on the composition of the population studied and the criteria used to define the condition. For example, it has been shown that the US black population has approximately twice the prevalence of hypertension compared with US whites (Cornoni-Huntley et al., 1989). Indeed, the prevalence of hypertension is influenced by population characteristics such as age, race, gender and socioeconomic status (Whelton et al., 1994). However, it is commonly reported that hypertension affects approximately 10% of the population. In only 5% of these hypertensive individuals can an underlying cause of high blood pressure (secondary hypertension) be identified and, if appropriate, specially treated before antihypertensive treatment is considered. Secondary hypertension can be due to a variety of causes and these are summarized in table I. For those people in whom a cause cannot be identified, the hypertension is designated essential or primary. Despite intensive research, there is considerable uncertainty about the pathophysiology of early essential hypertension. It is likely that mechanisms which initiate the increase in blood pressure are quite different from those which perpetuate it. There is general agreement that heredity plays an important role in the genesis of essential hypertension, but its expression appears to be influenced by environmental, dietary and other cultural factors.

This literature review deals firstly with genetic and environmental factors and their interaction in the pathogenesis of essential hypertension, and secondly with the significance of abnormalities in corticosteroid metabolism as *conditio sine qua non* for high blood pressure.

Table I: Disorders causing secondary hypertension.

- **Congenital or hereditary problems**
  - Coartation of the aorta (with other cardiac defects)
  - Congenital renal artery disease
  - Polycystic disease (usually presents in later life)
- **Drug induced problems**
  - Oral contraceptives (5% of women)
  - Carbenoxolone (mimics primary aldosteronism)
  - Liquorice (mimics primary aldosteronism)
  - Oral corticosteroids (systemic or topical)
- **Endocrine disease**
  - Phaeochromocytoma (may be associated with neurofibromatosis)
  - Primary aldosteronism
  - Cushing's syndrome
  - Acromegaly
  - Hyperparathyroidism
  - Some inherited steroid hydroxylation and oxidation-reduction defects
- **Renal disease**
  - Renin secreting tumours
  - Glomerulonephritis (particularly IgA nephropathy)
  - Pyelonephritis (unilateral or bilateral)
  - Diabetic nephrosclerosis
  - Polycystic disease (Mendelian dominant)
  - Renal artery stenosis (fibromuscular hyperplasia in young or atheroma in old patients)
  - Renal transplantation
  - Radiation nephritis
- **Vasculitis**
  - Progressive systemic sclerosis
  - Polyarteritis nodosa
  - Systemic lupus erythematosus
  - Pulseless disease

1.1.1 Definition and epidemiology of high blood pressure

Blood pressure is a continuously distributed variable with no dividing line between normal, high and low. For this reason, an epidemiological definition of hypertension must be arbitrary. Evans and Rose (1971) defined hypertension as that level of blood pressure where investigation and treatment do more good than harm. Thus, high blood pressure is not a disease in the usual sense of this term. It is a quantitative deviation of blood pressure from normal, which is not associated with symptoms but which predicts risk for future morbidity and mortality. The main problem in defining hypertension is the difficulty of precise measurement of blood pressure. The conventional methods can be unreliable because of technical inaccuracies, inherent variability of blood pressure (circadian variations of blood pressure) and tendency for blood pressure to increase in the presence of a physician (white-coat hypertension) (Pickering, 1994). Nevertheless, the Joint National Committee for Detection, Evaluation and Treatment of High Blood Pressure, 1993 (JNC V) defined cut-off points in order to facilitate the diagnosis of hypertension. It is recommended that blood pressure measurements be made by a mercury sphygmomanometer, using the Korotkoff technique and that the values are based on an average of two or more readings on two or more occasions in individuals not taking antihypertensive medications and not acutely ill. When the average systolic and diastolic blood pressure is above an arbitrary cut off point (table II), the patient is classified as hypertensive.

Results from longitudinal studies have shown that elevated blood pressure is associated with a high incidence of cardiovascular disease, stroke and heart failure (Smith, 1977; MacMahon et al., 1990; Stamler et al., 1993). Approximately 65% of deaths in hypertensive patients are due to cardiovascular disease (Kannel et al., 1985; Beaglehole, 1990). The Framingham study focused attention on systolic blood pressure elevation which carries a risk of cardiovascular disease similar to or greater than the

Table II: JNC V definitions for adults aged 18 years or older

	Systolic pressure	Diastolic pressure
Normotension	<140 mmHg	<90 mmHg
Hypertension	>160 mmHg	>95 mmHg
Borderline	falling between these limits	

risk associated with raised diastolic blood pressure (Kannel et al., 1981). Although over the past two decades diastolic blood pressure has helped to determine the level of blood pressure above which treatment reduces the risk of complications, isolated systolic hypertension is of particular importance and has special significance for the elderly population (VA Cooperative Study, 1970; Mann, 1992; Bennet, 1994). However, the total risk for hypertensive patients also comprises the effects of other factors. At any level of blood pressure, risk of death or of a cardiovascular event increases with high blood lipid levels, cigarette smoking, obesity, diabetes and left ventricular hypertrophy (as result of prolonged high blood pressure). The association of hypertension and cardiovascular mortality and morbidity is likely to be causative. Concordant data from different disciplines support the idea of a cause and effect relationship.

It was first believed that a single cause could explain the aetiology of essential hypertension. This idea derived from Goldblatt's demonstration that partial renal clamping could induce hypertension. Moreover, single causes such as primary aldosteronism, Cushing's syndrome, phaeochromocytoma and coarctation of the aorta are well known and now classified in the field of secondary hypertension (see above). Only in the early 1970s did epidemiological studies confirm that there was probably not a single cause of hypertension but that both genetic and environmental factors interact to cause the disproportionate rise of blood pressure with age which constitutes essential hypertension. However, there is no clear evidence that genetic differences can explain genetic variations in blood pressure. Migration studies helped to separate racial or genetic characteristics from environmental factors in the aetiology of hypertension (Beaglehole et al., 1978; Elford et al., 1990; Salmond et al.,1989; Ward et al., 1980). For example, the Japan-Honolulu-San Francisco study (Robertson et al., 1977) of Japanese men residing in Japan, Hawaii and California suggested that westernization may be responsible for weight and cholesterol differences among

Japanese but did not relate to level of blood pressure. There is evidence that the relevant factor in the high prevalence of hypertension in Japan is salt consumption. By contrast, the Ireland-Boston diet Heart Study (Kushi et al., 1985) showed higher blood pressure levels among Irishmen in Boston compared with their brothers who had stayed in Ireland. The Boston subjects were also heavier and had higher cholesterol levels. Rise in blood pressure with age seems to relate to communities with a high standard of living; such communities also tend to be obese, to have a high salt intake and to be relatively sedentary.

1.1.2 Genetic factors in hypertension

The study of the genetics of hypertension includes many research goals which have in common the aim of relating phenotypic information to a variety of genetic and environmental markers, of separating inherited from environmental influences, and of determining whether genetic effects are monogenic or polygenic.

Evidence in support of heredity as an aetiological factor in hypertension is derived from studies of familial aggregation of blood pressure in natural and adopted children and from animal experiments. Aggregation of a phenotypic trait among people who share genes is a strong indicator that it is genetically controlled, provided that a shared environmental influence can be excluded (Mongeau et al., 1986; Ward, 1990). Studies suggest a stronger correlation between the blood pressures of monozygotic twins than between dizygotic twins (Mather et al., 1961; Miall, 1971). Stamler et al. (1979) reported that the prevalence of hypertension among offspring of hypertensive patients is twice that observed in offspring of normotensive parents. Bianchi et al. (1979) estimated that children with two normotensive parents have a 3% probability of developing hypertension compared with a 45% probability if both parents are hypertensive. Further evidence for an important genetic component comes from the finding that the correlation between parents

and adopted children is smaller than that between natural parents and children (Annest et al., 1979a, b). In this type of study, environmental influences are better controlled. Table III summarizes the familial correlations of blood pressure between spouses, blood relatives, and parents with adopted children. Hunt et al. (1986) reported that the predictive strength of family history as a risk factor seems to vary with the degree of familial hypertension that is present (e.g., the risk doubles with one hypertensive first degree relative and increases nearly fourfold with two such relatives).

The explanation that is generally felt to fit the data best is that the ability to respond to environmental differences by developing hypertension is inherited polygenically and that the development of raised blood pressure is a function of the number of relevant genes inherited. This has been termed genetic heterogeneity (Dal Palu' & Pessina, 1985).

While the genetic contribution to hypertension remains unclear and by no means proven, the overwhelming evidence from epidemiological studies for an environmental factor or factors is demonstrated by the relationship of blood pressure levels with social class, relative weight, physical activity, alcohol intake, salt intake and geographic location. It is interesting to note that epidemiological studies have strongly implicated intrauterine environmental factors in the risk of hypertension in later life (Law et al., 1993). Low birthweight and large placental weight are potent predictors of hypertension at all age (Barker et al., 1990). Several environmental factors could be responsible for this such as maternal smoking (Spira et al., 1975), hypoxia (Kroger & Arias-Stella, 1970; Godfrey et al., 1991) and maternal malnutrition (Barker et al., 1990). Edwards et al. (1993) suggested that prenatal exposure of the fetus to glucocorticoid may not only retard intrauterine growth but could also affect the development of the fetal vasculature and its responses to pressor agents. Whatever the factor/s involved during intrauterine development,

Table III: Familial blood pressure correlations.

Comparison of pairs	Number	Correlation coefficient Systolic	Diastolic
Spouses	1433	0.08	0.06
Parent-adoptee	379	0.03	0.09
Parent-offspring	831	0.18	0.16
Sibling-sibling	2618	0.18	0.14
Dizygotic twins	264	0.25	0.27
Monozytic twins	248	0.55	0.58

Reproduced from Williams et al. (1988)

it appears that hypertension develops and persists after the removal of the primary cause, possibly because of irreversible changes in vascular structure (Lever & Harrap, 1992).

There are many potential physiological vehicles for translating genetic and environmental factors into rises in blood pressure. Among these are: intracellular ion transport, dietary salt intake, intravascular volume and kidney function, cardiovascular morphology and physiology and cardiovascular reactivity.

Several studies focused on the relationship between salt sensitivity and severity of hypertension (Parfrey et al., 1981; Intersalt, 1988) and it has frequently been suggested that a significant relationship exists between salt sensitivity of blood pressure and familial disposition to hypertension (Pietinen et al., 1979; Murakami et al., 1992). Tobian and Binion (1952) were the first to postulate raised intracellular sodium as a feature of essential hypertension. Since then, many other studies have confirmed this finding and shifted the research goals to studies of the pathways by which sodium may enter the cell. These include sodium-proton exchange, sodium-sodium exchange and sodium-potassium-chloride cotransport. Defects on ouabain-sensitive sodium pump (Na^+/K^+ ATPase), sodium-potassium cotransport and sodium-lithium countertransport (another form of sodium-proton exchange) have been reported in the normotensive relatives of subjects with essential hypertension and in patients suffering from essential hypertension (Goldsmith et al., 1991; Wehling et al., 1991b). However, it has been variously reported that the rate of red blood cell Na-K cotransport is elevated (Adragna et al., 1982; Smith et al.,1984), depressed (Garay et al., 1982; Lijnen et al., 1984) or similar (Cooper et al., 1983) in normotensive subjects with family history of hypertension compared with subjects without family history. It has been suggested that the above mentioned sodium transport abnormalities are markers of the hypertensive process. They may cause hypertension or they may represent a compensatory

mechanism resulting from a blood pressure increase. This effect may be defective in some people.

By contrast, contradictory data are reported on dietary salt intake and blood pressure. One explanation of this inconsistent relationship is that subjects vary in their genetic susceptibility to dietary sodium (Watt & Foy, 1982). There is some evidence (Watt et al., 1985) although incomplete, that the offspring of subjects with a family history of high blood pressure show a greater rise in blood pressure in response to sodium than those without such a family history. It is likely that the finding of an abnormality of sodium transport in essential hypertensive patients (Blaunstein, 1984) may reflect an inherited difficulty in the ability of the kidney to excrete sodium (MacGregor, 1985; Bianchi et al., 1983) and that the abnormality of salt sensitivity and cell ion transport may be related.

Studies of patients with mild or borderline hypertension have reported an abnormal pressure response to various pressor stimuli (e.g., cold pressor or mental stress test) (Julius & Conway, 1968; Anderson et al., 1987; Borghi et al., 1988). It has also been reported that in subjects whose blood pressure overreacts to stress, there is a higher than expected prevalence of family history of hypertension (Light & Obrist, 1980). In a population analysis of 340 hypertensive subjects in the Utah study, Smith et al. (1987) estimated that the degree of increase in blood pressure during mental arithmetic was approximately 50% heritable after adjusting for age, height, weight, number of total and correct subtractions, and baseline blood pressure.

Although the importance of genetic factors in the pathogenesis of essential hypertension is clear, information so far available has not yet clarified its pattern of inheritance. Two approaches of study have been suggested (Camussi, 1988). The first aims to identify intermediate phenotypes that display some form of Mendelian inheritance and seem relevant in explaining some pathophysiological finding in hypertension.

This is followed by the identification of candidate genes, their number, the frequency of particular alleles and their mode of action, in the control of variation in the intermediate phenotypes. The second approach studies the genetic or molecular difference of the genes or primary gene products (eg., mRNA, proteins) and attempts to see whether their eventual polymorphism among hypertensives and normotensives is relevant in the pathogenesis of hypertension (eg., all gene coding the known vasoactive substances that play some role in hypertension have been studied with molecular biology techniques, see below).

Strains of rat provide a valuable animal model of genetic hypertension which permit easy control of experimental conditions, show an early development of high blood pressure, ease of breeding, and genetic homogeneity. A number of strains of rat with inherited hypertension are available of which the spontaneously hypertensive rat (SHR) has been the most intensively studied. Dahl et al. (1962) selectively bred another type of rat for their blood pressure response to a high salt diet. Two strains were established, one sensitive and the other resistant to the hypertensive effects of a high salt diet. Another well established animal model for essential hypertension suitable for both genetic and physiological studies is the Milan hypertensive strain (MHS) of rat (Bianchi et al., 1974). This strain has an inbred normotensive control and hypertension develops in a well-defined fashion: pre-hypertensive phase, followed by the development of high blood pressure and established hypertension (more details on these genetic model of rat hypertension will be given below). The study of these rat models of genetic hypertension has allowed the identification of DNA markers for some biochemical variables in hypertension. This provided evidence, sometimes contradictory, for the existence of gene polymorphisms which relate to hypertension. The next section attempts to summarize the abundant data obtained by molecular biology techniques in the identification of candidate genes for the study of essential hypertension.

1.1.3 Candidate genes in hypertension

Over the past decades, molecular genetic techniques have become available for identifying gene differences even without knowledge of the primary gene product or the biochemical mechanism of the disease. This approach - genetic linkage - combines the identification of families in which the phenotype of interest follows a clear pattern of inheritance with the use of molecular biology techniques to search for a DNA-based genetic marker that is so close to the aberrant gene that it has a high probability of being inherited with the disease (Keating, 1992; Lindpaintner, 1992). The genetic marker can be any variation, or polymorphism, in the DNA which can be revealed using restriction endonucleases. These enzymes cleave DNA at precise sites to produce a pattern of fragments of characteristic lengths. Variation in the length of these DNA fragments occur because of the presence of polymorphisms and are called restriction fragment length polymorphisms (RFLP). A polymorphism can be used as a genetic marker regardless of whether it is causally related to the disease phenotype (Watkins, 1988).

Genetic linkage analysis has also been applied in hypertension research, even though blood pressure levels in families rarely follows single gene inheritance patterns and is likely to be influenced by several definable single gene contributions, i.e. polygenic blending. These effects are modified by shared family environment and other environmental effects (Lifton & Jeunemaitre, 1993) (see earlier). Although this reduces the likelihood of a tight linkage, statistically significant linkage provides valuable guidance to aetiology.

Although the genetic linkage study has been the most extensively applied approach to the study of the heritability of hypertension, other techniques have also been used such as: a) association studies, where a difference in the distribution of alleles at a marker locus in case controls is sought, b) direct search for mutations in candidate genes by the

polymerase chain reaction, and c) test of gene effects *in vivo* in animal models by transgenic techniques (Dzau et al., 1989; Bader et al., 1992; Kreutz et al., 1992).

Obvious candidate genes in essential hypertension are those involved in physiological systems that are known to affect the tonic regulation of blood pressure (Williams, 1989). Over the past few years, RFLP analysis has been a useful tool to explore genes encoding renin, haptoglobin, myosin, neuropeptide Y, the Na-H antiporter, atrial natriuretic peptide (ANP), vasopressin and renal kallikrein.

The renin-angiotensin cascade has a legitimate and well known pathophysiological place in the aetiology of hypertension and genes encoding renin-angiotensin cascade components were an obvious choice (Dzau, 1988a, b; Soubrier et al., 1988). Samani et al. (1989a) showed a 650 base pairs deletion of a tandem repeat sequence in the first renin gene intron in SHR compared to WKY. Moreover, higher renin mRNA levels were showed in young and adult SHR when compared to WKY (Samani et al., 1989b). Rapp et al. (1989) showed a significant association between a renin gene marker and hypertension in the Dahl rat strain of sodium-sensitive hypertension but similar analyses in the spontaneously hypertensive rat have been inconclusive (Lindpaintner et al., 1990; Kurtz et al., 1990). Similarly, human population studies failed to associate a renin gene polymorphism with hypertension, thus deflating the initial enthusiasm.

Angiotensin converting enzyme (ACE) is another component of the renin-angiotensin cascade which may be a pathophysiological candidate in hypertension. Hilfbert et al. (1991) using linkage analysis in crosses between spontaneously hypertensive stroke prone (SHR-SP) and Wistar-Kyoto (WKY) rats, identified two genes, BP/SP1 and BP/SP2, that contribute significantly to blood pressure variation in the F2 generation. Comparison of the human and rat genetic maps indicates that BP/SP1 could reside on human chromosome 17q in a region that also

contains the ACE gene. However, results of studies on human essential hypertension gave conflicting results. Jeunemaitre et al. (1992a) found no linkage between an ACE polymorphism and hypertension, while Zee et al. (1992) reported a significant association between these factors. Although the link between ACE gene and hypertension is not established, Cambien et al. (1992) and Tiret et al. (1993) showed a direct association between an insertion (I)/deletion (D) polymorphism in intron 16 of the human ACE gene and the risk of myocardial infarction, a cardiovascular event for which hypertension is one of many risk factors.

By contrast, Jeunemaitre et al. (1992b) have established a direct involvement of the locus of the angiotensinogen gene in human hypertension and the same group have reported that a molecular variant of angiotensinogen may be associated with pre-eclampsia (Ward et al., 1993). Moreover, a significant linkage and association of the angiotensinogen gene locus to essential hypertension has recently been reported in a study which considered 63 white European families with two or more members suffering from hypertension (Caulfield et al., 1994).

West et al. (1992) looking for candidate genes in hypertension, chose two genes coding proteins with vasoconstrictive action (renin and neuropeptide Y) and two genes associated with familial hypertrophic cardiomyopathy (β heavy chain of cardiac myosin and haptoglobin) but none was associated with hypertension.

Lifton et al. (1991) in a genetic linkage study of the human gene encoding Na-H antiporter also excluded mutations at this locus as a contributing factor in the pathogenesis of hypertension.

Since the phosphoinositide signalling system mediated by the activation of phospholipase C (PLC) regulates cellular calcium levels and the activities of protein kinases, the role of PLC in the genesis of hypertension has become a focus of attention (related to muscle tone and responsiveness) (Aqel et al., 1987; Koutouzov et al., 1987; Turla &

Webb, 1987). Thus, Yagisawa et al. (1991) identified eight RFLPs (six in the PLC-β, one in the PLC-δ and one in the PLC-τII locus) between SHR and WKY genomes but no conclusion concerning the relationship between these RFLPs and hypertension has been drawn.

Watt et al. (1992) using the "four corner" approach, suggested that young people with higher than average pressure, whose parents have a high pressure, are more likely to possess a particular RFLP for the glucocorticoid receptor gene (but not for the ANP gene) than young people with lower than average pressure. Barley et al. (1991) tested whether any relationship existed between allelic polymorphisms at the gene loci for either renin or ANP and blood pressure. They showed significant ethnic RFLP differences at both loci and provided evidence for a possible link between variations within or close to the renin gene and elevated blood pressure in Afro-Caribbeans.

The environment may affect the expression of genes that are not directly involved in blood pressure regulation. For example, some rats and mice have an increased susceptibility to high environmental temperature and respond with greater than normal rises in blood pressure. In cross-breeding studies, of F2 generation of such mice, the abnormal thermosensitivity appeared to be determined by a specific locus cosegregating with blood pressure (Tms) (Malo et al., 1989). The best candidate genes for this thermosensitivity are the heat stress protein genes, a major representative of which is heat shock protein 70 (Hsp70) (see later). Hamet et al. (1992) using a Hsp70 probe, identified a RFLP of Hsp70 after digestion of genomic DNA with BamHI in rats. This RFLP cosegregates with 15 mmHg of blood pressure in recombinant inbred strains of Brown-Norway and spontaneously hypertensive rats. Although the authors could not define the precise functional significance of this observation, they pointed out that the area of the genome in which the Hsp70 gene is located is of interest since it contains other genes potentially relevant to environmental interactions (Hamet, 1992), such as

the steroid 21-hydroxylase gene (enzyme involved in the adrenal steroid synthesis) and TNF alfa gene (a growth factor which is likely to be involved in smooth muscle cell proliferation during hypertension). Moreover, Hsp70 is a protein bound to the cortisol receptor (Pratt, 1993; Scherrer et al., 1990) and seems to be relevant in the regulation of the effectiveness of cortisol. For this reason Hamet (1992) suggested that increased levels of Hsp70 may enhance the potency of cortisol, resulting in insulin resistance, which is believed to be one of the hallmarks of hypertension (Ferranini et al., 1990; Williams, 1994).

Over recent years, abnormalities of corticosteroid metabolism as possible pathogenetic mechanisms in essential hypertension have been identified. Corticosteroids such as aldosterone and cortisol can be both directly and indirectly implicated in the control of the vascular tone. The following sections review the metabolism and actions of corticosteroids and how abnormalities can be the cause of secondary and essential hypertension.

1.2 STEROIDS AND BLOOD PRESSURE CONTROL IN HUMANS

Despite the rarity of secondary hypertension, it is logical to suppose that the mechanisms which raise blood pressure in this minority of patients might contribute to the pathophysiology of essential hypertension in a more subtle way. Although the classification of disorders causing secondary hypertension includes renal, congenital or hereditary, drug-induced problems, the study of endocrine disease has attracted particular attention. The involvement in blood pressure control of the renin-angiotensin system and the adrenal cortex hormones has been extensively studied.

1.2.1 Corticosteroid metabolism and physiology

The adrenal glands are extraperitoneal organs at the upper poles of the kidneys. They are made up of cortex and medulla which have separate embryological origin, mesoderm and ectoderm respectively. The classical view of the microscopic morphology of the adrenal cortex identifies 3 layers of cells arranged as concentric shells. From the outer layer; these are the zonae glomerulosa, fasciculata and reticularis. The zona glomerulosa produces aldosterone and 18-hydroxycorticosterone as its major steroids, and is present under the capsule in ill-defined form that constitutes about 15% of the cortex. The cells of this zone have a relatively small cytoplasmic volume with a small amount of lipid. The cortisol-producing zona fasciculata comprises about 75% of the cortex. Its cells contain more cholesterol and cholesterol esters, giving them a vacuolated or clear appearance in stained sections. Cells of the zona reticularis make up the inner zone and also produce cortisol, though at a much lower rate than the fasciculata cells. They are relatively free of lipids and have a granular, compact appearance. This zone also produces ACTH -regulated adrenal androgens (dehydroepiandrosterone and its sulfate, androstenedione and testosterone in the rat) and oestrogens

(Hyatt, 1987; Nussdorfer, 1986).

All corticosteroids are C21 derivatives of the pregnane series which derive from cholesterol and its esters. Cholesterol, either from circulating lipoprotein or synthesised *de novo*, is stored in esterified form in intracellular lipid droplets. Steroid synthesis starts with the hydrolysis of cholesterol esters and the sterol is then transferred to the inner mitochondrial membrane. Following hydroxylation at 22R and 20α, the cholesterol side chain is lost as isocaproic aldeide first and as acid second following oxidation. The C21 steroid which this produces, pregnenolone, passes from the mitochondria to the smooth endoplasmic reticulum. A series of enzyme reactions leads to the formation of steroids with two main activities, glucocorticoid and mineralocorticoid (refer to Fig. 1).

The major mineralocorticoid, aldosterone, is synthesised in the zona glomerulosa via a series of hydroxylations of progesterone. These hydroxylations are catalysed by mixed function oxidase ("hydroxylases") enzymes, which contain cytocrome P450, with the formation of 11-deoxycorticosterone (DOC), corticosterone, and probably 18-hydroxycorticosterone as intermediates. The unique enzyme of the glomerulosa is aldosterone synthase which catalyses the formation of aldosterone from DOC (Vinson et al., 1992).

The zona fasciculata has two biosynthetic pathways: a) the 17α-hydroxysteroid pathway which involves the hydroxylation of progesterone to 17α-hydroxyprogesterone, 11-deoxycortisol and finally to cortisol; b) the 17-deoxy pathway where progesterone is converted to DOC by 21-hydroxylase, and then DOC is hydroxylated at the 11 and 18 position to form corticosterone or 18-hydroxy DOC respectively. 11β-Hydroxylation of DOC occurs in the mitochondria and is catalysed by a single enzyme, 11β-hydroxylase.

Cortisol is the most important glucocorticoid in normal human subjects, but corticosterone at high concentration, in some adrenal

diseases, may also exhibit glucocorticoid activity . The rat adrenal has no 17-hydroxylase acitivity and corticosterone is the most important glucocorticoid in this species. Approximately 95% of cortisol is bound to proteins such as transcortin or corticosteroid-binding globulin (CBG) and albumin; the metabolic effects are mediated by the remaining unbound (or free) steroid (Pardridge, 1987; Tait & Tait, 1991).

Aldosterone and DOC are the principal mineralocorticoids. Aldosterone binds loosely to CBG and albumin so that 30-40% of the measured circulating aldosterone concentration is free. DOC is virtually totally bound to CBG with only 5-8% appearing as free DOC in the circulation.

Glucocorticoid effects are usually linked with the control of intermediary metabolism which includes stimulation of protein catabolism (Simmons et al., 1984) and hepatic glycogenesis and gluconeogenesis (Hers, 1985), with decreased glucose uptake and utilization in peripheral tissues. Glucocorticoids have direct effects on adipose tissue metabolism, enhancing the tendency for triglyceride accumulation by stimulating lipoprotein lipase activity in mature adipocytes (Pedersen et al., 1994). Glucocorticoids also inhibit growth (Smith et al., 1986; Schuchard et al. 1993), decrease calcium absorption in the gut and increase calcium loss in the urine causing a negative calcium balance (Singer, 1987), and modulate the humoral and cellular components of the inflammatory response (Bailey, 1991).

Mineralocorticoids are usually associated with changes in water and electrolyte metabolism. Aldosterone increases the reabsorption of sodium in the kidney and in secretory epithelia, thus reducing the sodium content of urine, saliva, sweat, gastric juice, and faeces. In the kidney tubules, an increased proportion of filtered sodium is exchanged for potassium or hydrogen ion which leads to antinatriuresis, kaliuresis and increased urine acidity with a metabolic alkalosis. After long-term treatment the kidney "escapes" from the sodium retaining actions of aldosterone but continues to stimulate the potassium secretion. This may

relate to a compensatory mechanism activated both by extracellular fluid volume expansion as a result of sodium retention and changes in renal haemodynamics (Knox et al., 1980; Hall et al., 1984). Atrial natriuretic peptides seems to be involved; high levels are found during escape from the sodium retaining effects of both aldosterone and DOC (Metzler et al., 1987; Granger et al., 1987).

Glucocorticoid and mineralocorticoid actions are mediated by intracytoplasmic proteins (corticosteroid receptors). It is necessary to define the actions of corticosteroids in terms of the receptors that mediate them. This is discussed in a later section (see below).

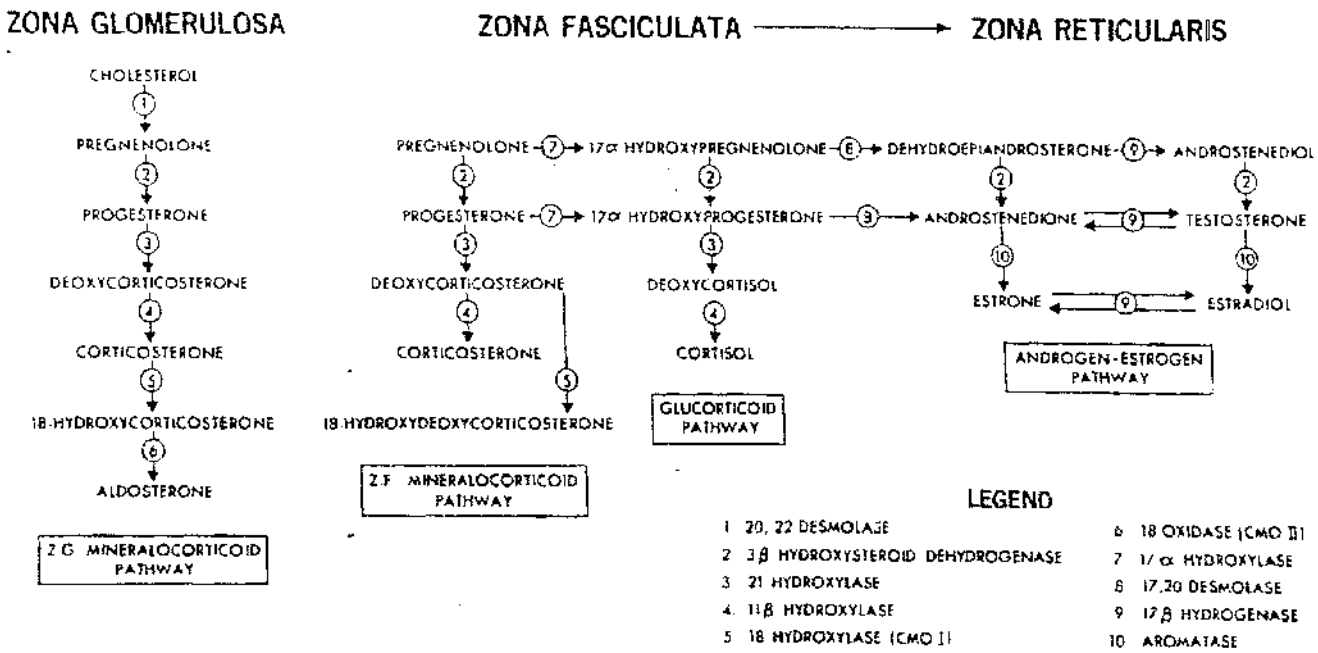

Fig. 1: Biosynthetic pathways of steroid hormones (reproduced from Irony et al., 1989)

Glucocorticoid secretion

The secretion of cortisol is largely controlled by adrenocorticotropic hormone (ACTH). This peptide hormone is synthesized in the corticotroph cells of the anterior pituitary, deriving from a large precursor, pro-opiomelanocortin. ACTH secretion is promoted by several factors, corticotropin releasing factor (secreted by the hypothalamus), arginine vasopresin and certain neurotransmitters, notably epinephrine (Dallman et al., 1987; Jacobsen & Sapolsky, 1991). The major physiological stimulus to ACTH secretion is "stress" both emotional and physiological. A negative feedback mechanism is present where glucocorticoids act on both the hypothalamus and adenohypophysis to inhibit CRF and ACTH secretion. Acutely, ACTH acts on the adrenal gland both by increasing the secretion rate of corticosteroids and by causing vascular changes which result in an increase in the rate of blood flow through the gland. ACTH and therefore cortisol are secreted with a distinct circadian rhythm with peak levels between 06.00-09.00 hours, falling during the day to reach a minimum in the late evening. The Fig. 2 describes the mechanism for the regulation of glucocorticoid secretion.

ACTH regulates the adrenal steroidogenic pathways as well as being an adrenal growth factor. Its action is exerted through increments of adenylate cyclase activity, with subsequent activation of side chain cleavage enzyme complexes which transform cholesterol into pregnenolone. Although ACTH is the principal regulator of both 17-deoxysteroids and 17α-hydroxysteroids, there is growing indirect evidence that other factors such as endogenous opiates (De Souza & Van Loon, 1982), prostaglandins (Rolland et al., 1981) and growth hormone (Castro-Magana et al., 1983) may regulate the responsiveness of the adrenal gland to ACTH.

Bovine and human adrenal fasciculata-reticularis cells contain specific angiotensin II receptors, most of which are of the subtype AT1 (Ouali et al., 1992), and *in vitro* angiotensin II (AII) induces a rapid

stimulation of cortisol secretion. *In vivo* infusion of AII in man has yelded contradictory results (Rayyis & Horton, 1979; Mason et al., 1979; Calogero et al., 1991), while *in vitro* AII causes a small increase in cortisol secretion by isolated adrenal cells and potentiates the acute steroidogenic effects of low ACTH concentrations (Parker et al., 1983; Pham-Huu-Trung et al., 1986). Lebrethon et al. (1994) have recently examined the effects of ACTH and AII on cultured human adult fasciculata-reticularis specific function. The authors reported that both ACTH and AII stimulated the expression of the mRNA of cholesterol side-chain cleavage enzyme ($P450_{scc}$), 17α-hydroxylase ($P450_{17\alpha}$) and 3β-hydroxysteroid dehydrogenase although the effects of ACTH were significantly greater than those of AII.

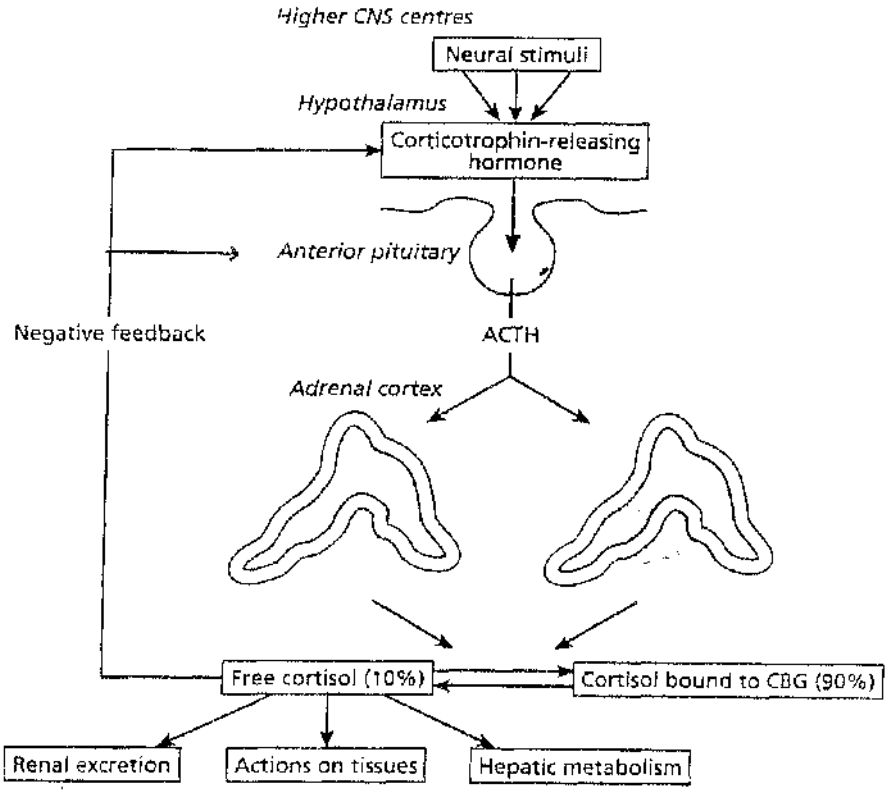

Fig. 2: The hypothalamic-anterior pituitary-adrenal axis.

Mineralocorticoid secretion

The most important stimulus to aldosterone secretion is the alteration of electrolytes and fluid status. Responses are mediated through a complex interaction between several factors, stimulatory and inhibitory, such as the renin-angiotensin system (see table IV). This system which has been mentioned earlier (reviewed by Quinn & Williams, 1988; Mitchel & Romen, 1989) begins with the secretion of renin from the juxtaglomerular cells of the kidney in response to reduction of renal perfusion or sodium load, and to sympathetic stimulation. Renin cleaves angiotensinogen - an α2-globulin secreted by the liver - to angiotensin I in the blood stream. This decapeptide is converted to the active hormone, the octapeptide angiotensin II by angiotensin-converting enzyme (ACE) in the lung and elseswhere. Angiotensin II has three main actions: 1) arteriolar vasoconstriction, 2) stimulation of aldosterone secretion, 3) reabsorption of sodium by direct action on the proximal tubule at the S-1 tubular segment.

Although the renin-angiotensin system is the most important mechanism for the control of aldosterone secretion, many other factors are also involved. Small increaes in plasma K^+ concentration specifically stimulate aldosterone synthesis by the zona glomerulosa. Hypophysectomy or hypopituitarism may also affect basal aldosterone output. There is evidence that a pituitary factor is necessary to maintain the adrenal responsiveness to various stimulators of aldosterone secretion, such as infusion of exogenous angiotensin II. It is likely that this factor is ACTH. Acute administration increases plasma aldosterone levels, while long term ACTH excess inhibits aldosterone secretion (Connell et al., 1987). In addition to those factors that exert physiological control, there are many others whose physiological relevance remains unclear. Table IV summarizes them in relation to whether they stimulate or inhibit aldosterone secretion.

Like cortisol, aldosterone is secreted with a clear circadian

rhythm, the mechanism of which is unclear (Katz et al., 1975). This is maintained in absence of ACTH, does not correlate with plasma sodium and potassium, and does not fully relate to renin. Although the rhythm is abolished in anephric subjects, it persists during administration of angiotensin converting enzymes inhibitors (DeForrest et al., 1979). Since β-blockers abolish the circadian rhythm of aldosterone, a neural mechanism is suggested (Cugini et al., 1977).

Table IV: Factors influencing aldosterone secretion. All, ACTH and K are the major "regulators" of aldosterone secretion. The influence of Na^+, independent of the renin-angiotensin-system, has contradictory results. The only negative regulator with a likely physiological role is ANP. ANP effects on blood pressure and volume are further enhanced by inhibition of both renin and aldosterone secretion

Stimulatory	**Inhibitory**
Angiotensin II	ANP
ACTH (acute)	Dopamine
K^+	Mg^{++}
Na^+	Somatostatin
Serotonin	ACTH (chronic)
POMC	
α-γ MSH	

1.2.2. Glucocorticoid-induced hypertension

High blood pressure is common in clinical cases of endogenous hypersecretion or exogenous administration of glucocorticoids. Thus, hypertension is a common feature of Cushing's syndrome, the clinical features of which occur as a result of prolonged abnormally high concentrations of glucocorticoids in the circulation. The disease is relatively rare and this is therefore an uncommon cause of hypertension in the general population. The prevalence of hypertension in Cushing's syndrome varies with the cause of hypercortisolism but is undoubtedly high, probably between 75-85% (Ross & Linch, 1982; Walker & Edwards, 1992). Hypercortisolism may either be ACTH-dependent (Cushing's disease *per se*, where there is ACTH hypersecretion from the pituitary gland, and ectopic ACTH syndrome where there is autonomous secretion of ACTH by a tumor of non-pituitary origin) or non-ACTH-dependent (autonomous secretion of cortisol from an adrenal adenoma or carcinoma, or from bilateral primary adrenocortical nodular dysplasia).

Routine biochemical investigations are rarely of diagnostic value in the diagnosis of Cushing's syndrome, although certain abnormalities may suggest it. Serum sodium concentration is normal in most patients but occasionally it is elevated. Serum potassium and bicarbonate levels are usually normal but hypokalemia and alkalosis are frequent with the ectopic ACTH syndrome or adrenal carcinoma. Serum calcium and phosphorus concentrations are almost invariably normal but hypercalciuria occurs in approximately 40% of the patients. Impaired glucose tolerance and elevations of triglyceride and cholesterol levels occurs in the majority of the patients. Studies of the renin-angiotensin system in Cushing's syndrome have reported normal (Brown et al., 1965; Ritchie et al., 1990), suppressed (Mantero et al., 1978) or ocasionally increased (Krakoff, 1973) plasma renin activity. This inconsistency seems to reflect the expected variability in mineralocorticoid expression in the different forms or Cushing's syndrome. Some of the biochemical and

clinical features of glucocorticoid excess may also be found in a variety of conditions referred to as pseudo-Cushing's syndrome where the clinical features can occasionally be present in the absence of biochemical evidence of hypercortisolemia (i.e. primary obesity and glucocorticoid hypersensitivity) (Iida et al., 1990). Excess cortisol secretion without Cushing's syndrome is found in conditions of glucocorticoid resistance secondary to defective glucocorticoid receptor function (Arai & Chrousos, 1994).

In experimental studies in normal human subjects, high doses of both ACTH and cortisol raise blood pressure within 3-5 days (Whitworth et al.,1983; Connell et al., 1987; Whitworth, 1992). Connell et al. (1987) reported that this ACTH effect on blood pressure is entirely a consequence of cortisol excess. Administration of either cortisol or ACTH in subjects on normal sodium intake led to profound anti-natriuresis, kaliuresis, suppression of renin and aldosterone, and a distinct rise in blood pressure. There was also evidence of expansion of extracellular fluid volume (ECFV) and plasma volume - i.e. mineralocorticoid-like effects- and alteration of both glomerular filtration rate and renal vascular resistance (Connell et al., 1988). It has been hypothesized that changes in plasma volume and cardiac output might be important in the early stages of ACTH and cortisol-induced hypertension, and increased peripheral resistance is probably a necessary development in the long-term maintenance of high blood pressure (Scoggins et al., 1984).

In sheep, the effects of ACTH on blood pressure may depend not only on cortisol but also on the synergistic action of other cosecreted steroids such as 17α-hydroxyprogesterone and 17α-hydroxy-20-dihydroprogesterone (Coghlan et al., 1988).

ACTH-induced hypertension can also be induced in the rat (Haack et al., 1978). It is adrenal-dependent (Whitworth et al., 1990a) and seems to be due to ACTH-stimulated increases in corticosterone secretion.

Although blood pressure increase is a feature of high concentration of glucocorticoids (cortisol in man and corticosterone in rats) in both pathological and experimental conditions, in the rat it has been shown that only small doses of steroid are required to increase blood pressure. The infusion of small doses of the synthetic glucocorticoid dexamethasone (<5μg/rat per day), in male Spague Dawley rats, increases blood pressure within a few days without loss in body weight, although the extent of the increase is less than with mineralocorticoids (Tonolo et al., 1988). Interestingly, the dexamethasone low-dose infusion did not affect renin, aldosterone, plasma sodium and potassium concentrations but the concentrations of plasma atrial natriuretic peptide (ANP) decreased by 40-50%. The decrease of ANP contrasts with ANP increases in response to mineralocorticoid treatment in rats with DOC-induced hypertension (see below). Unlike mineralocorticoid-induced hypertension, the pressor effects of dexamethasone were ameliorated but not abolished by dietary sodium restriction and were unaffected by sodium loading.

Cardiovascular effects of glucocorticoids

The effects of glucocorticoids on the cardiovascular system can be summarized as follow:

1) Mineralocorticoid effects. Cortisol is a relatively weak mineralocorticoid but at high circulating concentration might act as mineralocorticoid (Wehling et al., 1992b) (see below).

2) Abnormalities of renin-angiotensin system. Glucocorticoids increase plasma renin substrate (angiotensinogen) synthesis (Hasegawa et al., 1973; Krakoff et al., 1975; Whitworth, 1987; Feldmer et al., 1991). This might lead to increased plasma renin activity and to elevated plasma angiotensin II if angiotensinogen concentration is rate-limiting.

3) Direct effects of glucocorticoid. It has been shown that various glucocorticoids have direct pressor effect without altering sodium

metabolism (Whitworth & Scoggins, 1990). It has been suggested that corticosteroids may have a pressor activity separate from either their glucocorticoid or mineralocorticoid activity, possibly acting via a "hypertensinogenic" receptor (Coghlan et al., 1976). This might be the vascular receptor.

4) Increased sympathetic nervous system activity and increased vascular sensitivity to catecholamines. Steroid treatment *in vivo* and *in vitro* potentiates the actions of epinephrine, norepinephrine, dopamine and serotonin (Kalsner, 1969; Russo et al., 1990; Saruta et al., 1986; Webb, 1982). This can be explained by reduction of extraneuronal catecholamine uptake and reduced metabolism by cathechol-o-methyl transferase, which is the distal regulator of catecholamine synthesis in chromaffin cells of the adrenal medulla. Moreover, *in vitro* studies showed that dexamethasone down-regulates the number of specific β2-receptor (Jazayeri & Meyer, 1988) and increases the number and affinity of specific α1-receptors (Haigh & Jones, 1990).

5) Altered prostaglandin synthesis. Glucocorticoids suppress prostaglandin synthesis by inhibiting release of arachidonic acid substrate from phospholipids by phospholipase A2 (Grunfeld et al., 1986; Sessa et al., 1990; Yasunari et al., 1988; Russo et al., 1990). It has been suggested that this also decreases the synthesis of vasodilators such as prostacyclins.

6) Altered interactions between vascular endothelial cells and underlying smooth muscle cells. Endothelial cell produce a potent, locally acting vasorelaxant, nitric oxide. Glucocorticoids inhibit the expression of the inducible nitric oxide synthase. The inhibition of the synthesis of this relaxing factor should tend to increase blood pressure (Radomski et al., 1990).

Fig. 3 is an attempt to summarise the integration of pathophysiological changes leading to hypertension due to glucocorticoid excess.

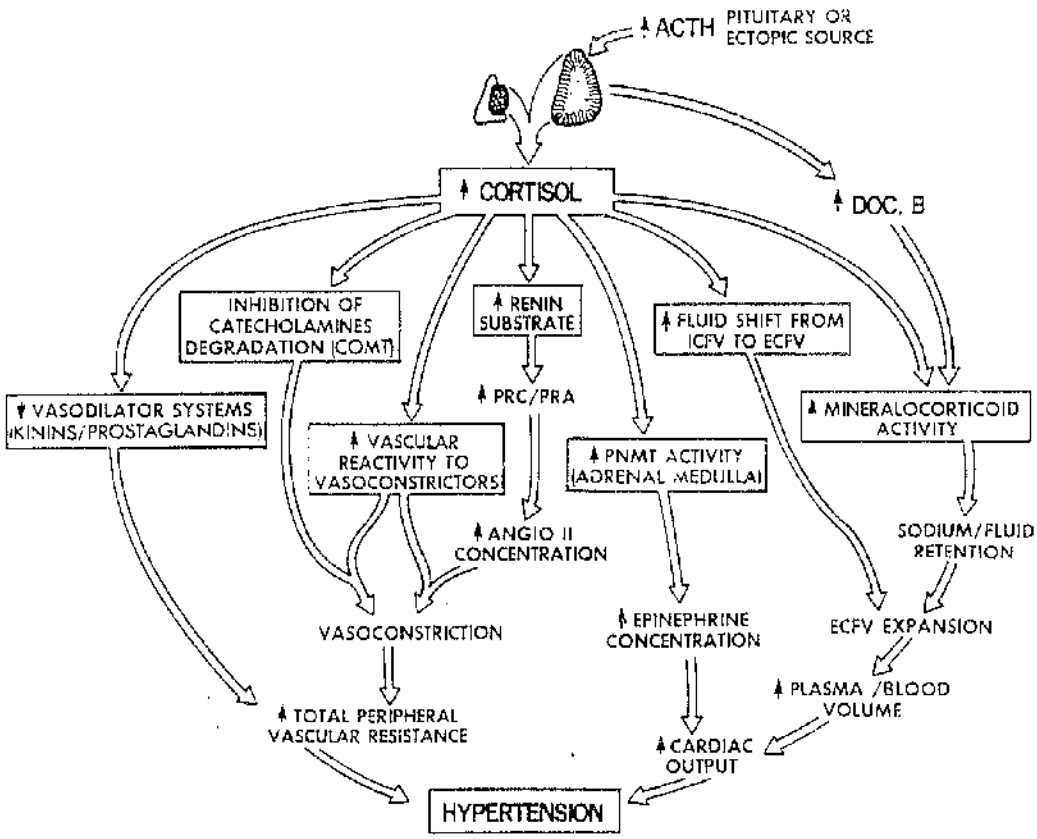

Fig. 3: Pathogenesis of hypertension due to glucocorticoid excess. ICV, ECFV, intra- and extracellular fluid (reproduced from Irony et al., 1989).

1.2.3 Mineralocorticoid-induced hypertension

Abnormally high levels of exogenous and endogenous mineralocorticoids cause hypertension.

A pathological increase of aldosterone in human subjects is a feature of a group of conditions termed primary hyperaldosteronism. This term is often applied to all patients with hypertension, hyperaldosteronism and concomitant renin suppression. Primary hyperaldosteronism is an uncommon cause of hypertension, the prevalence among hypertensive patients of which is less than 1%. This includes primary hyperaldosteronism due to a relatively autonomous adrenal adenoma (Conn's syndrome) or carcinoma and other forms of aldosterone excess with low plasma renin such as the bilateral zona glomerulosa hyperplasia and dexamethasone-suppressible hyperaldosteronism.

Aldosterone-producing adenoma (Conn's syndrome) is the commonest cause of primary hyperaldosteronism which accounts for approximately 75% of the patients. Plasma concentration of aldosterone has been reported to vary from high normal to several times the upper limit of the reference range. However, less variability has been observed when urinary aldosterone excretion rate was measured (Ferriss et al., 1978; Weinberger et al., 1979). Untreated patients show plasma sodium concentration at the upper end of the reference range and hypokalemia associated with low plasma renin and angiotensin II and a metabolic alkalosis. In a subset of patients, aldosterone secretion remains responsive to angiotensin II and the plasma renin activity is not markedly suppressed (Gordon et al., 1987).

Adrenocortical carcinoma is the cause of only 1-2% of primary hyperaldosteronism. In these patients, hypertension is usually associated with profound and often symptomatic hypokalemia and frequently there is evidence of glucocorticoid and androgen overproduction also (Arteaga et al., 1984; Isles et al., 1987).

In a subgroup of patients with features of primary

hyperaldosteronism, no adrenocortical adenoma is present and the gland may instead show various degrees of bilateral micronodular hyperplasia, or no discernible abnormality. Their biochemistry shows less markedly raised plasma aldosterone concentration while plasma renin and potassium concentrations are less fully suppressed. Similar adrenal pathology has been described at autopsy in patients who had been suffering from essential hypertension (Neville & O'Hare, 1985).

Dexamethasone-suppressible hyperaldosteronism is a rare familial autosomal dominant syndrome in which the biochemical features of primary hyperaldosteronism (hypokalemia and suppressed plasma renin activity) and raised blood pressure are corrected by administration of dexamethasone. Patients frequently present as children or young adults (Jamieson et al., 1993). This condition is further characterized by lack of aldosterone response to angiotensin II but exagerated response to ACTH (Connell et al., 1986; Ganguly et al., 1984), and abnormally elevated levels of 18-hydroxycortisol and 18-oxocortisol (Gomez-Sanchez et al., 1984; Gomez-Sanchez, 1985). No pituitary abnormality has been demonstrated, even though the abnormal adrenal function is clearly pituitary-dependent. The genetic defect in this condition appears to involve a mutation by which the regulatory region of 11-β-hydroxylase with 5' ACTH-responsive regulatory sequence is fused to the coding sequences of aldosterone synthase on chromosome 8 (chimaeric gene) (Lifton et al., 1992). This permits the expression of aldosterone synthase in the zona fasciculata so that aldosterone together with the characteristic 18-oxygenated derivative of cortisol can then be produced under ACTH control. However, the normal gene is also expressed in the zona glomerulosa so that the suppression of the chimaeric gene by dexamethasone restores angiotensin II-dependent aldosterone secretion. The disease can now be identified in affected kindreds by molecular biology techniques.

Mineralocorticoid hypertension can be observed in genetic defects of the enzymes involved in the adrenal steroidogenesis. Abnormal

hydroxylase activity can result in hypertension. A defect of the 17α-hydroxylase, inherited as an autosomal recessive trait, results in an inability to manufacture cortisol which results in an increase in ACTH stimulation of the adrenal cortex (Biglieri et al., 1966; Fraser et al., 1987). This causes increased progesterone, pregnenolone, DOC and corticosterone synthesis. DOC is a mineralocorticoid and very high plasma levels found in this syndrome cause sodium retention with hypokalemia. A reduced activity of 11β-hydroxylase, inherited as an autosomal recessive trait, results in defective conversion of DOC to corticosterone and of 11-deoxycortisol to cortisol in the adrenal cortex. This syndrome is also characterized by increased ACTH plasma levels which stimulate the adrenal to secrete a high level of DOC and 11-deoxycortisol (Fraser, 1983). In this case, corticosterone levels are low. The increased DOC secretion results in sodium retention, potassium loss and associated hypertension. In both defects plasma aldosterone is suppressed as a consequence of inhibition of the renin angiotensin system by sodium retention. Disproportionate increases in DOC or 11-deoxycortisol level in relation to corticosterone and cortisol, respectively, are suggestive of impaired 11β-hydroxylase activity, while altered progesterone:17α-hydroxyprogesterone ratios suggests reduced 17α-hydroxylase activity.

Administration of mineralocorticoids to man and animals raises blood pressure. The hypertensive effects of DOC were first described in rats. The acetate was used. The blood pressure response was shown to be quicker and greater if rats are unilaterally nephrectomized and given an high-sodium diet. This is called the DOCA-salt model of hypertension (Morton et al., 1990). Responsiveness varies between species and strains of rat. Long-Evans, Sabra normotensive, Wistar-Furth and Dahl salt-resistant strains are relatively insensitive to DOC compared with Sprague-Dawley, Sabra hypertensive, Dahl salt-sensitive and other Wistar strains (Ben-Ishay et al., 1972; Rapp et al., 1982; Bruner, 1992). Dogs and

rabbits are relatively insensitive whereas in human subjects and pigs, mineralocorticoid hypertension can be evoked by DOC without reduction in renal mass or modification in dietary sodium intake. Interestingly, depending on the dose of steroid and salt intake, rats can enter a malignant phase, whilst after a prolonged period of exposure to the steroid, blood pressure remains elevated even after stopping the treatment (Morton et al., 1990).

Mineralocorticoid effects in hypertension

Unlike glucocorticoid hypertension, mineralocorticoid hypertension is sodium dependent (Fraser & Padfield, 1985; Robertson, 1987). Plasma volume and total or exchangeable body sodium level and extracellular fluid volume are all increased above normal. The most frequent presenting biochemical feature is hypokalaemia together with a metabolic alkalosis. In the early stages of mineralocorticoid excess, hypertension is a function of an increased cardiac output but in chronic excess, cardiac output returns towards normal or low while total peripheral resistance is increased (Wenting et al., 1977). Altered sympathetic tone (Distler et al., 1979), increase sensitivity to angiotensin II (Zoccali et al., 1983; Gordon et al., 1987) and a direct action of corticosteroids on resistance blood vessels all lead to abnormal cation turnover, depolarization and vasoconstriction (Moreland et al., 1984; Gurwitz et al., 1982) which may account for the increased total peripheral resistance (refer to Fig. 4).

An additional mechanism may be the direct central action of aldosterone. Intracerebroventricular infusion of aldosterone produced hypertension in rats which could be reversed by infusion in the same site of a competitive aldosterone antagonist (Gomez-Sanchez, 1986; Gomez-Sanchez et al., 1992; Gomez-Sanchez, 1993). However, this should not be interpreted as evidence for a unique central site of action of mineralocorticoid in blood pressure control since intracerebroventricular

infusion of aldosterone in adrenalectomized animals fails to raise blood pressure.

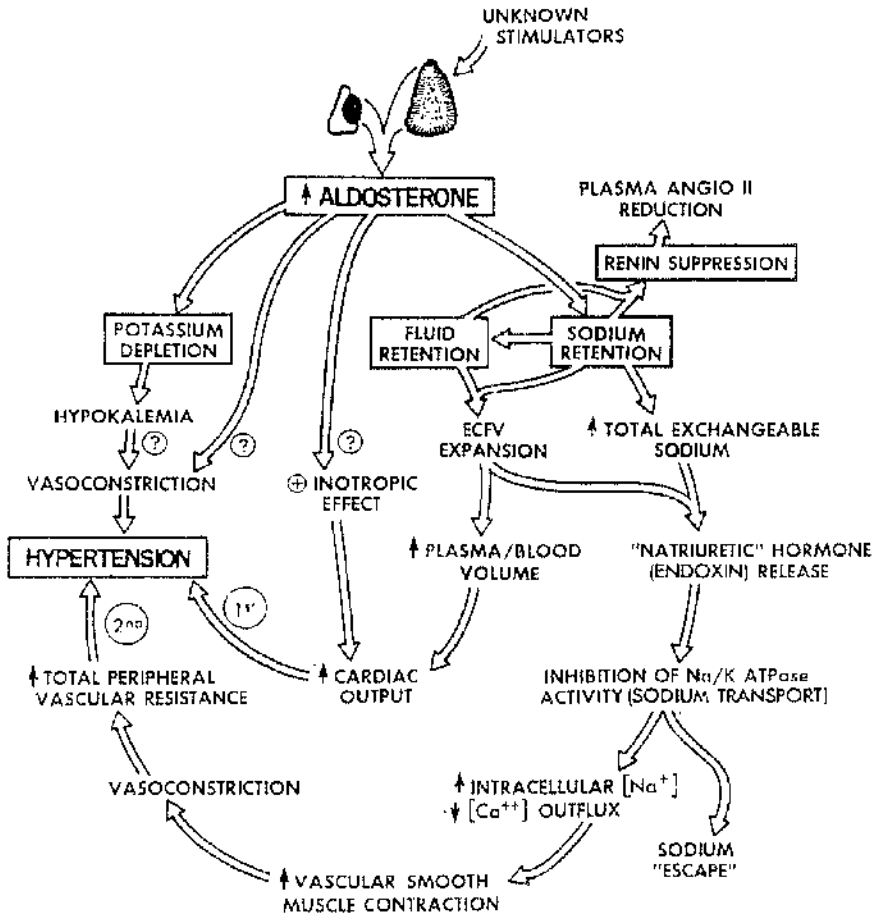

Fig.4: Pathogenesis of hypertension due to mineralocorticoid excess (from Irony et al., 1989).

1.2.4 Altered steroid metabolism in essential hypertension

Although several abnormalities in adrenocortical function in subjects with hypertension have been reported, the importance of corticosteroids in the genesis of this condition is not yet clear. Excess, even minor increases in secretion of either mineralocorticoids and glucocorticoids can raise blood pressure but excessive secretion, by definition, excludes the diagnosis of essential hypertension.

Several investigators have tried to identify unusual steroids or steroid metabolites which might be increased in hypertensive subjects. Increased 18-hydroxysteroids, such as 18-hydroxy-11-deoxycorticosterone and 19-nor-deoxycorticosterone have been reported in hypertensive patients but the results have not been convincingly confirmed (Gomez-Sanchez et al., 1985; Griffing et al., 1985). Kisch et al. (1976) reported an increased aldosterone response to angiotensin II in essential hypertensive patients. However, this seems to appear only in a subgroup of patients with essential hypertension (Dluhy et al., 1979; Williams et al., 1979) and it is likely to reflect an underlying abnormal activity of the renin-angiotensin system (Wisgerhof & Brown, 1978).

Bearing in mind the inherited hypertensive disorders of adrenal steroid synthesis due to abnormal steroid hydroxylase activity, some investigators have studied adrenal function in essential hypertensive patients during stimulation with ACTH. Honda et al. (1977) and De Simone et al. (1985) reported a partial defect of 11β-hydroxylase activity in some patients with essential hypertension using a greater than normal response of 11-deoxysteroid levels after ACTH stimulation as an indicator. However, neither study found an abnormal aldosterone response to ACTH. Unfortunately these studies are still inconclusive since other investigators (Guthrie et al., 1983) could find no differences in DOC response to ACTH in young people from the upper and lower deciles of blood pressure but who were not hypertensive.

Another approach has been to suggest that small changes in

corticosteroid concentrations contribute to the development of hypertension. Interestingly, Watt et al. (1992) showed higher levels of plasma cortisol, 18-hydroxycorticosterone and angiotensinogen in the adolescent offspring of hypertensive parents whose mean blood pressure was at the upper level of their group distribution when compared with those whose parents were normotensive and who had lower blood pressure.

These studies in general show that corticosteroids, both mineralocorticoids and glucocorticoids, are capable of causing hypertension and that this can result from relatively small increases in plasma concentrations persisting over long periods of time.

1.3 RAT MODELS OF GENETIC HYPERTENSION AND ABNORMALITIES OF THE STEROID METABOLISM

A number of strains of rats with inherited hypertension are available and some of these have abnormalities of corticosteroid secretion. Of these, the spontaneously hypertensive rat (SHR) has been the most intensively studied . In the SHR, the adrenal cortex has been reported to be hypertrophied compared with the normotensive control Wistar-Kyoto (WKY) and adrenalectomy prevents the development of hypertension (Brownie et al., 1990). The measurement of corticosteroid concentrations such as corticosterone, DOC and 18-hydroxy-DOC in SHR has been variably reported as increased, unchanged or decreased compared with controls. Dale et al. (1982) also reported an increase in 19-nor-DOC excretion which has not yet been confirmed. However, the use of SHR has been criticised because of the absence of a genetically appropriate normotensive control strain. It is known that among different sources of WKY, there are substantial genetic heterogeneities (Kurtz & Morris, 1987).

The Dahl salt-sensitive and resistant strains of rat provide a better model of genetic hypertension. At least part of the salt-dependent increase in blood pressure is due to inherited altered 11β-hydroxylase activity, producing an increased 18-hydroxy-DOC:corticosterone ratio *in vivo* and *in vitro*. Cross-breeding studies have shown that the preferential conversion of DOC to 18-hydroxy-DOC rather than corticosterone, which characterizes the salt-dependent rats, accounts for at least 16% of the increase in blood pressure (Rapp & Dahl, 1972). The salt-resistant and salt-sensitive rats possess different alleles of the 11β-hydroxylase gene, differing by five amino acid substitutions. The salt-resistant allele, by coding for an enzyme which synthesises proportionately less 18-hydroxy-DOC, is thought to protect the rat from salt-induced hypertension (Matsukawa et al., 1993).

Three distinct genetic strains of Lyon rat are available: hypertensive, normotensive, and hypotensive (Brownie et al., 1990). Five weeks old hypertensive animals have increased excretion of DOC, reduced excretion of corticosterone and unchanged 18-hydroxy-DOC, 19-nor-DOC and aldosterone compared with normotensive and hypotensive controls (Vincent et al., 1989). However, at 20 weeks old, DOC has returned to normal but there is higher excretion of corticosterone, lower aldosterone and unchanged 9-nor-DOC compared with controls. Whether this represent altered corticosteroid secretion or metabolism is still uncertain.

Abnormal steroid secretion has been demonstrated in Milan hypertensive strain (MHS) of rat (Ferrari et a., 1985; Mantero et al., 1983; Fraser et al., 1994). Study of the MHS has the advantage of a simultaneous normotensive genetic control strain, Milan Normotensive (MNS). Fraser et al. (1994) have recently reported higher secretion rates of corticosterone, 18-hydroxycorticosterone and aldosterone in the hypertensive group compared with the normotensive. These data have been interpreted as showing greater adrenocortical 11β-hydroxylase activity rather than aldosterone synthase activity in MHS compared to MNS rats. An understanding of the abnormalities in steroid metabolism is important because there are pathophysiological similarities between MHS and human essential hypertension (Bianchi & Ferrari, 1983).

1.4 THE STEROID RECEPTOR AND HYPERTENSION

The research on relationships between corticosteroid and hypertension has for years addressed the role of increased secretion or reduced clearance of steroids. In the recent years a link has been sought with altered steroid tissue metabolism (i.e., defect of 11β-hydroxysteroid dehydrogenase) or receptor and/or post-receptor function (e.g., glucocorticoid resistance syndrome). There is now abundant support for the first hypothesis and increasing evidence of the second.

This section reviews the mechanism of action of corticosteroids via their specific receptors and how abnormalities in receptor function and/or specificity can lead to hypertension.

1.4.1 Mechanism of action of steroids

Steroid action is a multistage process which acts through a common mechanism of ligand-dependent transcriptional modulation. The steroid enters the target cells, probably by simple diffusion, and binds to receptors in the cytosol, which undergoe structural changes associated with activation. Activation facilitates the transfer of the hormone-receptor complex to the nucleus where it binds to DNA at specific steroid response elements, modulating the transcription of genes (Allan et al., 1991; Carson-Jurica et al., 1990). This then leads to increased or decreased mRNA and protein synthesis. The different steps are summarized in fig. 5.

Over the past years, the cDNAs for all major steroid receptors have been cloned and sequenced (Carson-Jurica et al., 1990; Williams & Franklin, 1994). The amino acid sequences have then been deduced from the structure of the exons of the genes which code for them. In this way, it was discovered that, although the steroid receptors differ in length, their structure revealed remarkable homologies ('superfamily' of receptors) with each other and also with the receptors for 1,25-

dihydrocholecalciferol, thyroid hormone, retinoic acid, ecdysteroids (hormones controlling moulting in insects), and a number of receptors for which the ligand has not yet been identified (orphan receptors). The steroid receptor sequence homology shows three regions which are highly conserved among all members (see fig. 6). The strongest homology among receptors is in the region C1, a 66 amino acid sequence, which comprises the DNA binding domain. This domain forms two "zinc fingers" analagous to the structures formed by transcription factor IIIA, initially observed in the amphibian (Allan et al., 1991; Schule et al., 1988). Each finger is composed of 4 cysteines which coordinate one zinc atom. Domain swapping experiments suggested that the domain binding region is the sequence which specifically recognizes the cognate steroid response elements located in the genomic DNA adjacent to target genes (O'Malley, 1990; Freedman & Luisi, 1993). It has also been suggested that the first finger contains primary information for sequence specificity while the second stabilizes the receptor binding to its DNA response element.

The other two highly homologous regions, C2 and C3 in the C-terminal portion, consist of approximately 42 and 22 amino acids, respectively. These regions share less homology than do the C1 regions. They are hydrophobic and it is unclear whether they participate directly in the ligand binding, protein-protein structural interactions, or transcriptional activation. However, mutational analyses revealed that the entire C-terminal domain must be intact for high affinity ligand binding.

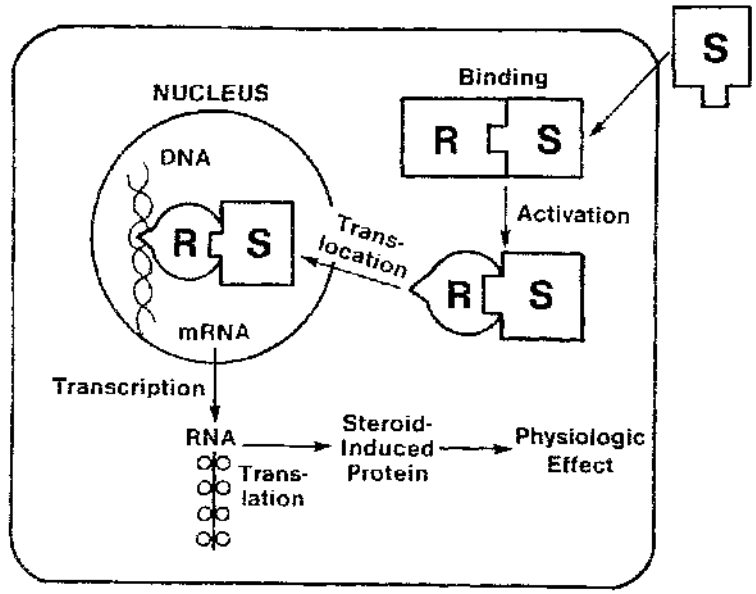

Fig. 5: Signal transduction pathway of steroid hormones. For the description of the different steps, see text.

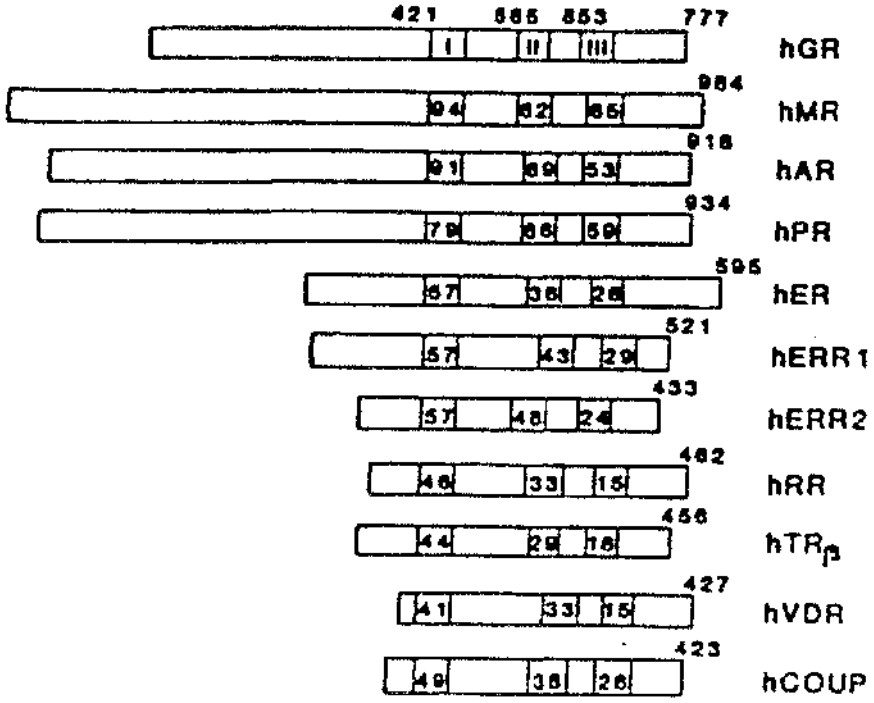

Fig.6: Sequence homologies of steroid receptor superfamily [human receptor for glucocorticoid (hGR), mineralocorticoid (hMR), progesterone (hPR), oestrogen (hER), oestrogen-related receptors (hERR1, hERR2), retinoic acid (hRR), thyroid hormone (hTRβ), vitamin D (hVDR) and the orphan receptor (COUP, chicken ovalbumin upstream promoter)]. Numbers inside the three major conserved regions indicate the percentage of amino acid homology with the hGR. The numbers to the right of each bar show the number of amino acid residues in that receptor (reproduced from O'Malley, 1990)

The amino-terminal region of the receptor proteins is a hypervariable region having low homology between receptors. The epitopes of most antibodies raised against steroid receptors are located in this region. It has been suggested that this region may contribute to the diversity of receptor specificity.

1.4.2 Corticosteroid receptors

In the target tissues, corticosteroid action is mediated by two distinct intracellular (cytoplasmic) receptors, currently referred to as the Type I or mineralocorticoid receptor (MR) which shows aldosterone selectivity, and the Type II or glucocorticoid receptor (GR) which shows selectivity for cortisol in human subjects and corticosterone in the rat. The classification of corticosteroid receptors into subtypes has been made possible, in part, by the synthesis of highly selective ligands. Dexamethasone and triamcinolone acetonide are high-affinity GR binders but the most recent synthetic corticosteroids, RU26988, RU28362, and RU38486, have higher specificity and affinity for GR. Steroids such as 9α-fluorocortisol and 19nor-progesterone are mineralocorticoid agonists, whilst spironolactone, ZK91587 and RU26752 are mineralocorticoid antagonists.

Mineralocorticoid receptors

Mineralocorticoid receptor sites may be characterised on the basis of the hierarchy of binding affinity shown for the different steroids.

Aldosterone > DOC > Corticosterone > Cortisol >Dexamethasone

Using different experimental approaches (biochemical studies, topography, and molecular biology), the high affinity aldosterone binding sites have been detected not only in classical epithelial target tissues such as kidney tubules (Lan et al., 1982; Grekin & Sider, 1980; Marver et al.,

1972) but also in non-epithelial tissues such as vascular smooth muscle cells (Meyer & Nichols, 1981; Kornel, 1993), septo-hippocampal region (Lowy, 1991; Reul et al., 1985; Reul et al., 1990), mammary gland (Quirk et al., 1983) and mononuclear leukocytes (Armanini et al., 1985). Binding affinities approximate to the physiological concentration of plasma aldosterone with dissociation constant (Kd) around 10^{-9} mol/l (Lan et al., 1982; Armanini et al., 1985) (for years the mineralocorticoid receptor was thought to have a higher Kd for glucocorticoids, see below).

The mineralocorticoid effector mechanism involves the binding of aldosterone to cytoplasmic receptor and the transcription of mRNA from which aldosterone-induced proteins (AIPs) are translated. It has been reported that the AIPs might act by increasing the permeability of the apical membrane to sodium, so that passive entry of sodium into the cells is enhanced and intracellular sodium concentration rises (Bastl & Sebastian, 1987). This then activates and/or induces the activity of the basolateral membrane Na^+/K^+ ATPase ('the sodium pump') (Garty, 1986) and also stimulates ATP synthesis to drive the pump (Law & Edelman, 1978).

However, it has recently been reported that a rapid increase of sodium flux in response to aldosterone may reflect a non-genomic, possibly a membrane action of aldosterone (Wehling et al., 1992a). Wehling et al. (1991a) demonstrated a high affinity binding site for aldosterone, but not cortisol and canrenone, in plasma membrane rich fractions from human mononuclear leukocytes with a calculated Kd for aldosterone of 0.04 nM. These data suggested a two-step model for mineralocorticoid action; firstly aldosterone binds to membrane receptors which trigger changes in membrane electrolyte transports systems within minutes (immediate activation of pre-existing Na^+/K^+ ATPase and sodium-proton exchanger) and secondly, binding to the cytoplasmic receptor initiating genomic mechanisms within hours/days (de-novo-synthesis of Na^+/K^+ ATPase molecules, etc.) (Wehling et al., 1992a).

Glucocorticoid receptors

The cloning of the human glucocorticoid receptor complementary DNA (cDNA) provided details of the receptor structure (Hollenberg et al., 1985). The GR gene is located on chromosome 5 and consists of 10 different exons (Encio & Detera-Wadleigh, 1991). Exon 1 is an untranslated region, whereas exons 2-9 are the coding regions. The cDNA encodes two types of proteins: 777-amino acid protein (α-glucocorticoid receptor) and 742-amino acid protein (β-glucocorticoid receptor). These proteins differ in their codons for the carboxy-terminal amino acids (50 for α and 15 for β) and 3'-untranslated regions. Figure 7 shows the human glucocorticoid receptor gene structure. The α form is more prevalent and binds steroid when expressed *in vitro* with the use of α-cDNA, whereas the β forms is less well represented, does not bind glucocorticoid when expressed *in vitro* and its function is unknown at the present .

Glucocorticoid receptors have been demonstrated in a multitude of organs in many species (e.g., kidney, thymus, adipose tissue, central nervous system, vascular smooth muscle cells, endothelium, etc.) (Feldman et al., 1978). Binding affinities fall within the normal range of plasma concentrations of free hormone (Kd around 30 nM) and are 5 to 10 times higher for synthetic glucocorticoids such as deaxamethasone and triamcinolone acetonide. In a series of competitive binding assays, the sequence of potency for Type II receptor in the rat was:

Dexamethasone > Corticosterone > Cortisol = aldosterone

Numerous data on binding characteristics of steroid hormones suggest that steroid hormone receptors contain a single binding site for their respective ligands (Baxter & Funder, 1979). A more complex receptor-ligand interaction may also exist. Using a synthetic steroid such as cortivazol (11α, 21-trihydroxy-6-16α-dimethyl-2'-phenyl-2'H-pregna-2,4,6-trieno [3,2-c]pyrazol-20-one 21 acetate) two glucocorticoid binding

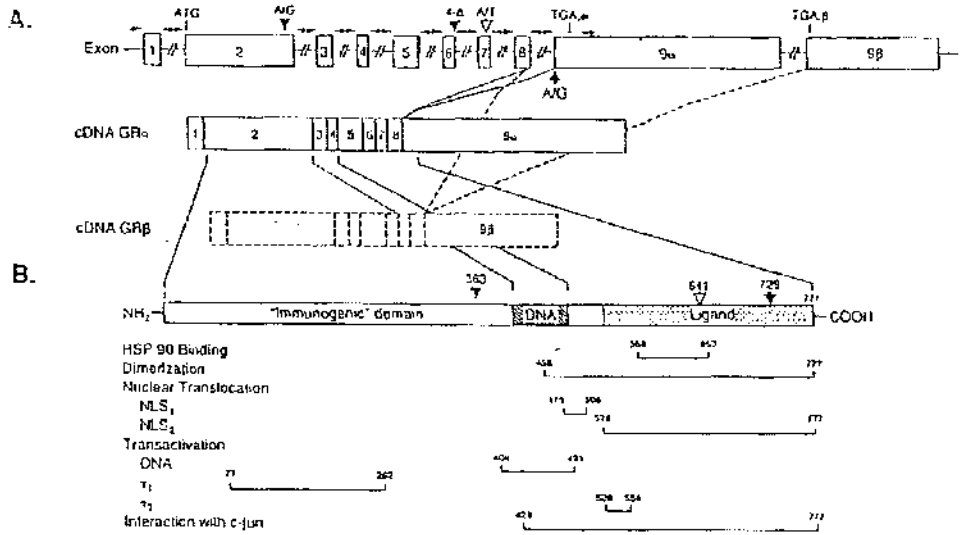

Fig.7: Schematic representation of genomic and complementary DNA and protein structure of the human glucocorticoid receptor. A) The gene consists of 10 exons. Exon 1 is untranslated, exon 2 codes tor the immunogenic domain, exon 3 and 4 for DNA binding and exon 5-9α for the ligand binding domain. GRβ does not bind to glucocorticoid. B) Representation of the 3 function domain in a linear model. Numbers correspond to amino acids in the primary sequence of the receptor. NLS, nuclear localization sequence (reproduced from Karl et al., 1993).

sites on the human GR were identified in human leukaemic T cell line CEM C7 (Schlechte et al, 1985). The binding studies using [^{3}H]-cortivazol showed a high (0.4 nM) and low (11 nM) affinity component with receptor concentration of 0.14 pM/mg protein and 0.30 pM/mg protein respectively and this compared to a single class of binding sites using [^{3}H]-dexamethasone with Kd 1.9 nM and receptor concentration of 0.46 pM/mg protein. Moreover competition studies of [^{3}H]-cortivazol with unlabelled dexamethasone showed that dexamethasone binds only to the low affinity site detected by the labelled ligand. However, the physiological importance of this finding is still in debate.

Although the cascade of events which leads to glucocorticoid induction of gene expression is more or less as reported above (refer to section 1.4.1), detailed studies of the GR identified a system of considerable complexity. The ligand-free glucocorticoid receptor is present in the cytosol as a 9 S complex with a molecular mass of 310 kDa (Muller & Renkawitz, 1991; Munck et al., 1990). The activated GR has a coefficient of sedimentation of 3.2 S (4 S form) with molecular mass of 90 kDa. Thus, it appears that the receptor activation involves the dissociation of a large multiprotein complex. Using antibodies against the GR, it was concluded that the 9S form contains only one receptor molecule and is a heteromer composed of one hormone binding GR and two non-hormone-binding proteins of 90 kDa (Smith & Toft, 1993). This associated protein has been identified as the 90 kDa heat shock protein (Hsp90) (Joab et al., 1984; Sanchez et al., 1985). Detailed analysis of the 9S complex showed that in addition to Hsp90, several other protein subunits are associated with the non-activated receptor such as Hsp56 (Tai et al., 1986; Rexin et al., 1992) and Hsp70 (Pratt, 1990) as well as the smaller peptide p23 (Pratt, 1993). Hsp90 plays a biologically important role in GR activity, stabilising the complex in the cytosol. The loss of Hsp90 by heat activation decreases the affinity for the ligand of approximately 100 times compared to the heterocomplex GR-Hsp90

(Nemoto et al., 1990a). Mutational analysis showed that deletion of amino acids 532-697, comprising two-thirds of the hormone binding domain, completely abolished the formation of 9S complex (i.e., Hsp90 binding) with consequent impairment of hormone binding (Pratt et al., 1988). It has been suggested that in the inactivated state, the GR is associated with two Hsp90 molecules which cover the DNA-binding domain. Dissociation from Hsp90 exposes the DNA-binding site on the GR (Carlstedt-Duke, 1988). The stoichiometry of the complex is one GR, two Hsp90, one Hsp70 and one Hsp59 (Rexin et al., 1991; Rexin et al., 1992).

The Hps90 is an ubiquitous, highly conserved protein (Lindquist & Craig, 1988) that is bound to all the steroid receptors and to the dioxin receptor (Pratt, 1990), and it is thought to perform a general chaperone function in the cell (Wiech et al., 1992; Hendrick & Hartl, 1993). The association of Hsp90 with steroid receptors is stabilized by molybdate, vandate and tungstate (Leach et al, 1979; Pratt, 1987; Pratt, 1990; Rexin et al., 1991). Hsp90 possesses ATPase activity and undergoes autophosphorylation (Nadeau et al., 1993). It has been suggested that the receptor is normally phosphorylated and that lack of hormone binding activity may be due to a dephosphorylated receptor (Munck et al., 1972; Munck et al., 1990). In cell-free assembly experiments, the GR heterocomplex formation was temperature- and ATP-dependent and appeared to be directed by an enzymatic process (Scherrer et al., 1990).

The glucocorticoid receptor bound to the cognate ligand, and dissociated from the Hsp, assembles to form a homodimer (Erikson et al., 1991; Wrange et al., 1989). This complex translocates into the nucleus by specific transport via the nuclear pore (Picard & Yamamoto, 1987). Inside the nucleus, ligand-bound GR exerts its genomic effects by binding to specific glucocorticoid responsive elements (GREs) on the chromatin by the Zn-fingers exposed in the DNA-binding domain (Miesfled, 1990; Freedman, 1992; Freedman & Luisi, 1993) (refer to Fig. 8). The GREs are in the vicinity of the regulated genes and are composed of a palindromic

sequence that consists of two hexameric half-sites separated by three bases. A functional concensus which imparts glucocorticoid-inducible gene expression is: 5'-GGTACAnnnTGTTCT-3' (n can be any nucleotide) (Beato, 1989; Carson-Jurica et al., 1990; Fuller, 1991; Williams & Franklin, 1994). Once the homodimer is bound to its responsive element, it can couple with another dimer or transcription factor. Different arrangements and composition of binding sites for transcription factors and GR within a regulatory unit lead to different activities in gene expression: synergistic and non-synergistic, associated to induction or repression, respectively (Miesfeld, 1990; Muller & Renkawitz, 1991; King, 1992) (refer to Fig. 9). In table V are summarized the genes whose transcription is under glucocorticoid modulation.

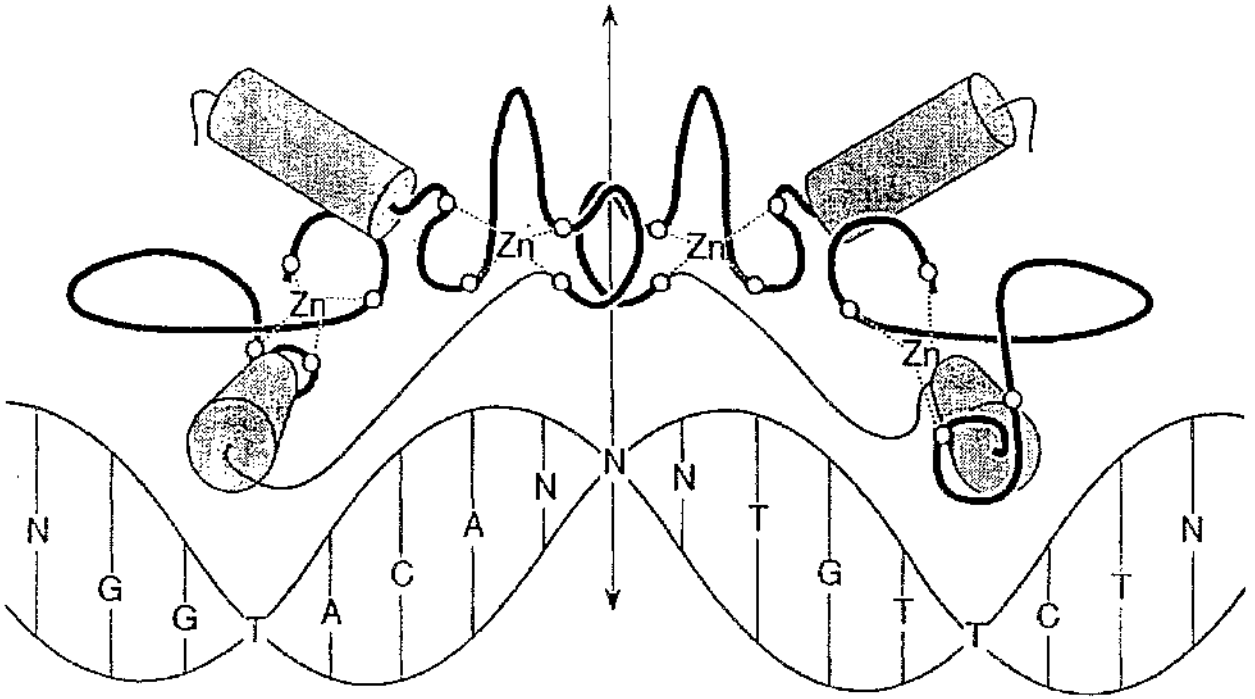

Fig. 8: Schematic model of the dimeric DNA binding domain bound to DNA. On top of the DNA receptor-binding sequence is represented the glucocorticoid receptor DNA-binding domain consisting of two 'zinc-finger' regions and two α-helical regions. It appears that one of the two α-helices within a receptor monomer serves as a recognition helix located in the major groove of the DNA (reproduced from Carson-Jurica et al., 1990).

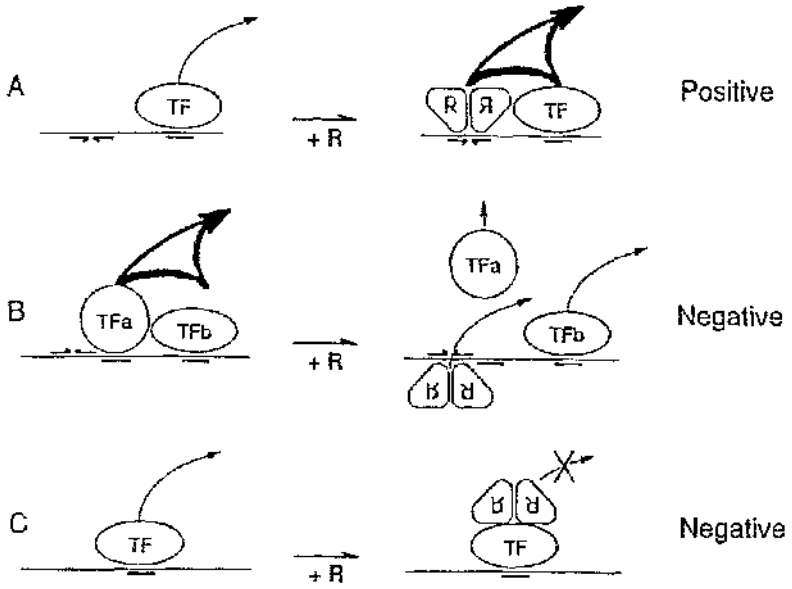

Fig. 9: Positive and negative transcriptional effects of the receptor. A) Binding of the receptor dimer immediately adjacent to a transcription factor leads to synergistic activation, the strength of transcriptional activation is indicated by the strength of the curve arrows, synergism by combined arrows. B) binding of the receptor to the GRE displaces a positive transcription factor from a synergizing complex of two transcription factors (TFa and TFb) and thereby represses gene activity; the remaining receptor dimer and TFb are bound in a non-synergistic arrangement. C) Binding of the receptor to a positive transcription factor via protein-protein interaction blocks the activation signal and thereby represses transcription (reproduced from Muller & Renkawitz, 1991)

Table V: Genes whose transcription is modulated by glucocorticoid. The proteins of the genes in bold might have physiopathological role in essential hypertension.

Glucocorticoid modulated genes

Positively	**Negatively**
ACE (Mendelson et al., 1982)	**Insulin**
ANP (Seidman et al., 1984; Gardner et al., 1986)	Lysozyme and other protein of the complement cascade (Lappin & Whaley, 1991)
Angiotensinogen (Ben-Ari et al., 1989; Clauser et al., 1989; Feldmer et al., 1991)	**Prostaglandin and prostacyclin** (Grunfeld et al., 1986)
Prorenin and renin (Morris et al., 1984; Glorioso et al., 1989)	**Glucocorticoid receptor** (Burnstein et al., 1991)
Angiotensin II type 1 receptor (Sato et al., 1994)	**Nitric oxide** (inducible) (Radomski et al., 1990)
Insulin receptor (McDonald & Golfine, 1988)	Prolactin (Whorwood et al., 1993)
Pepsinogen (Ichinose et al., 1990	
Prepro-Neuropeptide Y receptor (Larsen et al., 1994)	
11β-Hydroxysteroid dehydrogenase (Tannin et al., 1991; Stewart, 1994)	

It is also noteworthy that glucocorticoids alter the stability of specific mRNAs, an important function which has been observed with several genes, by an unknown mechanism (Kern et al., 1988; Boggaram et al., 1991). It has been suggested that the ligand-bound glucocorticoid receptor alters the transcription of proteins that regulate the stability of these mRNAs.

Because the presence and the quantity of GR in target cells correlates with the extent of hormonal responsiveness, this raises the possibility that mechanisms which regulate GR are pivotal in the control of hormone action. Endogenous glucocorticoid in patients suffering from Cushing's syndrome (Pardes et al., 1989) and administration of glucocorticoid to patients (eg., suffering from acute and chronic disorders) (Shipman et al., 1983), normal subjects (Schlechte et al., 1982) and rats (Antakly et al., 1990; Kalinyak et al., 1987) decreases GR activity in target cells and tissues while adrenalectomy increases GR mRNA and protein expression (Kalinyak et al., 1987; Reul et al., 1989). However, in the rat neither adrenalectomy nor administration of corticosterone significantly altered GR mRNA levels in the hypothalamus (Peiffer et al., 1991). Lowy (1991), studying the GR binding characteristic in adrenalectomized rats, showed an increased binding capacity in the hippocampus, frontal cortex and hypothalamus, but not in thymus, spleen, lymphocytes and pituitary gland. These observations suggested that firstly, GR regulation is part of a negative feedback loop partially controlled by glucocorticoid concentration and secondly, the autoregulation of the GR is likely to be tissue specific. Denton et al. (1993) comparing a glucocorticoid-sensitive (dex^s) human T-cell line 6TG1.1 with a glucocorticoid-resistant (dex^r) activation-labile cell line 3R7.6TG.4 and a dex^r cell line CEM-C1, demonstrated differential cell-specific autoregulation of human GR expression.

Dong et al. (1988), using hepatoma tissue cell culture and rat liver *in vivo*, showed a complex GR autoregulation which occurs at both

transcriptional and post-translational levels. No effect of glucocorticoids on GR mRNA half-life in hepatoma tissue culture cells (determined to be 4.5h both in presence and absence of 0.5ug of dexamethasone) was observed but GR protein half-life was reduced after steroid treatment (25h in absence of dexamethasone and 11h in presence of hormone). It appears then that the primary mechanism of down-regulation of receptor protein is at the level of mRNA. Since a negative autoregulation of GR mRNA expression has been demonstrated, there may be a post-translational regulation too. Burstein et al. (1991) suggested that the transcriptional response of the GR gene to glucocorticoid may occur via the interaction of GR with its own gene. They observed firstly that the human GR cDNA contains multiple copies of the original GRE TGTTCT and secondly that human GR cDNA shifted the sedimentation position of the receptor on sucrose density gradient (Tully & Cidlowski, 1990).

1.4.3 Corticosteroid receptors specificity

In vitro studies showed that corticosteroids bind to some extent to both receptor types but usually prefer one or the other. Very few steroids are absolutely specific in their effects: glucocorticoids such as cortisol may show mineralocorticoid activity and aldosterone potentially has glucocorticoid activity albeit at unphysiological high concentrations. A lack of specificity is indicated in Cushing's syndrome, where it has been suggested that the high plasma cortisol level can lead to hypertension via Type I receptors (Fraser et al., 1989). Several factors govern physiological tissue-response specificity:

a) Circulating levels of steroids. Aldosterone-mediated glucocorticoid activity is very unlikely because plasma aldosterone level is 2 to 3 orders of magnitude lower than that of cortisol. It follows, however, that cortisol may compete for type I mineralocorticoid receptors. The steroidal biological activity is held by the free rather than the protein-bound steroid which leaves the circulation and binds to the cellular

receptor. This presumably depends on the relative affinity of the different corticosteroids for albumin and transcortin (for review see Pardridge, 1987). In practice, the levels of free cortisol and corticosterone approximate to 1% of total circulating levels. However, the hypothesis of mineralocorticoid specificity conferred by the cortisol binding globulin (Funder et al., 1973; Stephenson et al., 1984) is not tenable since this specificity was retained in 10-days-old rats deficient in this protein (Sheppard & Funder, 1987a).

b) Receptor affinity (Baxter & Funder, 1979). Receptors have the ability to discriminate signal from noise. Therefore, they must have an appropriately lower affinity for potentially misleading signals (i.e., specificity). Hormonal receptor affinity is due to non-covalent binding, which involves electrostatic interactions including hydrogen binding, hydrophobic interactions and van de Waals forces. The formation of the hormone receptor complex is the rate-limiting step. The binding of hormone (H) and receptor (R) can be simply represented:

$$H+R \underset{k2}{\overset{k1}{\rightleftharpoons}} HR$$

where HR is the hormone-receptor complex, and *k1* and *k2* are the two kinetic constants. The equilibrium constants, *k1/k2* and *k2/k1* are designated association constant (Ka) and dissociation constant (Kd), respectively. Kd is, in practical terms, the concentration of free hormone required to give half maximal binding of hormone in any given system and is therefore a valuable expression of the avidity with which the receptor binds the hormone.

It has been shown that the ligand-binding domain region is conserved for a given type of ligand within the steroid receptor 'superfamily' but not conserved between receptor types. This fits both with the ligand being chemically unrelated and with the lack of cross-over binding between members of the group of steroids receptor 'superfamily' (Fuller, 1991). It has been suggested that the presence of particular residues in the binding domain is an important determinant of specificity.

Cysteine 638 and aspartate 641 in the GR lie within a relatively hydrophobic region which suggests that this region is a likely site of receptor ligand interaction (Carlstedt-Duke et al., 1988). Mutations at these residues are associated with reduced affinity for cortisol and decreased binding specificity (see earlier). Moreover, *in vitro* mutation of cysteine 656 of the rat GR causes an increased affinity for GR and a decrease in the relative affinity for cross-reacting steroids such as progesterone and aldosterone. Such a mutation has not been shown to occur in nature (Chakraborti et al., 1991). The proximity of two other cysteines to cysteine 638 is unique to the GR and it could be that these cysteine residues in GR play an important specificity-conferring role (Lopez et al, 1990).

Finally, although aldosterone and glucocorticoids show equivalent affinity for the mineralocorticoid receptor, Lombes et al. (1994) reported striking differences in the dissociation constants of aldosterone- and glucocorticoid-mineralocorticoid receptor complexes, as determined by direct *in vitro* binding assay. This would suggest that the preference of Type I receptor for aldosterone is an intrinsic feature of the receptor molecule.

c) Modulators. There is evidence that a variety of small, heat stable molecules may modulate receptor activity. Sumida et al. (1993) found that long chain polyunsaturated fatty acids decrease the affinity of the rat liver receptor for dexamethasone and suggest that they may act by strengthening the interaction between Hsp90 and the receptor protein. Interestingly, the binding of the glucocorticoid antagonist, RU486 is less affected, possibly suggesting that its interaction with the hormone binding domain is slightly different from glucocorticoid agonists. Two other modulators, characterised as amino-ether phosphoglycerides, have been identified which stabilise - i.e. reduce affinity for ligand - the receptor (Bodine & Litwack, 1988; Bodine & litwack, 1990). Their precise mode of interaction with the receptor protein has not yet been determined.

d) 11β-hydroxysteroid dehydrogenase (11β-HSD). *In vivo* studies of glucocorticoid binding in rat tissues involved with the transport of sodium showed that kidney, parotid and colon were "aldosterone-selective" and bound aldosterone in preference to corticosterone, whereas hippocampus and heart were "non-selective" and did not distinguish aldosterone and corticosterone (Sheppard & Funder, 1987a, b). Moreover, the *in vitro* expression of the cloned mineralocorticoid receptor showed identical binding affinity for aldosterone and cortisol (Arriza et al., 1987). The matter was intriguing and an explanation came from the clinical observation that cortisol acts as mineralocorticoid in both congenital and acquired deficiency of 11β-HSD (Edwards et al., 1988; Fraser, 1990; Stewart & Whorwood, 1992). As already mentioned, 11β-HSD catalyses the conversion of cortisol to its inactive metabolite cortisone in humans and corticosterone to 11-dehydrocorticosterone in rats. These 11-oxo-derivatives do not bind to the mineralocorticoid receptors, with the result that both cortisol and corticosterone are prevented from expressing their mineralocorticoid effect. This observation led to the proposal that 11β-HSD is normally responsible for protecting the type I receptor from exposure to cortisol in aldosterone-selective tissue such as the kidney (Edwards & Stewart, 1991; Monder, 1991). Thus, the target tissue specificity appears to be enzyme- rather than receptor-mediated, indicating that 11β-HSD is necessary to protect mineralocorticoid receptor from normally higher serum concentration of cortisol by its local inactivation, allowing aldosterone to regulate sodium homeostasis (Funder et al., 1988; Morris & Souness, 1992; Pearce, 1994). By contrast, aldosterone is protected from the enzyme action because of its 11-18 hemi-acetal structure.

Two types of 11β-HSD have been identified. The type 1 enzyme is mainly found in the liver, has an apparent molecular weight of 34000, requires NADP as co-factor and shows both reductase and dehydrogenase activity. It has also been shown that the NADP:NADPH ratio in the liver

favours reductase activity with conversion of cortisone to cortisol. The type 2 enzyme is found in kidney and placenta, has a molecular weight of approximately 40000, requires NAD as co-factor and converts cortisol to cortisone (dehydrogenase activity) exhibiting little or no reductase activity (Stewart, 1994).

11β-HSD is present in most tissues but its specific functions in the majority of these are unknown. In addition to the kidney, its actions have been studied in testis and brain. Depletion of cortisol by 11β-HSD may initiate testosterone production at puberty and affect testosterone production during adult life (testosterone synthesis is suppressed by glucocorticoids). Co-localization of 11β-HSD and glucocorticoid receptors in the brain may be important in controlling the specificity of corticosteroid interaction with GR and MR.

1.4.4 Can glucocorticoid receptor abnormalities cause hypertension?

Metabolic abormalities

Variation in 11β-HSD activity, as discussed above, could represent a novel mechanism in the pathogenesis of essential hypertension (Stewart & Edwards, 1991; Walker, 1993; Seckl & Brown, 1994). In a proportion of patients suffering from essential hypertension, it is possible that excessive activation of renal mineralocorticoid receptor occurs, since plasma renin activity is low in 20-30%. However, neither mineralocorticoid nor glucocorticoid is found in excess and it has been concluded that any abnormality of adrenal steroid secretion is not of sufficient magnitude to explain the rise in blood pressure. However, abnormally high sensitivity to cortisol might provide an explanation. Support for this idea comes from two observations:

a) administration of dexamethasone produced a paradoxical fall in blood pressure in a subgroup of essential hypertensive patients, possibly implicating cortisol or another ACTH-dependent steroid in pathogenesis

(Hamilton et al., 1979; Whithworth er al., 1989);

b) young adults at increased risk of hypertension have slightly but significantly higher levels of cortisol than controls (Watt et al., 1992);

c) subnormal activity of 11β-HSD in the presence of higher tissue steroid levels could be a possible hypertensinogenic mechanism. Data from several groups support the conclusion that 11β-HSD is impaired in some hypertensive patients (Walker et al., 1993; Kornel & Margolis, 1992; Soro et al., 1995).

However, the observation of defective 11β-HSD in essential hypertension could be relevant only if it can be linked with a mechanism which explains the rise of blood pressure. Thus, it would be reasonable to expect to find suppression of renin and hypokalemia and prolonged half life for [11α^{3}H]-cortisol (the half life of this compound is the most sensitive technique available to study the 11β-HSD activity *in vivo*) in essential hypertension as occurs in patients with congenital and acquired 11β-HSD deficiency. Because those abnormalities were not found, Walker et al. (1991) proposed that the rise in blood pressure in essential hypertension is due to defective activity of 11β-HSD in modulating corticosteroid actions on vascular tone at local sites in blood vessels. This hypothesis involves modulation of steroid access to the receptor rather than an abnormality in the receptor itself.

Receptor abnormalities

There are clinical situations in which abnormal glucocorticoid activity due to mutations in the glucocorticoid receptor gene causes high blood pressure. The syndrome of glucocorticoid resistance in man and animals is well-documented (Brandon et al., 1991). In man, impairment at the glucocorticoid receptor level is compensated by increased ACTH drive in an attempt to raise plasma cortisol concentration to an effective level (Chrousos & Karl, 1992; Arai & Chrousos, 1994). This is obviously due to an impairment of the negative feed-back mechanism by

glucocorticoid on the hypothalamus and anterior pituitary gland. The syndrome frequently includes hypertension with a hypokalaemic alkalosis characteristic of excess mineralocorticoid activity which has usually been attributed to abnormally high secretion of the ACTH-dependent, minor mineralocorticoid, DOC (Vecsei et al., 1989; Lamberts et al., 1992). An alternative explanation is that the increased mineralocorticoid activity might also be due partially to high cortisol concentrations interacting with Type I receptors (see fig. 10). Thus, it is likely that the higher local cortisol concentrations overide the 11β-HSD protective activity on Type I receptor (see earlier). Chrousos & Karl (1992) have listed the various biochemical and molecular abnormalities which cause glucocorticoid resistance. These include reduced receptor concentration, usually caused by nucleotide sequence deletions of varying length, and reduced receptor affinity caused by point mutations at or near the steroid binding domain. Werner et al. (1992) analysed 7 cases of glucocorticoid insensitivity in some detail. In addition to the affinity and concentration changes already described, they also found instances of increased thermolability of the receptor associated with an abnormally high rate of glucocorticoid receptor gene expression. Interestingly, a thermolabile glucocorticoid receptor was the only biochemical alteration reported in one patient whose only symptom was fatigue (Bronnegard et al., 1986). Mutations which alter the binding of the hormone-receptor complex to DNA have also been identified although in nonhuman primates rather than man (Brandon et al., 1991). Here the crucial change is the spacing between zinc fingers. The consequences for blood pressure control were not discussed. Adcock et al. (1993) studying a group of asthmatic patients insensitive to glucocorticoid treatment, reported that the ability of GR to bind to GRE and with other transcription

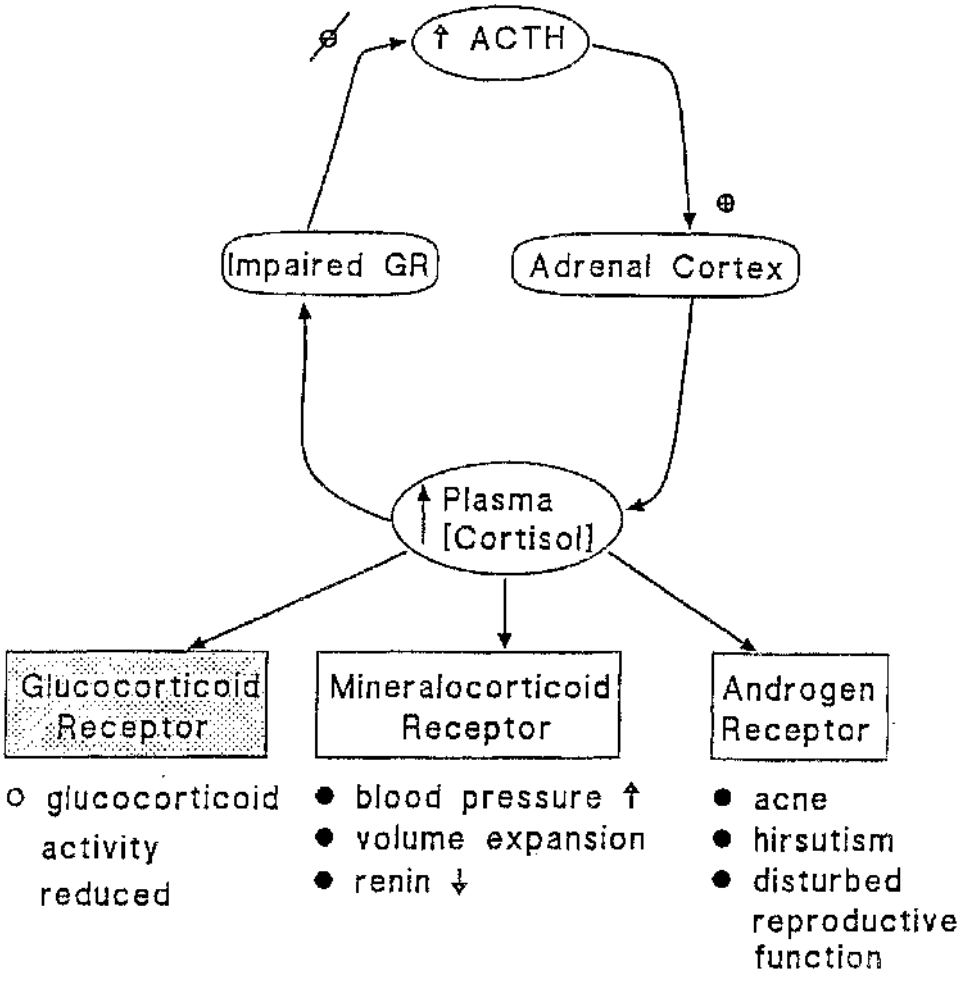

Fig. 10: Side effects of end-organ resistance to cortisol.

factors may be impaired in steroid-resistant patients. This study did not discuss blood pressure effects.

A stimulating observation came from the analysis of data obtained in the epidemiological study of Watt et al. (1992). Various genotypic and phenotypic variables were measured in a group of young subjects. Results were analysed on the basis of (i) whether or not their parents were hypertensive and (ii) where in their peer group range their personal blood pressure came. This "four corner" design revealed a tendency for offspring of higher blood pressure parents to have higher personal levels of blood pressure. Associated with this trend was the additional tendency of higher blood pressure in young subjects with hypertensive parents to have higher plasma cortisol concentrations. The difference was small but significant. In the same study, glucocorticoid receptor RFLP genotype was tested. The results strongly suggested a more frequent occurrence of one genotype (AA) in the higher blood pressure offspring of high blood pressure parents, although the size of the study precluded the result achieving conventional statistical significance. However, when results from all 4 groups were pooled, young subjects with the AA genotype had significantly higher blood pressure scores than those with the aa genotype (Kenyon et al., 1993). The results are presented in Fig. 11. The coexistence of increased cortisol levels and a possibly different receptor in the susceptible group could be interpreted in different ways. If the AA genotype is a marker for GR impairment then, as with primary cortisol resistance, hypertension results from excess mineralocorticoid activity. Against this interpretation is the lack of effect on plasma renin and potassium. If on the other hand, the AA genotype signifies increased affinity then increased receptor binding together with raised plasma cortisol should cause a glucocorticoid-type hypertension. This is in part supported from the finding of increased angiotensinogen. A third possibility is that RFLP is in close linkage dysequilibrium with a gene other than GR which is important in blood pressure regulation.

In this thesis, an experimental approach to the testing of these alternatives will be described. The possibility that abnormalities in type II corticosteroid receptor properties are contributory factors in two genetic models of hypertension will be discussed.

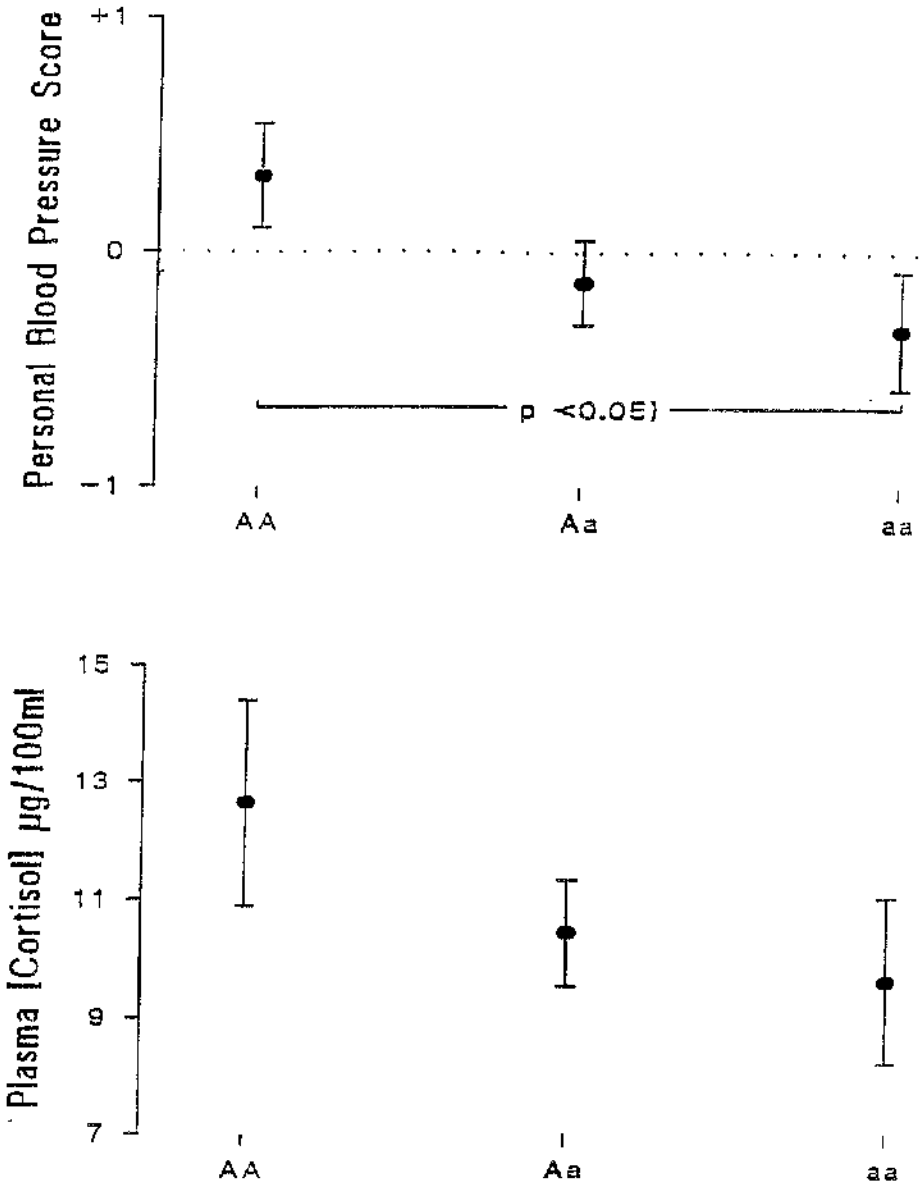

Fig. 11: Blood pressure and plasma cortisol in relation to the GR RFLP genotypes (reproduced from Kenyon et al., 1993).

1.5 SUMMARY AND AIM OF THE EXPERIMENTAL STUDIES

Current evidence suggests a familial component in the development of hypertension but the precise genetic abnormalities, obscured by interaction with many environmental influences, remain unknown. It seems likely that several rather than a single gene locus are involved and that among the systems affected are renal, ion transport mechanisms and a variety of neuroendocrine and endocrine functions. Of the endocrine influences, corticosteroid hormones, as well as being hypertensinogenic themselves, have the propensity to potentiate other cardiovascular control elements. Although various mechanisms link corticosteroids to the cardiovascular system, it is not known which one, if any, is of overriding importance in blood pressure control. Both mineralocorticoid and glucocorticoids acting in different ways are key factors in blood pressure control. The activity of the adrenal cortex and the way in which its steroid products interact with their target tissues, may vary in a genetically-determined manner. It also appears that some of the cardiovascular influences are expressed at concentrations of glucocorticoid hormones which are less than those required to produce symptoms of Cushing's syndrome.

Endogenous glucocorticoids, cortisol in man and corticosterone in rats, can bind to two types of receptors. The Type I or mineralocorticoid receptor evokes effects on salt and water metabolism, the Type II or glucocorticoid receptor is conventionally linked to effects on intermediary metabolism, though they can also cause changes in electrolyte metabolism. Normally, access of cortisol and corticosterone to Type I receptors is prevented by the activity of the 11-OHSD which converts cortisol to cortisone and corticosterone to 11-dehydrocorticosterone. It is important to note that an increase in blood pressure may be due to enhanced glucocorticoid activity either via Type I or Type II receptors. Moreover, the Ladywell study suggested that plasma cortisol levels may be significantly higher and that the

glucocorticoid receptor may be different in human subjects with a familial predisposition to hypertension.

The purpose of this thesis has been to investigate whether variations of Type II receptor function are associated with variations in blood pressure.

Do the different GR RFLP genotypes, AA and aa, represent receptors with different binding efficiency and is GR activity a significant factor in genetic hypertension?

Chapter 2 **MATERIALS AND METHODS**

2.1 MATERIALS AND GENERAL METHODS

2.1.1 Chemicals

Reagents were obtained from the following sources:

- Fisons, UK

Na_2HPO_4, NaH_2PO_4, NaCl, Na Citrate, NaOH, MgCl.

- Amersham International, Buckingham, UK

[1,2,4^{3}H]-dexamethasone (1.35 Tbq/mmol)

- Sigma Chemical Co, Poole, Dorset, UK

Unlabelled dexamethasone, estradiol, progesterone, dehydroepiandrosterone (DHA), cortisol, aldosterone, human recombinant lysozyme, Micrococcus *lysodeikticus*, polyethylenimine (PEI), crystal violet, cynogen bromide, sodium molybdate, EDTA, ammonium cloride, Tris/Hcl, Triton x100, proteinase K.

- Flow Laboratories, Rickmansworth, Herts, UK

Ficol-Hypaque (lymphocytes separation medium, 1077 g/l), RPMI-1640, GF/C filter paper.

- Gibco BRL, Paisley, UK

Foetal-calf serum (FCS), glutamine, penicillin, streptomycin, 96-well culture plates.

- ICN Biochemicals LTD, Immuno-biologicals Division, High Wycombe, UK

Bovine serum albumin (BSA).

- Farmachem, Strathleven, Scotland, UK

D-glucose, $NaHCO_3$ (analytical grades).

- Diagnostic Products Corporation, Los Angeles, CA, USA

Coat-A-Count cortisol and aldosterone kits.

- Northumbria Biological Ltd., Cramlington, UK

Bcl I restriction enzyme.

- Merck Ltd, Hunter Boulevard, Magna Park, Lutterworth, Leicestershire, U.K.

Acetic acid, chloroform, dipotassium hydrogen orthophosphate, ethanol, glycerol, potassium dihydrogen orthophosphate, sodium dodecyl sulphate (SDS).

- Astra Pharmaceutical, Sweeden

Budesonide.

2.1.2 Equipment

- β-counter, LKB Wallac 1217, Rackbeta, LKB-Produckter, AB, Bromma, Sweeden.

- IEC Centra 8R Centrifuge, Damon/IEC division, Needham HTS, Massachussets, USA.

- γ-counter, LKB or LKB 1280, LKB-Produkter as above.

- Polytron homogenizer, Kinematic, Lucerne, Switzerland.

- Microtitretek, Anachem, Luton, UK

- Spectrophotometer, LKB, LKB-Producter as above.

2.1.3 Buffers

- Phosphate-buffered saline (PBS): 0.85% NaCl and 6.7 mM sodium phosphate, pH 7.2

- Buffer A: 10 mM Tris-HCl, 2mM dithiothreitol, 1.5 mM EDTA, 0.1 M sodium molybdate, 10% (w/v) glycerol, pH 7.4

- Dextran-coated charcoal (DCC): 0.5% (w/v) of activated charcoal, 0.005% (w/v) dextran 770 in buffer A

- Sodium citrate solution: 3.9% Na citrate, pH 7.2

- Red cell lysing buffer: 0.155 M NH_4Cl, 10 mM K_2PO_4, KH_2PO_4 and 0.1 mM EDTA, pH 7.2

- White cell counting solution: 1% acetic acid, 0.01 crystal violet.

- Lysis mix: 0.32 M sucrose, 10 mM Tris, 5 mM MgCl, 1% Triton x100, pH 7.5

- Nuclei lysis buffer: 10 mM Tris, 0.4 M NaCl, 2 M EDTA, pH 8.2

- T.E. buffer: 10 mM Tris/HCl, 0.5 mM EDTA, pH 7.4
- 20X SSC: 3 M NaCl, 0.3 M Na citrate, pH 7.0

2.1.4 Other materials

- Male Sprague-Dawley (SD), Spontaneously hypertensive (SHR) and Wistar-Kyoto (WKY) rats (200-250 g) were from Harlan Olac Ltd, Bicester, Oxon, England, UK.
- Female Milan normotensive (MNS) and hypertensive (MHS) strains of rat (200-250 g) were from Prof. I.W. Henderson, Department of Zoology, University of Sheffield, Sheffield, England.
- RU486 was a gift from Roussel Laboratories, Paris, France.
- Glucocorticoid receptor c-DNA insert (OB7) was a gift from Prof. R. Evans, Salk Institute, La Jolla, CA, USA.

2.1.5 Protein measurement

The protein measurement was carried out by the method of Lowry et al. (1951).

2.1.6 Hormonal measurements

a) Serum and urinary cortisol and aldosterone concentrations were measured using Coat-A-Count kits. The methods are based on a solid phase ^{125}I-radioimmunoassay.

b) Urinary tetrahydrocortisol (THF), alloTHF and tetrahydrocortisone (THE) were kindly measured by Ms M. Ingram by gas chromatography-mass spectrometry analysis (after steroid extraction using the method of Shakleton et al. (1985) on a ITS40 mass spectrometer (Finnigan MAT, San Jose, Ca., USA) coupled to a Varian 3400 gas chromatograph (Varian Inc, Walnut Creek, Ca., USA).

The ratio of 11β-hydroxy- to 11-dehydro-metabolites was calculated using the following formula: THF + alloTHF/THE.

c) Plasma renin was kindly measured by Dr. J.J. Morton, MRC Blood

Pressure Unit, Western Infirmary, Glasgow, by direct radioimmunoassay of angiotensin I generated by sample incubation at 37^0.

2.1.7 Routine measurements

Routine serum and urine measurements were carried out at Clinical Biochemistry Department, Western Infirmary, Glasgow. The tests and respective methods are as described in table VI. Creatinine clearance was calculated from the following formula:

milliters of plasma cleared per minute: Ucr x V / Pcr

where Ucr and Pcr are concentration of creatinine in urine and plasma, respectively, and V is volume of urine in milliliters per minutes.

Glomerular filtration rate (GFR) was estimated using the serum creatinine concentration alone. If the endogenous production of creatinine remains constant then serum creatinine will be inversely proportional to GFR, therefore:

GFR (ml/min) = 1.2 [140-age (years)] x weight (kg)/serum creatinine (μmol/l)

Routine haematological profiles were carried out at the Department of Haematology, Western Infirmary, Glasgow by cytoflurimetric method.

Table VI: Routine urine and serum measurement with respective method used.

Serum

Cholesterol	Salkowski reaction
Triglyceride	Enzymatic colorimetric method
Sodium	Ion-selective electrode
Potassium	Ion-selective electrode
Calcium	o-Cresolphthalein complexone reaction
Creatinine	Jaffe' reaction

Urine

Sodium	Flame photometry
Potassium	Flame photometry
Creatinine	Jaffe' reaction
Calcium	o-Cresolphthalein complexone reaction

2.1.8. DNA extraction

Total genomic DNA was extracted from white cells using a sodium dodecyl sulphate (SDS)/proteinase K and phenol extraction method as described by Miller et al. (1988). Ten ml of whole blood (anticoagulated with EDTA) were resuspended with 50 ml of 'lysis mix' and incubated for 5 min at 0^0. This was followed by 10 min centrifugation at 700g. The pellets were resuspended in 3 ml of nuclei lysis buffer. The cell lysates were digested overnight at 37^0 with 0.2ml of 10% SDS and 0.1 ml of protease K solution (10 mg/ml in 1% SDS and 2 mM EDTA). When digestion was complete, 1 ml of saturated NaCl (approximately 6 M) was added to each tube and shaken for 15 min. 5 ml of phenol/chloroform (1:1, v/v) were added and the tubes centrifuged for 10 min at 700g. The precipitated protein pellet was left at the bottom of the tube, whilst the supernatant containing the DNA was transferred to a clean tube. Exactly two volumes of 95% ethanol at room temperature were added and the tubes inverted several times until the DNA precipitated. The DNA was dried at room temperature and resuspended in 0.5 ml of T.E. buffer. The DNA was allowed to dissolve 2 h at 37^0 before quantitating.

The amount of DNA was measured by spectrophotometry. The DNA was diluted 1:100 in distilled water and the optical density (OD) read at 260 and 280 nm. The OD 260/280 ratio was calculated to verify the purity of DNA samples. For an acceptable purity the ratio must be between 1.7 and 2.

2.1.9 Genotyping analysis for glucocorticoid receptor polymorphism

DNA (10 μg) was digested using the restriction enzyme Bcl I which recognizes the sequence:

5'-T↓ G-A-T-C-A-3'

3'-A-C-T-A-G ↑ T-5'

and cleaves at the positions shown by the arrows (Bingham et al., 1978).

Restriction fragments were separated by electrophoresis on 0.8% agarose gels and transferred onto Hybond-N nylon membranes by Southern blotting (Southern, 1975; Wenham, 1992), and fixed by baking at 80^0 for at least 2 h. Radioactively labelled glucocorticoid receptor c-DNA (OB7) insert (Hollenberg et al., 1985; Murray et al., 1987) was hybridised to the membrane in 7% SDS/0.5 M phosphate buffer (pH 7.0) at 65^0 overnight. The non-specific binding was washed off in 2X standard saline citrate (SSC)in 0.1% SDS at 65^0. The membranes were exposed to Hyperfilm MP, with intensifying screens, for at least 3 days at -70^0.

2.1.10. Skin vasoconstriction test

The Stoughton-McKenzie vasoconstriction assay (McKenzie & Stoughton, 1962) as modified by Place et al. (1970) was used to determine the variability of the effect of the synthetic glucocorticoid, budesonide. Budesonide solutions were made up freshly in 95% ethanol at concentrations of 0.1, 3.9, 15.6, 62, 250, 1000 µg/ml in coded vials and were used within 24 h of preparation. 5 µl volumes were applied in random order to 7 x 7 mm squares outlined by silicone grease on the flexor aspect of the forearm. After it had been allowed to dry, the test area was covered with polyester film for 18 h. The degree of blanching was assessed after removal of the occlusive dressing and scored by an independent observer on a scale of 0=none, 1=mild, 2=definite, 3=intense blanching.

The dose-dependent curve was plotted for each individual and the median effective dose (EC50) for budesonide calculated.

2.2 METHOD DEVELOPMENTS

2.2.1 White blood cells separation

Glucocorticoid receptors are found in nearly all tissues (Feldman et al., 1978; McGimsey et al., 1991), including kidney, brain and circulating white blood cells. Leucocytes have frequently been used as a peripheral model to study GR regulation in clinical disease states associated with alterations in cortisol levels (Murakami et al., 1979; Schlechte et al., 1982; Pardes et al., 1989; Tanaka et al., 1991; Girardin et al., 1991). Leucocytes have the following advantages: a) ease of isolation, b) do not need to be cultured, c) maintenance of integrity and physiological properties of plasma membranes and nuclei, d) free from contaminating extracellular proteins.

For these reasons, white blood cells were used to test the hypothesis that glucocorticoid receptor binding characteristics are abnormal in hypertension.

Cell preparation

Peripheral blood obtained from fasting healthy volunteers was collected into citrate and platelet-rich plasma was removed by low speed centrifugation (600g for 5 min). The remaining cells were diluted 1:3 with PBS and fractionated on a Ficoll-Hypaque gradient according to the method of Boyum (1968). This separates mononuclear leucocytes (ML) at the top of the gradient from a mixture of polymorphonuclear cells (PMN) and red blood cells at the bottom. The layer of mononuclear cells was removed and washed three times with PBS at room temperature by centrifugation and resuspension to reduce platelet contamination. The final ML pellet was resuspended in RPMI-1640 medium containing 10% fetal calf serum, penicillin, streptomycin, and glutamine (unless differently stated). The ratio of mononuclear cells to platelets was 3:1 and

the ratio of lymphocytes to monocytes was 15:1. Monocytes monolayers from this suspension of ML were prepared in 96-well tissue culture plates and maintained at 37^0 in a humidified atmosphere of air with 5% CO_2. A purified population of lymphocytes was obtained by collecting those cells that had not adhered to the tissue culture plates after 90-min incubation. Lymphocyte suspensions were washed by centrifugation (400g for 10 min) and resuspension in medium before being transferred to 96-well plates.

PMN were obtained from the bottom of the Ficoll-Hypaque gradient; contaminating red cells were lysed as described by Murakami et al. (1978). The red cells were mixed with two parts of PBS. This suspension was then mixed with 8 volumes of chilled lysing buffer. After the colour of the red cell suspension had changed from bright red to dark red, the cell suspension was centrifuged at 450g for 10 min at 4^0. The supernatant was discarded and PMN were recovered. The recovered cells were washed three times by centrifugation (400g for 10 min) and resuspension in PBS. Finally, the PMN pellet was resuspended in RPMI and maintained in culture as described for monocytes and lymphocytes.

The viability of all cell types was consistently higher than 95%, as evaluated by trypan blue dye exclusion. There were no changes in cell number or viability during any incubation procedure.

2.2.2 Glucocorticoid receptor assay in mononuclear leucocytes

Introduction

Research on glucocorticoid receptor binding has been hindered by complicated laboratory protocols. In developing this method, two features were sought: a) to have an assay which allows ease of processing of a high number of samples, b) to reduce the volume of blood required. These requisites were partially satisfied by the whole-cell competitive binding assays reported in literature. However, previously published protocols had in common firstly, the need for large amount of expensive disposables apparatus and secondly, laborious washing steps to remove the unbound steroids from the incubation mixture. To avoid this a microtitre assay system has been developed which is a modification of the whole-cell competitive binding assay previously described by Schlechte et al. (1982).

Methods

a) *Cell preparation*

Human mononuclear leucocytes (HML) were prepared as described in section 2.2.1. After the washing stages, the cells were resuspended in RPMI-1640 (supplemented with 700 mg glutamine), to obtain a final concentration of approximately 3-5x10^7 cell/ml. Cells were diluted 1:20 with crystal violet solution and counted under an optical microscope using the 4 outer quadrants of a haemocytometer.

b) *Whole cell GR assay*

Glucocorticoid receptor binding characteristics were measured by homologous competition for specific [^{3}H]-dexamethasone-binding sites under steady state conditions (Schletche et al., 1982).

Aliquots (100 µl) of cell suspension were added to 96-well microtitre plates containing 50 µl of [^{3}H]-dexamethasone and 50 µl of unlabelled dexamethasone in duplicate. The final concentration of [^{3}H]-dexamethasone was 2 nM and the final concentration of unlabelled

dexamethasone was 2 nM and the final concentration of unlabelled dexamethasone ranged from 0 to 150 nM. The unlabelled steroids were dissolved in ethanol and the final ethanol concentration was always less than 0.01%. Non-specific binding was measured by incubating the cells with 1000-fold excess of unlabelled dexamethasone. The plates were incubated for 3 h at 24^0. After incubation, HML were harvested on Whatman GF/C filter paper and washed with ice-cold PBS containing polyethylenimine (0.1%) (Bruns et al., 1983) using a Titertek cell harvester. Filters were treated with 1% Triton X-100 and the radioactivity from lysed cells was measured by scintillation spectrometry.

Binding constants [dissociation constant (Kd) and binding capacity (Bmax)] were calculated from Scatchard plots which were fitted using the computer program Ligand (Munson & Rodbard, 1980).

To standardize the experimental conditions, preliminary experiments of cell number, dose and time dependency and hormone specificity were performed.

Results

a) *Time course of ^{3}H-dexamethasone specific binding*

To measure the optimum time for measuring binding characteristics, $4x10^7$ cells/ml were incubated for between 0 and 240 minutes at 24^0. Analysis for specifc binding (SB=TB-NSB, where TB is the total binding, and NSB the non-specific binding) was done in triplicate for each time period. The results are shown in Fig. 12.

b) *Cell number dependency*

In the assessment of receptor number, "binding" and "cell number" are of major importance for a reliable study of GR binding. For this reason, cell number should be maximal without compromising the capacity of the cell harvester and with minimal non-specific binding. Optimal specific binding was achieved with approximately 3.0-5.0 x 10^6 cells. The results of incubating with various concentrations of cells is illustrated in Fig. 13.

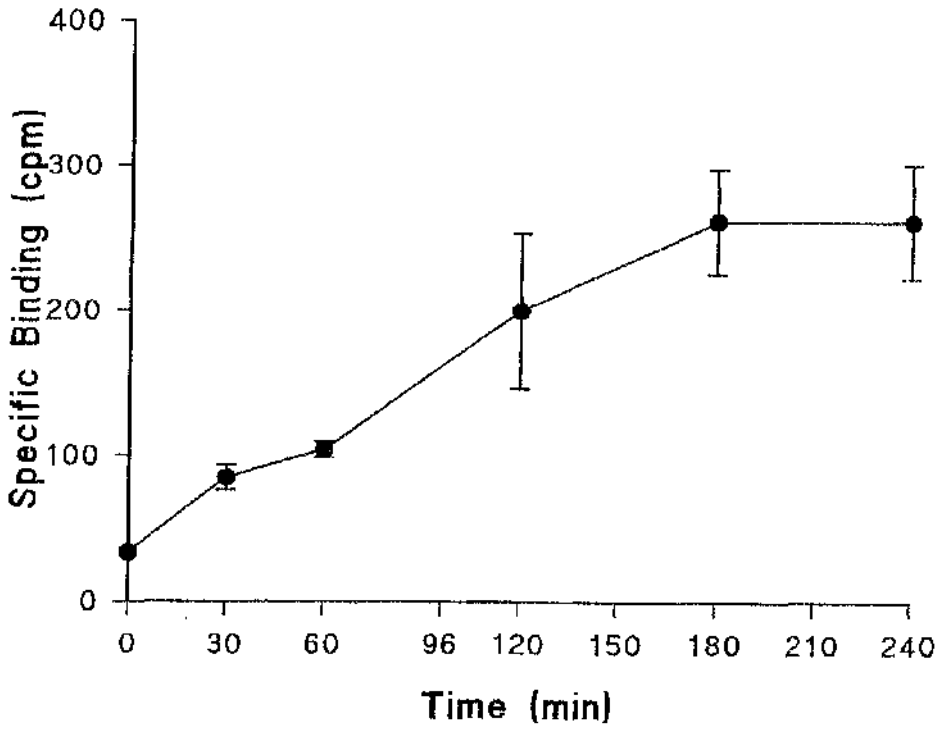

Fig. 12: Time course of binding of dexamethasone to glucocorticoid receptor in HML. Using the analysis of variance, there is no evidence of further binding after 180 min. Values are means ±SE of 3 replicates.

c) *Dose dependency*

^{3}H-dexamethasone specific binding was competed for by several concentrations of unlabelled dexamethasone (range, 0-150 nM). A threshold competition occurred at 0.6 nM unlabelled steroid. Fig. 14a and 14b show a representative binding and Scatchard plot analysis of one volunteer. Scatchard plots were linear with coefficients (r) predominantly between 0.68 and 0.90.

The GR binding characteristics were measured in 26 volunteers. Kd values ranged from 2.8-12.5 nM (mean±SEM, 6.6±0.45), and Bmax values ranged from 1701-6994 sites/cell (mean±SEM, 3974±567). In 18 of these individuals, GR Kd and Bmax were measured on a subsequent occasion. The coefficient of variation within individuals was 6% for Kd and 15% for Bmax (Braunsberg & James, 1961).

d) *Hormone specificity*

Specificity was tested by incubating the HML for 3 h at 24^{0} in the presence of several different steroids. The relative capacities of the steroid to compete with ^{3}H-dexamethasone for binding sites was as follows: RU486 = dexamethasone >cortisol > aldosterone > progesterone >E_2 > DHA. DHA did not compete (refer to Fig. 15).

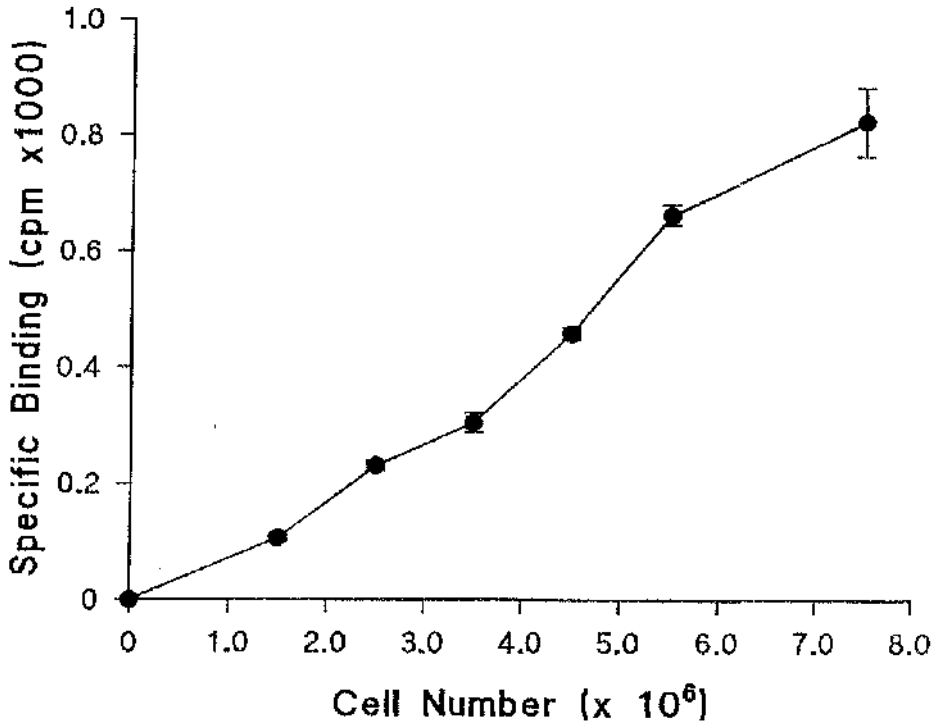

Fig. 13: Glucocorticoid receptor binding versus cell number (HML). Values are means ±SE of 3 replicates.

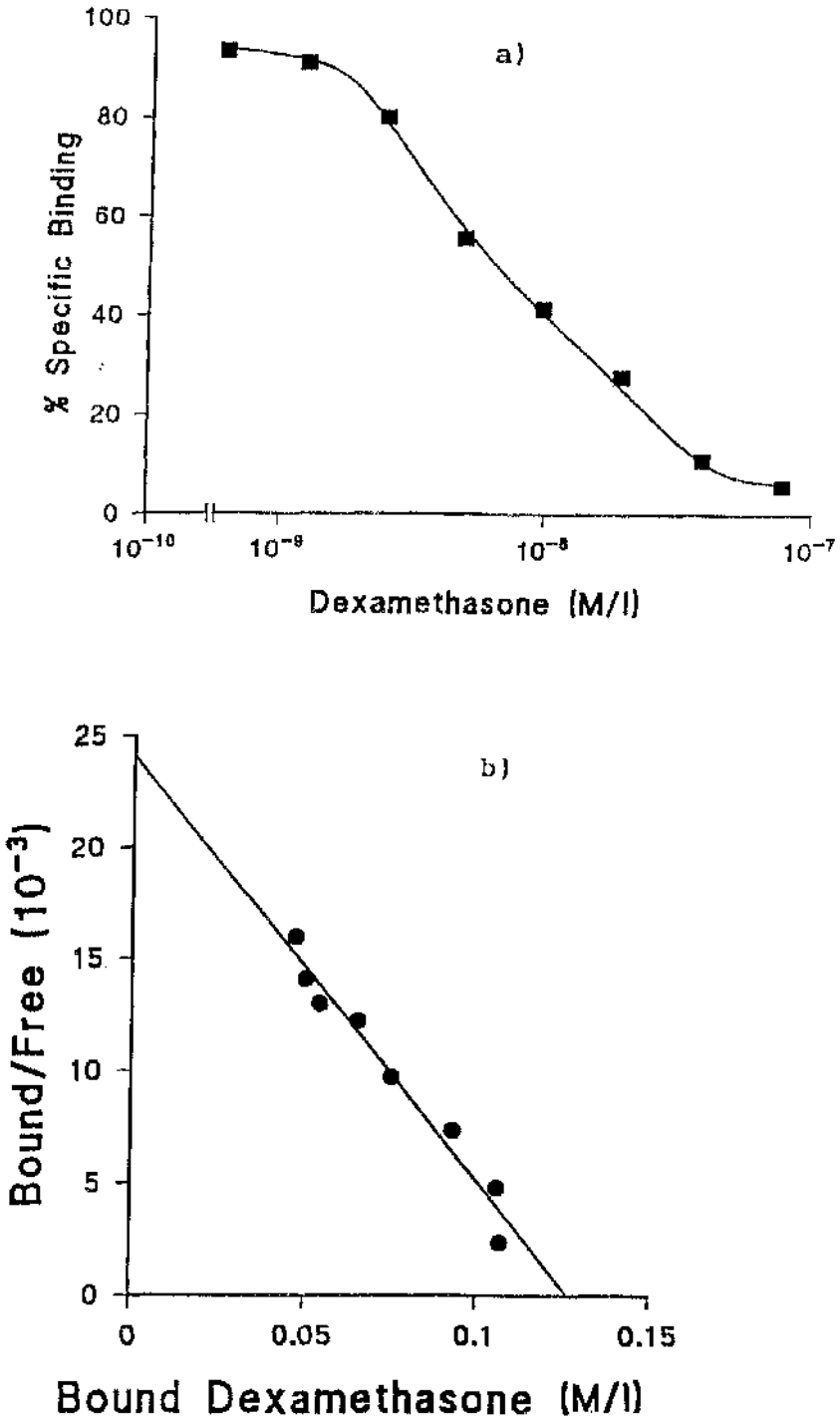

Fig. 14 a and b: Representative dose-dependent curve (a) and Scatchard plot (b) of dexamethasone binding to HML. The data are consistent with a single class of binding site.

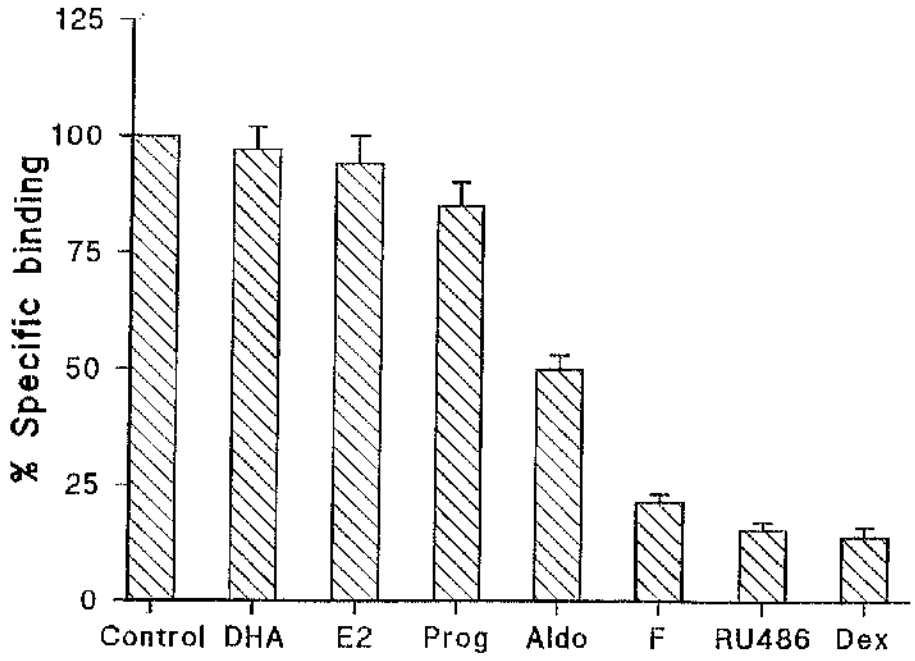

Fig. 15 : Comparison of the effect of dehydroepiandrosterone (DHA), oestradiol (E_2), aldosterone (Aldo), progesterone (Prog.), RU486, cortisol (F) and dexamethasone (Dex.) with untreated control (solid bar) on ^{3}H-dexamethasone specific binding in HML during 3 h incubation. Final concentration of all steroids was 1 μM; values, expressed as a percentage of controls, are means ±SE of 3 different preparations of cells.

Discussion

A previously published, whole-cell competitive binding receptor assay method has been modified into a microtitre system. This reduces the blood requirement and laboratory expenditure considerably and enables the simultaneous processing of multiple samples and probes. Thus speed of analysis is greatly increased. Because many factors can influence results, e.g. cell number, cell storage, time of blood withdrawal, temperature of incubation medium (data not shown), it is important to monitor them carefully. Using this protocol, this information was easily acquired.

The Kds and receptor densities measured in the ML of 26 healthy volunteers with this system were within the range published by Schlechte et al. (1982) and other published data at the same incubation temperature.

In summary, the microtitre system is a simple and reliable *in vitro* method of assessing glucocorticoid receptor binding characteristics in ML obtained from a relatively small blood sample and yields consistent results. For these reasons, it has been used in subsequent studies of whole-cell GR binding measurement.

2.2.3 Dexamethasone lysozyme inhibition assay: *in vitro* responsiveness to glucocorticoid

Introduction

One of the aims of this study was to establish a sensitive assay to test *in vitro* responsiveness to glucocorticoid. Variability in glucocorticoid responsiveness has been implicated in the aetiology of depressive illness (Schlechte & Sherman, 1985), obesity (Weaver et al., 1992), hirsutism (Werner et al., 1992), cardiovascular disease (Watt et al., 1992) and, on rare occasions, glucocorticoid resistance has been recognised as a primary disorder (Chrousos et al., 1982). It follows, therefore, that for both therapeutic and diagnostic reasons, a method of determining glucocorticoid sensitivity is necessary.

Glucocorticoid receptor studies of white cells and fibroblasts have identified some individuals with defective ligand binding (Werner et al., 1992) and, more recently, those with reduced receptor binding to DNA (Adcock et al., 1993), but these studies do not reveal subsequent defects of translation and transcription.

Two *in vitro* physiological responses of white blood cells have been used widely to assess steroid resistance in asthmatic, leukaemic and elderly critical care patients (Corrigan et al., 1991a; Bell et al., 1983; Tanaka et al., 1992; van Rijen et al., 1993). Glucocorticoids reduce the rate of proliferation of lymphocytes treated with phytohaemagglutinin and also prevent the uptake of 3-O-methyl-D-glucose by HML. Both methods are open to criticism. Generally, inhibition of proliferation is only achieved with supraphysiological concentrations of glucocorticoid hormones. 3-O-methyl-D-glucose uptake, although a more sensitive indicator, may lack specificity.

The present part of the project has validated a method of assessing glucocorticoid responsiveness based on the control of lysozyme gene expression. Inhibition of the expression of this gene by glucocorticoids

was first demonstrated in chicken macrophages (Steiner et al., 1987) and then in human monocytes (Lappin & Whaley, 1991). Using primary culture of white blood cells, the effect of hormone and cell type on sensitivity and specifity of the lysozyme response to glucocorticoids has been investigated by means of IC_{50} values and GR binding characteristics.

Methods

Cell preparation and GR assay were as described in sections 2.2.1 and 2.2.2.

Aliquots of cell suspension (0.5-1 x 10^6 cell) were cultured in a 96-well culture plate at 37^0 in 5% CO_2 for a maximum of 84 h, but routinely for 72 h. After centrifuging the plates, a 100-µl aliquot of supernatant was removed from each incubation well and stored at -70^0. Lysozyme activity in the supernatant was measured photometrically by the lysis of Micrococcus *lysodeikticus* (Strunk et al., 1980) using human recombinant lysozyme as a standard. Each sample was assayed in triplicate.

To standardise experimental conditions for lysozyme release from white cells, preliminary experiments of time-dependency, dexamethasone inhibition dose-dependency and hormone and cell specificity were performed. In one experiment with HML, lysozyme activity in the incubation medium and that in cells were measured simultaneously. After removing the incubation medium for assay, cells were centrifuged and washed with PBS. Cellular lysozyme was released by lysing the cells in distilled water.

Linear regression and correlation were calculated, and Student's *t* test was used for the comparison of variables.

Results

a) *Time course*

Mononuclear cells were cultured in the absence and presence of

1 μM dexamethasone. Samples of incubation medium were collected at intervals up to 84 h of incubation. As shown in Fig. 16, inhibition of lysozyme by dexamethasone was significant ($P<0.001$) after 24 h and reached a stable maximal inhibitory effect by 72 h. After a 72-h incubation, the intracellular lysozyme activity was 245±8 compared with 479±23 μU/cell in the incubation medium. With dexamethasone treatment, activities were 189±5 and 245±8 μU/cell, respectively. These data indicate that dexamethasone regulates lysozyme activity by inhibiting *de novo* synthesis, rather than by controlling the release of intracellular stores. In subsequent experiments, lysozyme activity was measured in incubation medium at 72 h.

b) Cell Specificity

Previous studies of glucocorticoid-sensitive lysozyme release were performed with monocytes (Lappin & Whaley, 1991) rather than HML as in the time course study (see above). Monocytes are the minority cell type in HML and yet lysozyme release from HML (expressed per unit cell) was similar in cultures of HML and monocytes alone. It follows that lymphocytes in HML must also release lysozyme under the control of glucocorticoid hormones. However, if HML rather than purified lymphocytes or monocyte samples are to be used to screen samples, it is important to confirm that all cell types produce enzyme and that enzyme activity in all types is regulated by glucocorticoids. Therefore, responses to dexamethasone were compared in HML, lymphocytes, monocytes, and PMN. Cells were incubated with and without 1 μM dexamethasone and lysozyme activity was measured in medium after 72 h. PMN released most lysozyme (Fig 17). However, the degree of inhibition by dexamethasone was similar for all cell types (HML, 50.2±1.5%; lymphocytes, 57.2±4.4%; monocytes, 58±1.8%; PMN, 47.8±2.2%). Bearing in mind that HML are easier to prepare than other cells and that the yield of HML from blood is greater than that of any other cell type, HML were used in subsequent experiments.

c) Glucocorticoid specificity

To test for glucocorticoid specificity of lysozyme inhibition, HML were incubated for 72 h in the presence of different steroids. At 1 μM, cortisol and dexamethasone were equally effective, but DHA, estradiol, progesterone, and even aldosterone had no significant effect (Fig. 18). Paradoxically, RU486, supposedly a GR antagonist, reduced lysozyme release. When comparing the concentration-dependent effects of dexamethasone and RU486, dexamethasone was 1000 times more effective. Dexamethasone showed threshold inhibition at 0.6 nM (Fig. 19). The antagonistic effects of RU486 were apparent in HML treated with various concentrations of dexamethasone. At 1 μM RU486, the IC_{50} and threshold values for the inhibitory effects of dexamethasone were shifted from 1.2 nM to more than 1 μM and from less than 1.0 to 19 nM, respectively.

d) Subject variability of GR binding characteristics and inhibition of lysozyme release by dexamethasone

The IC_{50} of dexamethasone for inhibition of lysozyme release was measured in 26 volunteers. Values ranged from 1.3-8.1 nM (mean±SEM, 3.8±0.3). In six of these individuals dexamethasone sensitivity was measured on a subsequent occasion. The coefficient of variation was 12% (Braunsberg & James, 1961). The apparent glucocorticoid receptor Kd values measured in HML, but not Bmax, were positively correlated with IC_{50} values for the effects of dexamethasone on lysozyme activity (refer to Fig. 20; $r=0.57$; $P<0.005$). Neither Bmax nor Kd correlated with cortisol concentrations in the plasma of blood used to isolate cells.

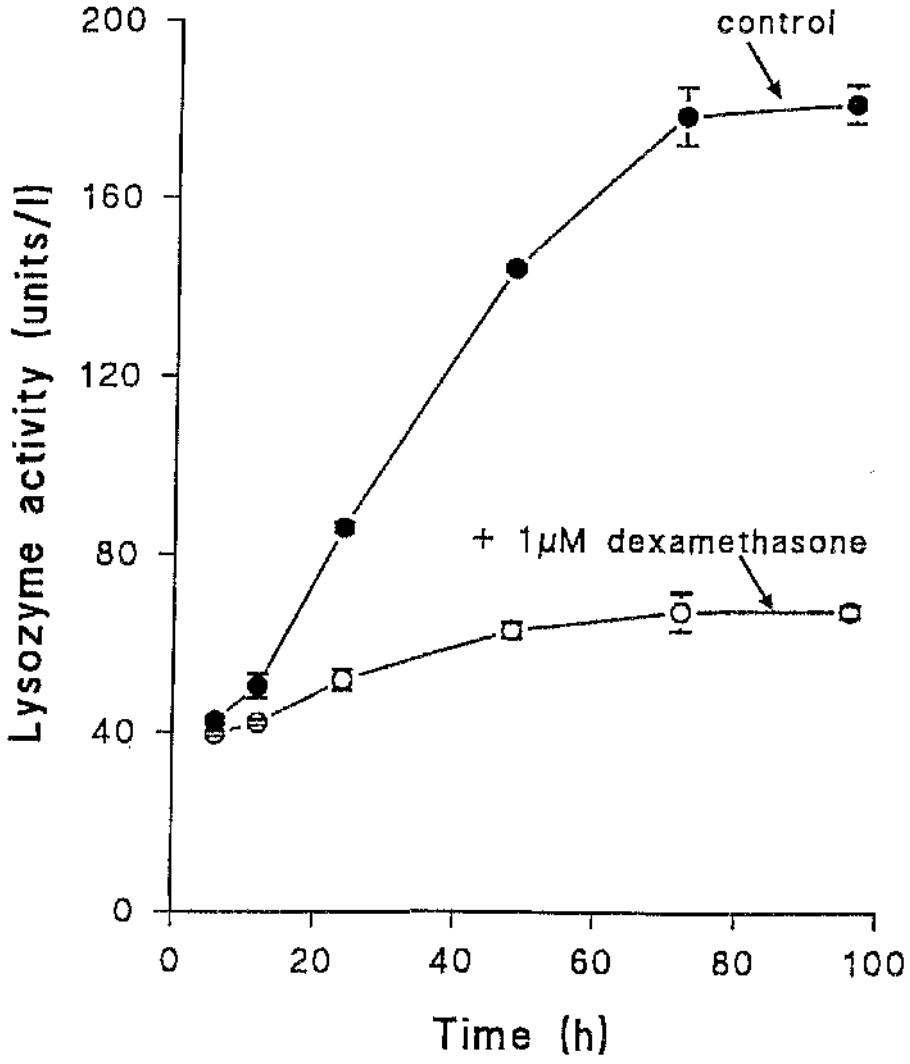

Fig. 16: Lysozyme activity in medium of HML cells after incubation with (open circle) or without (closed circle) 1 µM dexamethasone for various periods of time. Values are means ±SE of 4 replicates.

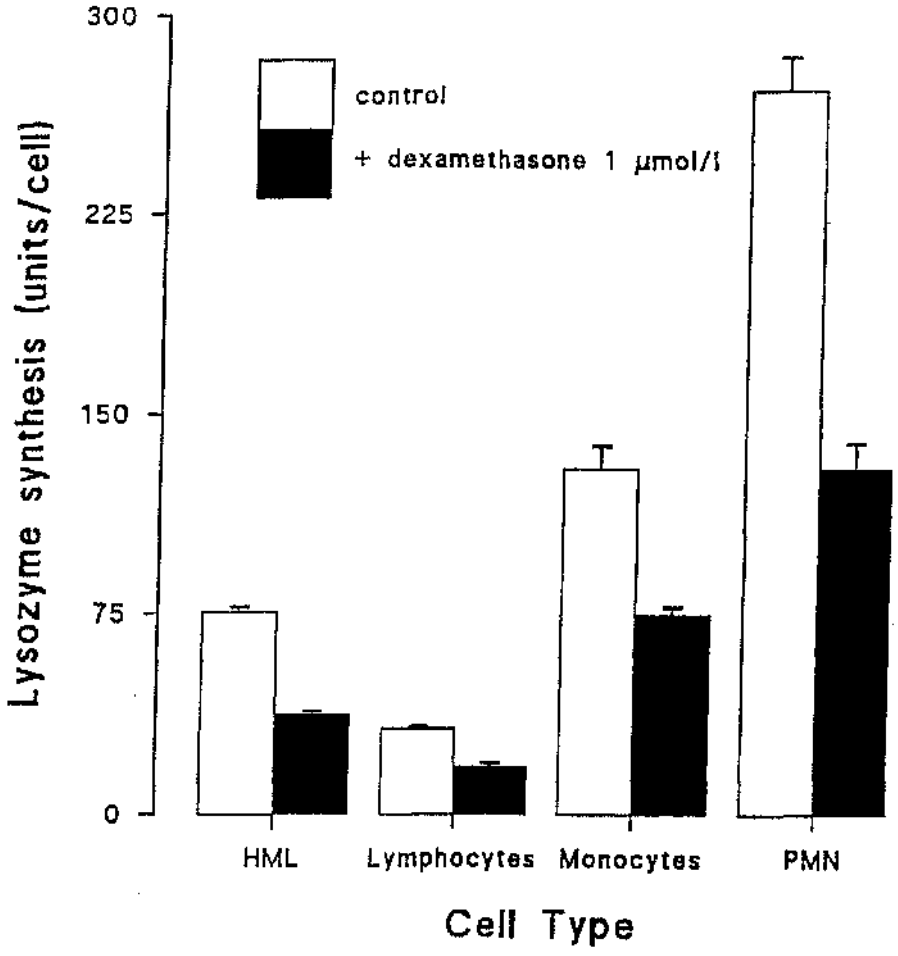

Fig. 17: Lysozyme activity in media of different white blood cell types after 72 h incubation with and without 1 µM dexamethasone. Values shown are means ±SE of cells from 5 volunteers.

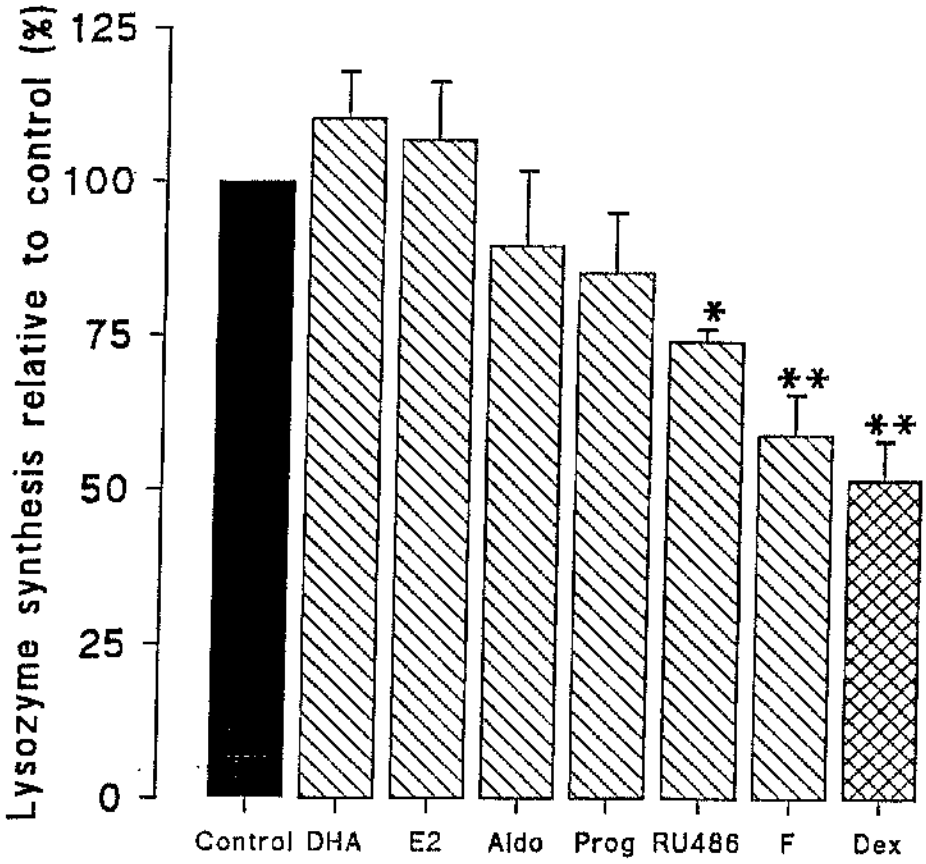

Fig. 18: Comparison of the effects of dehydroepiandrosterone (DHA), estradiol (E_2), aldosterone (aldo), progesterone (prog), RU486, cortisol (F) and dexamethasone (dex) with untreated controls (solid bar) on lysozyme released from HML during 72 h of incubation. Final concentration of all steroids was 1 μM; values expressed as a percentage of controls, are means ±SE of 6 different preparations of cells. Statistical significance of inhibition compared with controls were determined by paired t-test and are indicated by * ($P<0.05$) and ** ($P<0.01$).

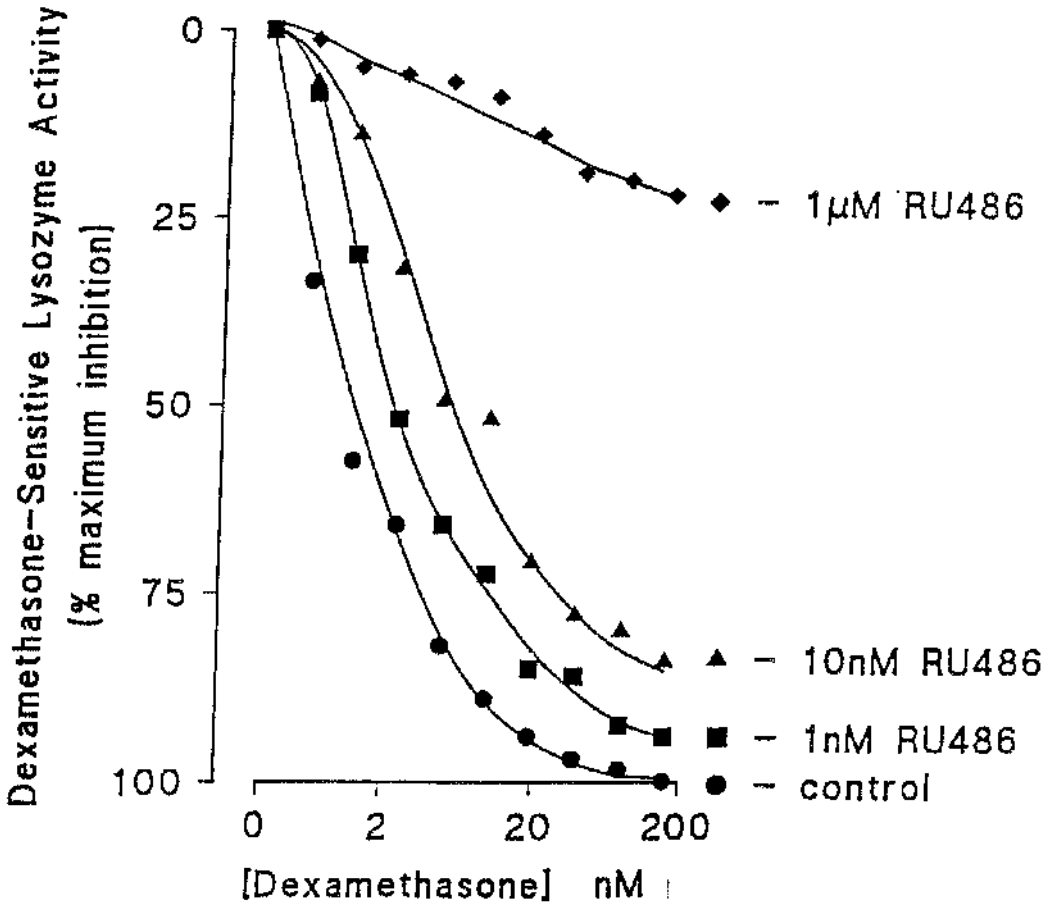

Fig. 19: Concentration-dependant antagonistic effects of RU486 on dexamethasone-sensitive lysozyme release from HML. Values shown are means of triplicate incubations.

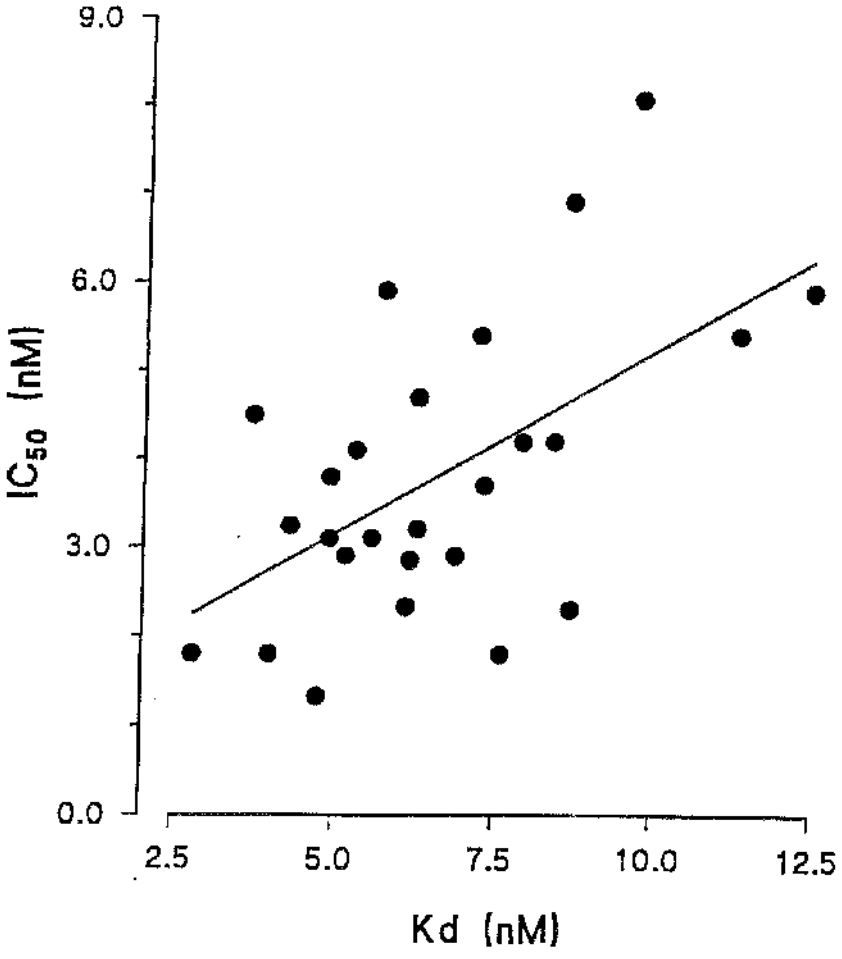

Fig. 20: Correlation between IC50 values for the inhibitory effects of dexamethasone on lysozyme activity and Kd values for dexamethasone binding in HML from 26 volunteers ($r=0.57$; $P < 0.005$, slope = 0.41).

Discussion

Lysozyme is a ubiquitous enzyme whose expression and release from macrophages are an integral part of the immune response. In keeping with the immunosuppressive effects of glucocorticoids, dexamethasone has been shown to inhibit lysozyme activity. This effect appears to be due to inhibition of lysozyme synthesis rather than a decrease in enzyme release, as dexamethasone reduced enzyme activity in both incubation medium and cell lysate. This confirms previous studies with monocytes showing glucocorticoid-dependant inhibition of lysozyme gene transcription (Lappin & Whaley, 1991). This study has also shown that all white blood cells release lysozyme and that this release in all types is glucocorticoid sensitive. Although the amount of enzyme synthesized varies among cell types, the degree of glucocorticoid inhibition is constant. This obviates the need for rigorous cell purification. HML are prepared in high yield from a small sample of blood; 10-20 ml are sufficient for a complete dexamethasone response curve.

Previously, inhibition of cell proliferation has been used widely as an index of glucocorticoid sensitivity. This inhibitory effect, measured as decreased incorporation of radioactive thymidine or uridine, is best seen in mononuclear cells from peripheral blood after treatment with a mitogenic stimulus such as the lectins Concavalin-A and phytohemoagglutinin (Neifeld et al., 1977). As with the lysozyme response, the proliferative response of all white blood cell types is inhibited. The effect of glucocorticoids appears to be as specific as that for the present lysozyme response; androgens, oestrogens, and progestogens in both assay systems are ineffective at concentrations below 1 μM (Neifeld et al., 1977). The use of the lysozyme assay offers three potential advantages over the mitogen method: increased convenience, sensitivity, and reproducibility. The end point for the lysozyme assay is a decrease in turbidity of a suspension of Micrococcus *lysodeikticus*, as described by Strunk et al. (1980). This has been readily

adapted to microtiter assay plates, so that a large number of small samples can be quickly processed using a multiscanner spectrometer. In contrast to methods that measure the antiproliferative effects of glucocorticoids, cells do not require mitogen treatment to elicit inhibitory effects on lysozyme release. Although this is a minor inconvenience in proliferation assays, stimulation with lectins also involves induction of GR, which, in turn, will influence sensitivity. Under the right conditions with the right degree of stimulation, the IC_{50} for dexamethasone's antiproliferative effect corresponds to that expected for an event mediated by a receptor with a Kd for dexamethasone in the nanomolar range. More often, however, the antiproliferation test has been applied more crudely with steroid concentrations greater than 100 nM (Corrigan et al., 1991b).

It also appears that the proliferative response to lectins varies between individuals, so that either more than one concentration of mitogen are tested (van Rijen et al., 1993) or the concentration of steroid required to inhibit proliferation bears no relation to GR characteristics. For example, a recent study compared receptor binding characteristics and the antiproliferative effects of dexamethasone in steroid sensitive and steroid-resistant asthmatic patients. Binding affinity and inhibition of cell proliferation were both reduced in the resistant patients, but the two variables did not correlate with each other (Corrigan et al., 1991b). This lack of agreement may reflect the inexactitude of the proliferation test. To demonstrate antiproliferation in all individuals with cells obtained from a modest sample of blood, a wide range of steroid concentrations with few intermediate points was needed. This assay was, therefore, unable to discriminate 2- to 3-fold differences in antiproliferative activity to correspond with the range of values for dexamethasone binding affinity in steroid-resistant and -responsive patients. Given that the lysozyme assay has a coefficient of variation of 12%, the values in normal individuals fall in the nanomolar range, and the binding affinity and inhibitory effect of dexamethasone on lysozyme activity are correlated,

the present assay may be of greater value in the study of glucocorticoid receptor abnormalities than the antiproliferative method.

Recently, another *in vitro* method of assessing glucocorticoid responsiveness has been described (Tanaka et al., 1992). The uptake of a nonmetabolized sugar, 3-O-methylglucose, by monocytes is inhibited by dexamethasone. No prestimulation of cells is required for this simple reproducible qualitative method. An advantage of the glucose uptake assay over the lysozyme assay is that it is quicker. Glucose uptake is inhibited in a dose-dependant manner by glucocorticoids, but perhaps because of the shorter incubation period, the sensitivity of the response is slightly less than the effect on lysozyme release. In comparing glucose uptake and dexamethasone binding in different blood samples, only the effect of a maximally effective concentration of dexamethasone on glucose uptake was investigated. Not surprisingly, inhibition of uptake with 1 μM dexamethasone correlated with receptor number rather than affinity. The lysozyme method is perhaps a more specific indicator of glucocorticoid activity than the uptake method. Progesterone at a concentration of 1 μM had no effect on lysozyme synthesis. The lack of an effect of aldosterone is surprising in view of the known affinity of the GR for this steroid. However, contrasting effects of mineralocorticoid and glucocorticoids have been described previously (Kenyon et al., 1984). Similarly, the partial agonist effects of high concentrations of RU486 on lysozyme activity, although unexpected, are not without precedent (Laue et al., 1988; Van Voorhis et al., 1989). It has been demonstrated that in the presence of dexamethasone, RU486 is a potent glucocorticoid antagonist at concentrations that exhibit no agonist activity when added to HML alone.

In summary, the lysozyme inhibition assay is a simple, robust, *in vitro* method of assessing glucocorticoid responsiveness based on the inhibitory effects of dexamethasone on lysozyme activity in cultures of HML obtained from a small blood sample. The specificity of the response

for glucocorticoid hormones is at least as good as that of any other method; mineralocorticoid, oestrogens, androgens, and progestogens are inactive, whereas the glucocorticoid antagonist RU486 markedly inhibited the dexamethasone response. The assay is reproducible and is not subject to variations caused by stimulation of cell proliferation. This test has been applied in the present studies to investigate potential GR polymorphisms. This study has been recently published (Panarelli et al., 1994, see page 228).

7.4 Glucocorticoid receptor binding measurement from rat liver cytosol

Introduction

Although the mononuclear leucocyte is a convenient tissue to measure GR binding characteristics in man, it has serious limitations for studying small animals. From an individual rat, only 9-10 ml of blood can be withdrawn which yields an insufficient number of ML. Thus, ML from several rats must be pooled to achieve the required cell number for a reliable measurement of GR binding (see section 2.2.2).

Glucocorticoid receptors are abundant in liver cytosol preparations (Howell & Lefebvre, 1989: Howell et al., 1990). The rat liver is easy to isolate and its homogenization yields a large amount of cytosolic protein for the measurement of receptor binding.

The assay has been developed using the normotensive Sprague-Dawley (SD) rat.

Methods

a) Tissue preparation

Six SD rats were stunned, decapitated and the liver perfused *in situ* with ice-cold 0.154 M NaCl solution. Livers were removed and minced in 3 volumes of ice-cold buffer A and the minces were homogenised briefly using a Polytron. The homogenate was centrifuged at 20,000g for 20 min. and the supernatant centrifuged at 105,000g for 1 h. All manipulations were carried out at 5^0. The final supernatant (cytosol) was used in binding studies.

b) Binding studies

Binding constants for dexamethasone, corticosterone, aldosterone and the glucocorticoid antagonist, RU486 were estimated by measuring homologous and heterologous competition for specific ^{3}H-dexamethasone binding sites. Aliquots of liver cytosol (final concentration of 2 mg protein/ml) were equilibrated overnight at 5^0 with 3 nM ^{3}H-dexamethasone and various concentrations of unlabelled ligands

(after dissolution in ethanol, the desired concentrations were obtained by a serial of dilutions with buffer A; final ethanol concentration was always below 0.01%). Non-specific binding was measured by incubating the liver cytosol with a 500-fold excess of unlabelled dexamethasone. Bound and free hormone fractions were separated by incubation with an equal volume (v/v) of charcoal suspension for 10 min and centrifuged at 450g for 10 min. Radioactivity was measured in aliquots of supernatant by liquid scintillation spectrometry.

Preliminary experiments of cytosolic concentration and time dependency were carried out.

Binding constants (Kd and Bmax) were calculated using the Ligand curve-fitting programme (Munson & Rodbard, 1980).

c) Effects of temperature on ^{3}H-dexamethasone specific binding

To investigate the thermostability of the bound ^{3}H-dexamethasone-receptor complex, cytosol was equilibrated overnight with ^{3}H-dexamethasone (±500 nM unlabelled dexamethasone) at 5^0 and then transferred to 24^0 and 37^0 water baths. Aliquots were removed at intervals.

The effects of incubation at 37^0 on receptor binding constants were also investigated by measuring homologous competition for ^{3}H-dexamethasone after 30, 60 and 120 min equilibration.

Results

a) *Receptor affinity and concentration*

The cytosolic protein concentrations and time-dependency results are shown in Fig 21 and 22. They indicate that 2 mg/ml protein is the lowest concentration at which high specific binding can be detected and that at 16 h incubation at 5^0, there is complete equilibrium of the receptor-ligand binding.

The binding curves for the various steroid ligands and the affinity constants are shown in Fig 23 and 24, respectively. ^{3}H-dexamethasone

binding was reduced to about half the control by equimolar unlabelled dexamethasone. In contrast, 50% reduction of ^{3}H-dexamethasone binding was caused by a 5-fold excess of unlabelled corticosterone and 40-fold excess of aldosterone. The antiglucocorticoid RU486 was also less effective (data not shown because they obscure the corticosterone results) with 6-8 times lower potency when compared to dexamethasone. The relative ^{3}H-dexamethasone binding site affinities for the ligands was: dexamethasone = RU486 > corticosterone > aldosterone.

b) Temperature sensitivity of receptor binding

The ^{3}H-dexamethasone-receptor complex was incubated overnight at 5^{0} before being transferred to higher temperatures. At 24^{0}, binding was constant over a period of 3 h (Fig. 25). At 37^{0}, binding declined rapidly to near zero values at 1 h. To test whether this decline in specific binding at 37^{0} reflected a change in affinity and/or a change in receptor concentration, binding characteristics were measured during longer term incubation at this higher temperature. Both receptor affinity and receptor capacity decreased with time at 37^{0} (Fig. 26).

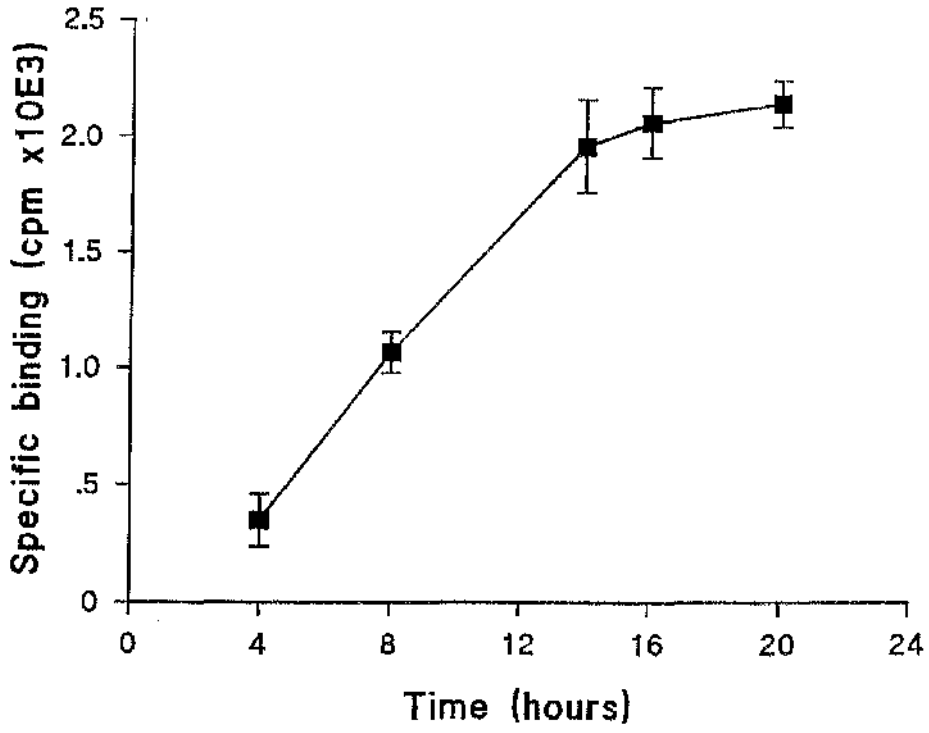

Fig. 21: Time-dependant [^{3}H]-dexamethasone specific binding in liver cytosol from SD rats at 5^0. Values are means ±SE of 3 replicates.

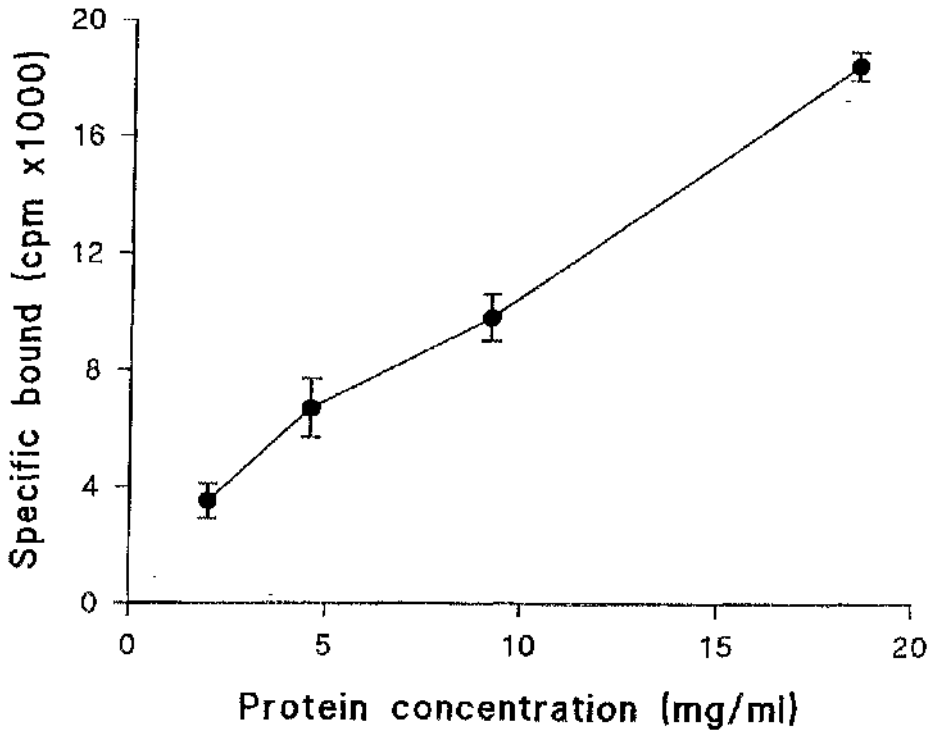

Fig. 22: [^{3}H]-dexamethasone specific binding in liver cytosol from SD rats *versus* protein concentration. Values are means ±SE of 3 replicates.

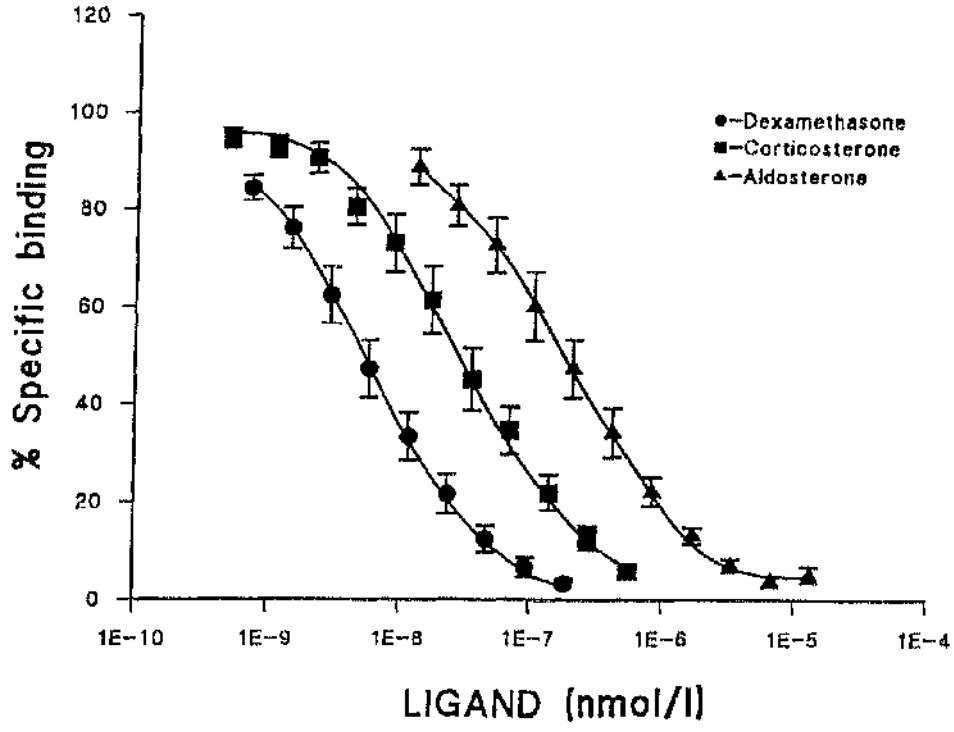

Fig. 23: Specificity of [^{3}H]-dexamethasone specific binding in liver cytosol from SD rats (means ±SE;n=6).

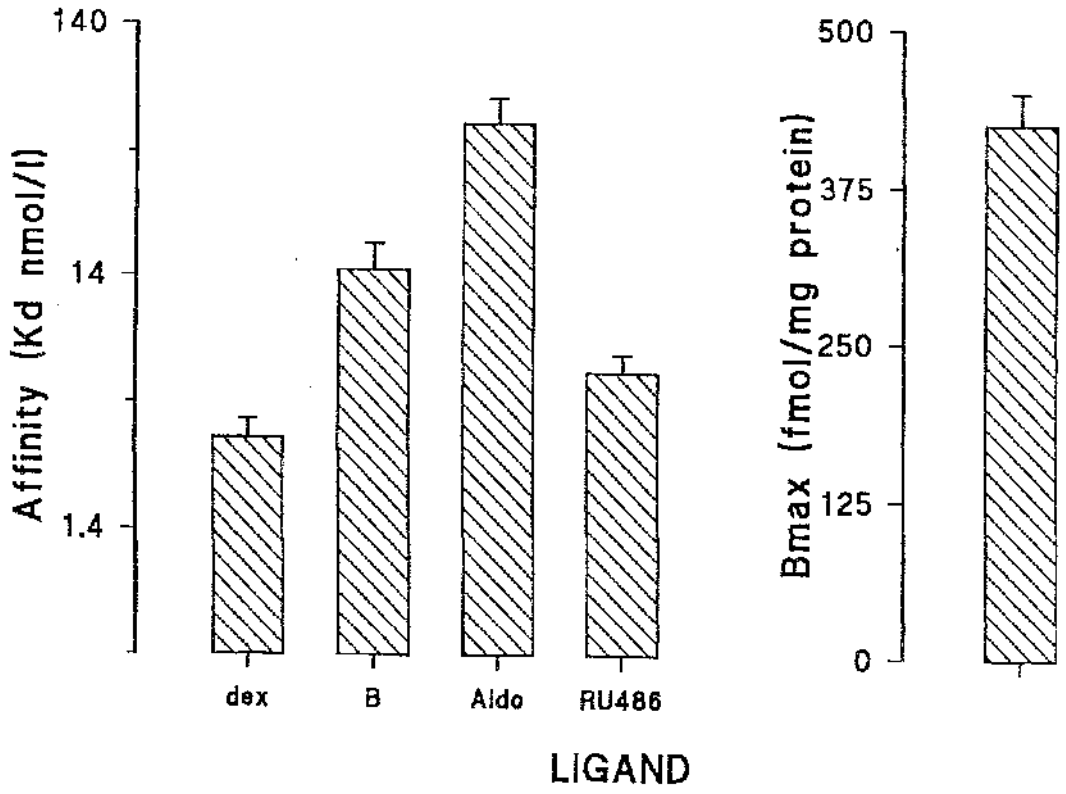

Fig. 24: Comparison of affinity constants for various ligands and binding capacity glucocorticoid receptors in liver cytosol from SD rats (dex: dexamethasone, B: corticosterone, Aldo: aldosterone). Values are means ±SE of 6 experiments.

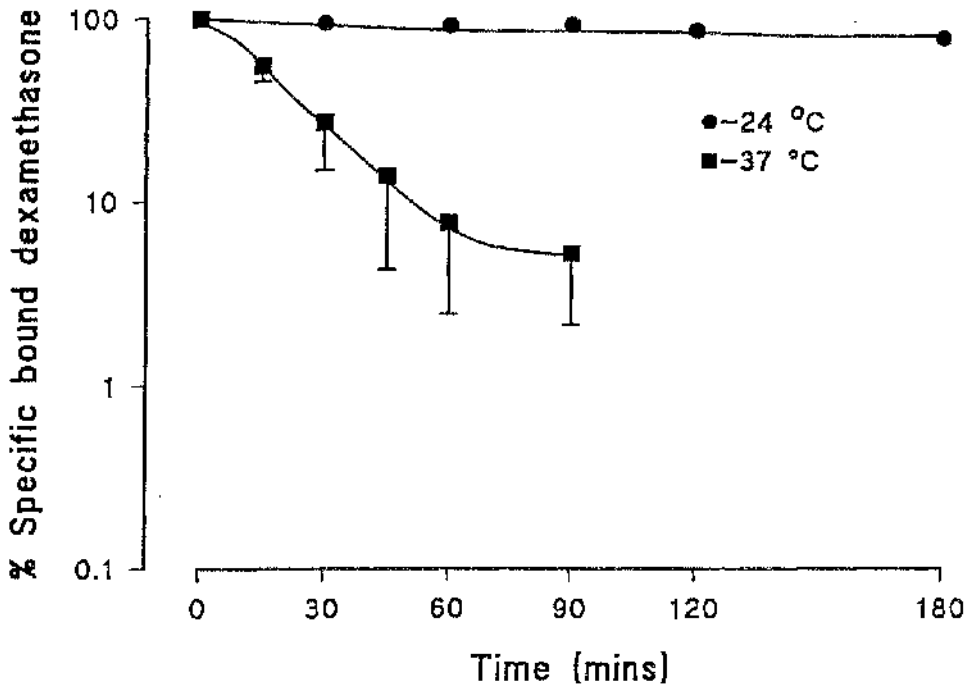

Fig. 25: Thermostability of glucocorticoid receptor complex in liver cytosol from SD rats (means ±SE; n=6).

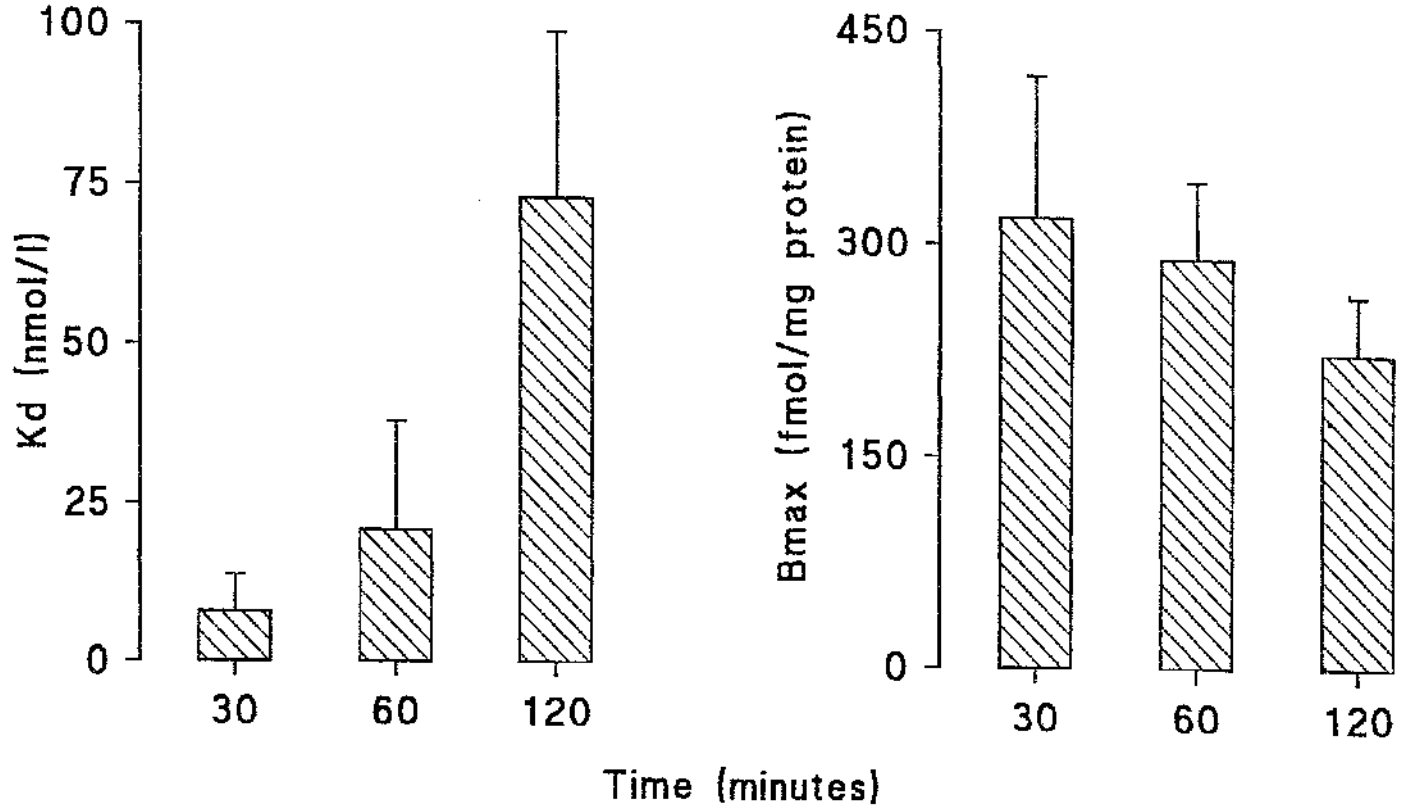

Fig. 26: Effect of prolonged incubation at 37^0 on glucocorticoid receptor binding characteristics in liver cytosol from SD rats (means ±SE; n=6)

Discussion

The aim of this method development was to devise a detailed protocol to study GR binding characteristics in several rat models of genetic hypertension which had previously been shown to have an adrenal component in the pathogenesis of their high blood pressure (see section 3).

According to literature reports, 16 h incubation at 5^0 had been shown to be necessary for a satisfactory liver cytosol glucococorticoid receptor binding (Howell & Lefebvre, 1989). As the cell number is important for a reliable binding assay, several protein concentrations were tested. As shown in Fig. 22, a concentration of 2 mg/ml was optimal and this made possible to carry out binding studies with multiple ligands in individual rats.

The cross-competition binding studies were set up to study the affinity of different ligands for ^{3}H-dexamethasone specific sites and to identify whether these affinities might differ between strains of rat. As shown in Fig. 23, in the hierarchy of differences dexamethasone has the highest affinity. In order to avoid degradation of the receptor-ligand complex both at 5^0 and 37^0, the incubation buffer (buffer A) contained a high concentration of sodium molybdate. This stabilizes the glucocorticoid receptor-Hsp complex, maintaining a state of high affinity of the GR (Leach et al., 1979). Despite the high concentration of sodium molybdate, the specific ^{3}H-dexamethasone binding after 60 min incubation declined rapidly at 37^0, less so at 24^0 and hardly at all at 5^0.

Reduced binding by prolonged incubation at 37^0 could be attributed to protein denaturation or transformation of the receptor from a high to a low affinity state. As already discussed, glucocorticoid receptor affinity depends on whether the receptor is complexed with heat shock proteins (Pratt, 1993) (for details see section 1.4.2). Testing whether the decline in specific binding observed in the thermal stability experiments reflected a change in GR capacity and/or affinity, the

competition studies of labelled binding after prolonged incubation at 37^0 showed a receptor affinity decline in a time-dependant fashion. Although this was not dramatic in the Sprague-Dawley, it should be an useful tool to test receptor binding where the underlying biochemical alteration may be receptor thermolability (Bronnegard et al., 1986; Werner et al., 1992).

In summary, a protocol to study glucocorticoid receptor in rat liver cytosol has been developed. The cross-competition, thermal stability and thermolability studies provide a comprehensive range of tests to identify a) any of the biochemical abnormalities which characterize an impaired glucocorticoid receptor (e.g.,as reported in glucocorticoid resistance syndrome) and b) an increased receptor affinity for steroid ligands. This protocol has been used in a study accepted for publication (Panarelli et al., 1995, see page 228).

Chapter 3 GLUCOCORTICOID RECEPTOR BINDING MEASUREMENT IN RAT MODELS OF GENETIC HYPERTENSION

Abnormalities of steroid metabolism and secretion in rat models of genetic hypertension have been reviewed in the first chapter. Several observations suggested that glucocorticoid hormones might contribute to the higher blood pressure in these rats:

a) rats are particularly sensitive to blood pressure raising effects of glucocorticoids (Tonolo et al., 1988);

b) the adrenal glomerulosa is necessary to initiate and sustain blood pressure in many strains of spontaneously hypertensive rat;

c) in some strains of rat, including Okamoto SHR but particularly the Milan rat, plasma corticosterone concentrations are elevated.

The efficacy of a hormone depends not only on its concentration at target tissue level but also on the efficiency and concentration of its receptor mechanism. Several endocrine abnormalities are characterised by normal or supernormal hormonal levels but with a defect in the receptor activity (e.g. primary cortisol, insulin and androgen resistance). It is similarly possible that in genetic hypertension in the rat, a receptor abnormality could be responsible. The following experimental section has investigated whether two widely used animal model of genetic hypertension have a defect in glucocorticoid receptor binding.

3.1 Alteration in the binding characteristics of glucocorticoid receptor in mononuclear leucocytes from spontaneously hypertensive rats (SHR) as compared to Wistar Kyoto rats (WKY)

3.1.1 Introduction

A number of strains of rats with inherited hypertension are available of which the spontaneously hypertensive rat (SHR) has been the most intensively studied. Intact adrenal glands are crucial to the rise in blood pressure but the essential component of adrenocortical function has not been identified (Brownie et al., 1990). Measurement of plasma adrenal steroid hormone levels have yielded conflicting results.

This part of the study measured glucocorticoid receptor binding characteristics in mononuclear leucocytes from SHR compared to WKY in order to obtain preliminary information on glucocorticoid receptor activity.

3.1.2 Methods

Adult male SH and WKY rats were anaesthetized with sodium phenobarbitone. Blood samples (7-10 ml) were taken from the aorta and anticoagulated with sodium citrate. Anticoagulated blood was centrifuged at 600g for 10 min. Cells at the interface between red cells and plasma were collected and pooled with others of the same strain (samples from 4-5 SH or WKY rats). These pooled samples were mixed with PBS. Mononuclear leucocyte separation and whole cell GR assay were as described in section 2.2.1 and 2.2.2, respectively.

3.1.3 Results

The affinity of the mononuclear leucocyte glucocorticoid receptor for the synthetic glucocorticoid dexamethasone and the number of binding sites per cell are shown in Fig. 27. The SHR cells had a significantly higher affinity for the glucocorticoid. Fewer sites per cell were observed

but this was of borderline significance.

3.1.4 Discussion

Glucocorticoid administration at very low doses raises blood pressure in genetically normotensive rats in 2-4 weeks (Tonolo et al., 1988) by a mechanism which is not completely understood but may involve changes in adrenergic receptor (Russo et al., 1990; Haigh & Jones, 1990) and phospholipase A2 activities (Bailey, 1991). In the SHR, widely used as a model for essential hypertension in man, many genetic loci are probably implicated in the rise of blood pressure (Rapp, 1982; Ikeda et al., 1991; Jacob et al., 1991; Hilbert et al., 1991; Ben-Ari et al., 1989). However, intact adrenocortical function is essential to the development of hypertension and glucocorticoid and mineralocorticoid components are required (Ruch et al., 1984; Morris& Kenyon., 1983). Kenyon et al. (1993) reported that basal plasma concentration of corticosterone was higher in young SHR and maximal stimulation with ACTH achieved a much higher concentration than in control WKY rats. It was suggested that these differences were due to increased responsiveness to ACTH. Others have suggested that pituitary ACTH activity is greater in SHR.

In mononuclear leucocytes from SHR, glucocorticoid receptors had a higher affinity but there were fewer receptor than in cells from WKY rats. Similar results were described in stroke-prone SHR by Laux et al. (1989). If the receptor is identical in all target tissues, this implies that tissues, such as brain and vascular smooth muscle, which are more important in blood pressure control, are also altered. A possible explanation of this finding could be a mutation in the glucocorticoid receptor gene.

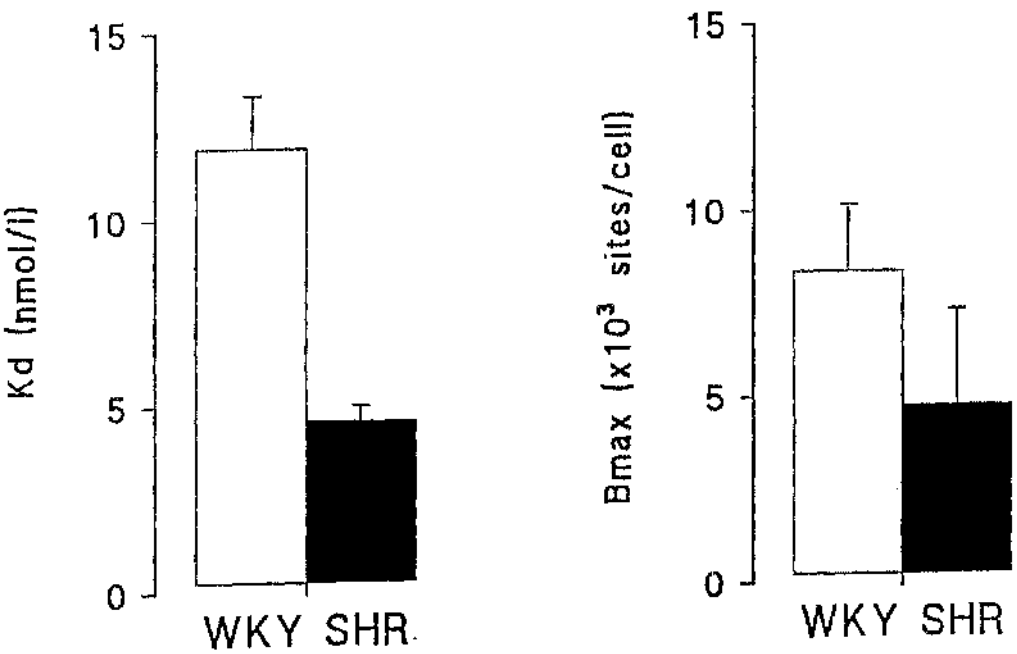

Fig. 27: Glucocorticoid receptor characteristics in WKY and SHR rat mononuclear leukocytes. Values are means ±SE of 4 separate ML pools.

The second important point is the observation that the number of receptors per cell may be lower in mononuclear cells from SHR compared to the control WKY. This may represent a down-regulation mechanism due to the higher affinity of the receptor to steroids as indicated by the lower Kd values. In normal human subjects, excess glucocorticoid hormone causes a fall in leucocyte receptor site number coupled with a rise in affinity, similar to that found in SHR in this study (Schlecthe et al., 1982). The down-regulation of the receptor is due to a fall in transcription rate and an increase in receptor protein turnover but not that of mRNA (Dong et al., 1988). However, no such changes were reported during normal diurnal variation in human glucocorticoid levels, nor were any changes detected in patients with Cushing's syndrome (Junker, 1983).

In summary, this preliminary study suggested altered glucocorticoid receptor binding characteristics in SHR compared to WKY. However, the main technical limitation was the need to pool blood sample from several rats of the same strain in order to obtain a sufficient cell number to carry out the whole cell GR assay. Further study of receptor specificity and affinity in individual rats was desirable. As reported in section 2.2.4, a protocol to study different aspects of the GR binding characteristics has been developed using liver cytosol from Sprague-Dawly rats. This was applied to a more detailed study of possible binding abnormalities in SHR.

3.2 Differences in temperature-sensitive receptor binding of glucocorticoids in spontaneously hypertensive and normotensive Wistar Kyoto rats

3.2.1. Introduction

In the previous section, it was shown that leucocytes from SHR bound the synthetic glucocorticoid dexamethasone with greater affinity than those from WKY rats. The next part of the study was designed to assess the specificity, temperature sensitivity and thermolability of GR in individual rats using liver cytosol from SHR and WKY.

3.2.2 Methods

Tissue preparation, binding studies and effects of temperature on ^{3}H-dexamethasone specific binding in SHR and WKY were as described in the section 2.2.4.

Statistical comparisons were made by Students' *t* test for paired data.

3.2.3 Results

The effect of dexamethasone, RU486, corticosterone and aldosterone competition for specific ^{3}H-dexamethasone binding sites at 5^{0} are shown in Fig. 28 (RU486 data are not plotted because obscure corticosterone results); the affinity constants for these three steroids are compared in Fig. 29. Dexamethasone showed the highest binding affinity and aldosterone the lowest. There were no differences between SHR and WKY preparations at this temperature either in affinity or concentrations of receptors.

Preliminary studies indicated that equilibrium was achieved quicker at 37^{0} (30-60 minutes). However, binding affinity was significantly less at 37^{0} than at 5^{0} in both species. Moreover, with prolonged incubation at 37^{0}, binding decreased even further despite the

presence of molybdate. This decrease was not a function of the number of receptors, rather the affinity for dexamethasone was reduced. When comparing SHR and WKY, the decrease in affinity for dexamethasone in WKY was greater than that in SHR. Differences in affinity between strains achieved significance at 30 and 60 minutes of incubation (Fig 30). WKY rats had a higher concentration of binding sites (457±144 fmol/mg protein) than SHR (326±70 fmol/mg protein) but this was not significant.

The thermostability of the dexamethasone-receptor complex was assessed in a further experiment. After overnight equilibration at 5^0, the dexamethasone-receptor complex dissociated only slowly when the temperature was raised to 24^0; approximately 80% of basal binding persisted at 3h. At 37^0, specific binding decreased rapidly and virtually zeroed at 90 min. However, there were no differences in dissociation rate between SHR and WKY cytosol preparations (refer to Fig. 31).

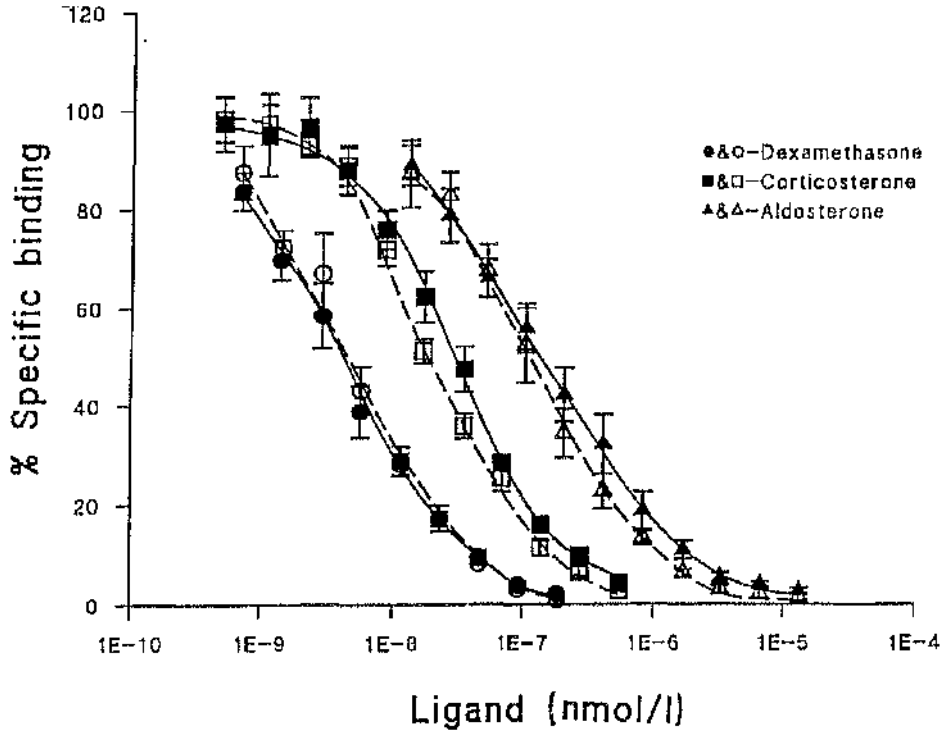

Fig. 28: Specificity of ^{3}H-dexamethasone specific binding sites in liver cytosol from SH (open symbol) and WKY (solid symbols) rats (values are means ±SE; n=6).

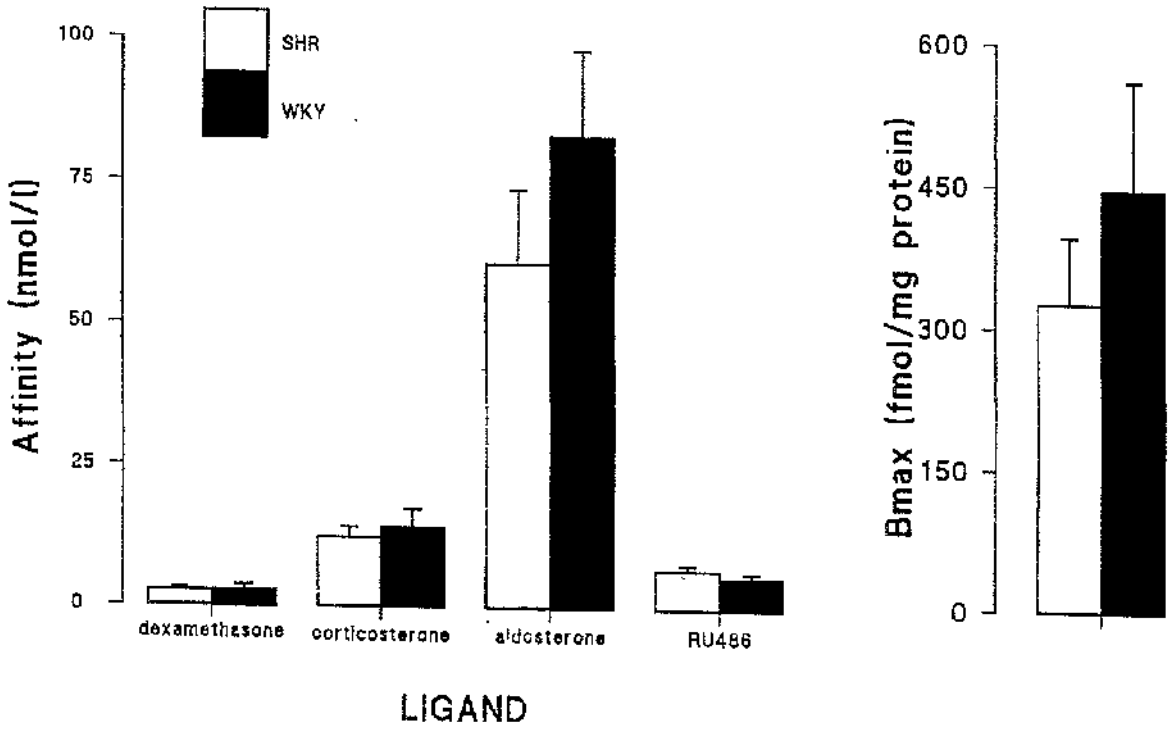

Fig. 29: Comparison of affinity constants for various ligands and binding capacity of glucocorticoid receptor in liver cytosol from WKY and SH rats (values are means ±SE; n=6).

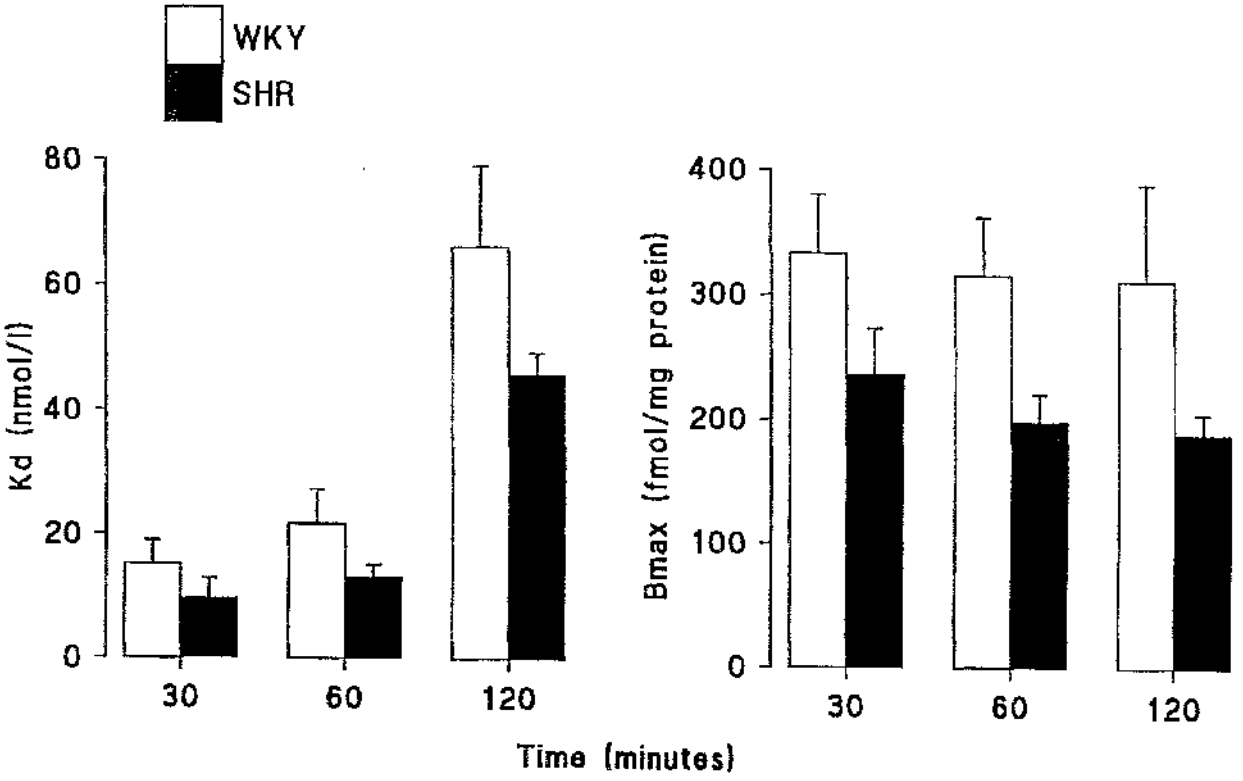

Fig. 30: Effect of prolonged incubation at 37^0 on glucocorticoid receptor binding characteristics in liver cytosol from SHR and WKY rats (values are means ±SE; n=6).

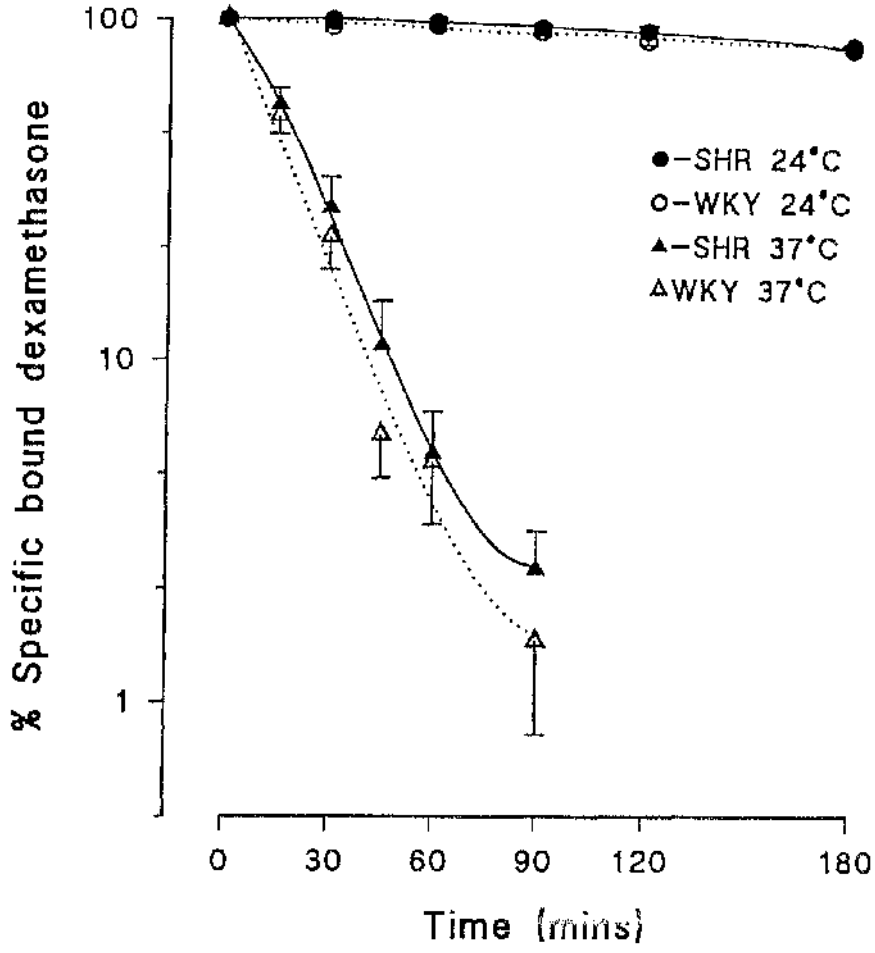

Fig. 31: Thermostability of glucocorticoid receptor complex in liver cytosol from WKY and SH rats (values are means±SE; n=6).

3.2.4 Discussion

Hypertension in SHR depends at least partly on the activity of the adrenal cortex. While glucocorticoid (i.e. corticosterone) levels have frequently been reported to be higher than in WKY rats, no such elevation of the principal mineralocorticoid, aldosterone has been found (Brownie et al., 1990). This is suggestive of a glucocorticoid-type hypertension. The previous section described a higher dexamethasone-binding affinity in the leucocytes of SHR compared to WKY rat cells when measured at 24^0. This confirmed a previous observation by Laux et al., 1988 in stroke-prone SHR. SHR leucocytes showed fewer binding sites, possibly due to down-regulation by the higher corticosterone levels mentioned above. The resultant of high agonist concentration, higher affinity but fewer binding sites in terms of real glucocorticoid activity is difficult to compute.

With the small number of leucocytes available from individual rats, detailed studies of the leucocytes receptor are not possible and a more abundant source is necessary. Therefore, the liver cytosolic receptor was studied. At 5^0, no differences between SHR and WKY rat preparations was observed. Moreover, there were no distinguishable differences in receptor specificity.

Clear differences could however be demonstrated at the higher, physiological temperature of 37^0. Dexamethasone-binding affinity at 30 min was markedly reduced in both SHR and WKY preparations compared with overnight incubation at 5^0. At 37^0, SHR cytosol had a higher binding affinity and a lower concentration of binding sites than WKY cytosol, confirming previous results in leucocytes at 24^0. Affinity deteriorated with time in both preparations but that of the SHR cytosol remained higher than that of WKY cytosol. This difference was not due to different rates of steroid-receptor complex dissociation; both preparations showed identical dissociation rates at both 24^0 and 37^0.

The agonist-free glucocorticoid receptor is part of a heterocomplex with several heat shock proteins (Hsp) and other smaller

molecules. The Hsp are necessary for the correct folding of the receptor protein and are important in determining its binding characteristics (details discussed in section 1.4.2). Although the stabilising influence of molybdate was present in the incubation medium, a plausible explanation of these results is that at the higher temperature, the complex dissociates and that this occurs more slowly in the SHR. This in turn might be due to differences in Hsp and/or interaction with the receptor protein between strains and thus account for the higher residual binding affinity of the SHR preparation at 37^0.

Other authors (Malo et al., 1989; Hamet et al., 1992) have described the phenomenon of temperature sensitivity in genetically hypertensive rats and mice and have demonstrated that this cosegregates with blood pressure in F2 and backcross populations. They have also identified a gene polymorphism for Hsp70 between SHR and WKY rats which might account for the observed differences in thermosensitivity of dexamethasone binding. However, a later detailed study of cosegregation of the polymorphism with blood pressure in F2 SHR x WKY rat crosses failed to find any association between these variables (Lodwick et al., 1993). This study has been accepted for pubblication (Panarelli et al., 1995, see page 228).

3.3 Evidence of impaired glucocorticoid receptor affinity in the Milan hypertensive rat

3.3.1. Introduction

Among the more recent inbred strains of rat which develop hypertension in a genetically-determined manner is the Milan hypertensive rat (MHS) and its simultaneously selected normotensive strain (MNS). Detailed studies (Baer & Bianchi, 1978; Bianchi et al., 1984) have shown that hypertension in the Milan rat cosegregates with smaller kidneys containing smaller nephrons and that hypertension can be transferred to MNS by transplanting them with MHS kidneys. Also, clear differences in erythrocyte and proximal tubular cell electrolyte metabolism have been identified between strains.

Adrenocortical structure (Mantero et al., 1983) and function is also different between strains. Corticosterone secretion rate and plasma concentration are higher in MHS than MNS (Bianchi et al., 1984; Ferrari et al., 1985; Mantero et al., 1983; Stewart et al., 1993) and, in older rats, aldosterone excretion rate is also raised. In keeping with the suggestion of greater mineralocorticoid activity, MHS have higher plasma volume and total body sodium content and suppressed renin (Fraser et al., 1994).

The relationship between raised corticosterone secretion and hypertension is unclear. If, as has been suggested (Fraser et al., 1994), the increase is due to 11β-hydroxylase activity, then in an otherwise normal system, increased glucocorticoid secretion should be compensated by reduced ACTH secretion. Alternatively, high corticosterone secretion rate is perhaps sustained because glucocorticoid receptor affinity is impaired in MHS. This latter hypothesis has been tested in this section. If true, one might expect that normal glucocorticoid activity is maintained but that the excess plasma corticosterone concentrations needed to satisfy impaired Type II receptor might lead to inappropriate occupancy of Type I receptors, causing the high volume, high sodium hypertension

characteristic of the MHS rat. A precedent exists in the hypertension of human subjects with primary cortisol resistance due to mutations of the glucocorticoid receptor gene; this is also accompanied by salt and water retention (Arai & Chrousos, 1994) (see also section 1.4.4). The affinity of the liver cytosol glucocorticoid receptor for several ligands was therefore compared between MHS and MNS rats. Since impairment of receptor binding may be secondary to thermal instability of the receptor complex (Werner et al., 1992), comparison was made of differences in binding over a range of temperatures.

3.3.2 Methods

Tissue preparation, binding studies and effects of temperature on ^{3}H-dexamethasone specific binding in MHS and MNS were as described in the section 2.2.4.

Means ± standard errors (SE) were calculated for each group. Data from individual MHS or MNS cytosol prepared and analysed on the same day with the same reagents and incubation conditions were compared by Student's *t* test for paired data.

3.3.3 Results

a) Receptor affinity and concentration

The binding curves for the various steroid ligands are shown in Fig. 32 and the affinity constants in Fig. 33. Corticosterone and aldosterone compete less effectively at 5^{0} for specific ^{3}H-dexamethasone binding sites in the liver cytosol of MHS compared with MNS rats. RU486 was also less effective (data not shown because they obscure the corticosterone results). For all ligands, including dexamethasone, affinity was significantly lower in MHS liver than in MNS liver but there was no difference in glucocorticoid receptor concentration. The relative affinities for the ligands were similar between strains (dexamethasone > corticosterone = RU486 > aldosterone) but the discrepancy between the

MHS and MNS preparations was greatest for the lower affinity ligand. This is illustrated in Fig. 34 where the slope of the regression of MNS affinity on MHS affinity for the ligands was significantly less than 45^0. Thus for dexamethasone, the highest affinity ligand, the affinity constant was approximately 40% higher in MHS liver than in MNS liver. For aldosterone with the lowest affinity, the difference was approximately 340%. Thus, at 5^0 MHS rat hepatic glucocorticoid receptors have a lower affinity than those from MNS rats.

b) Temperature sensitivity and thermolability of receptor binding

The ^{3}H-dexamethasone-receptor complex was equilibrated overnight at 5^0 before being transferred to higher temperatures. At 24^0, binding was constant over a period of 3 h (Fig. 35). At 37^0, binding declined rapidly to near zero values at 1 h. There was no difference between MHS and MNS preparations. To test whether this decline in specific binding at 37^0 reflected a change in affinity and/or a change in receptor concentration, binding characteristics were measured during long term incubation at this higher temperature. Both receptor affinity and receptor capacity decreased with time at 37^0 but there was no significant difference between MHS and MNS preparations (Fig. 36).

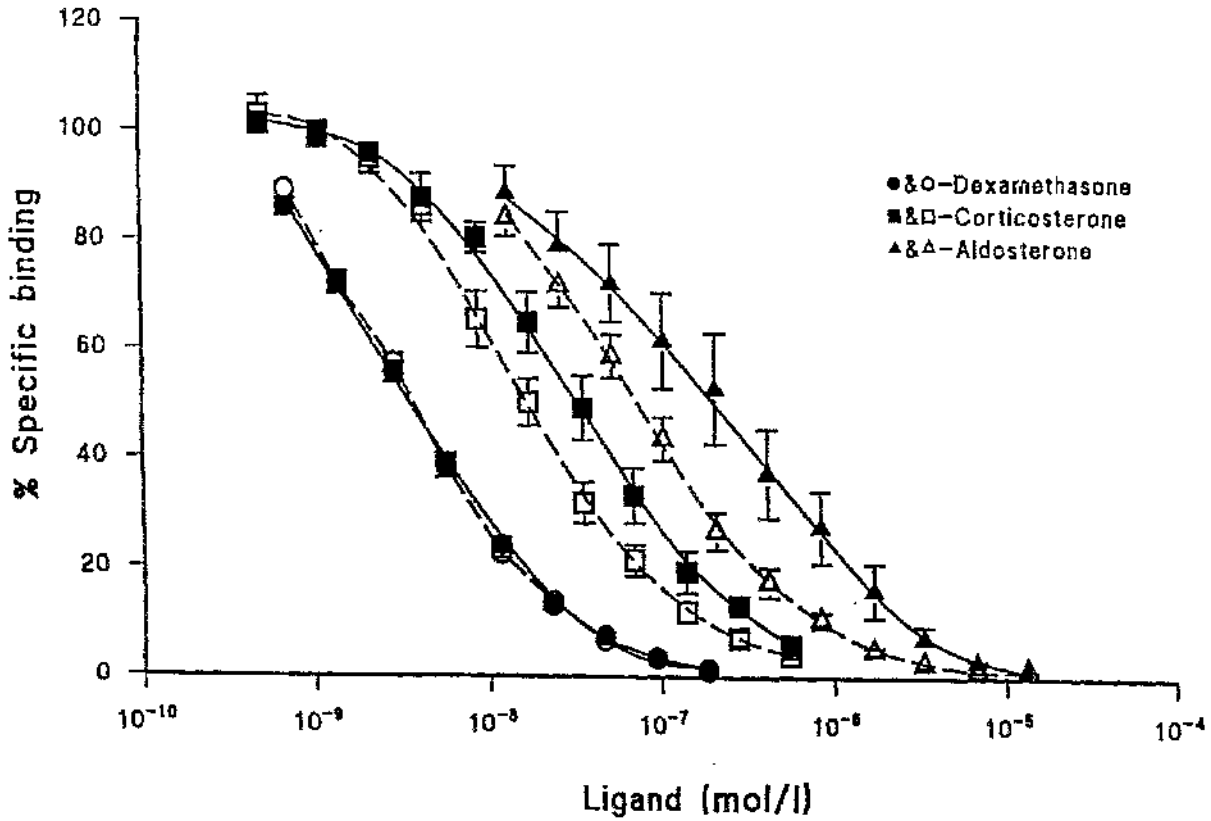

Fig. 32: Competition for specific ^{3}H-dexamethasone binding sites in liver cytosol from Milan Hypertensive (solid symbols) and Normotensive (open symbols) strains of rat (values are means ±SE; n=7).

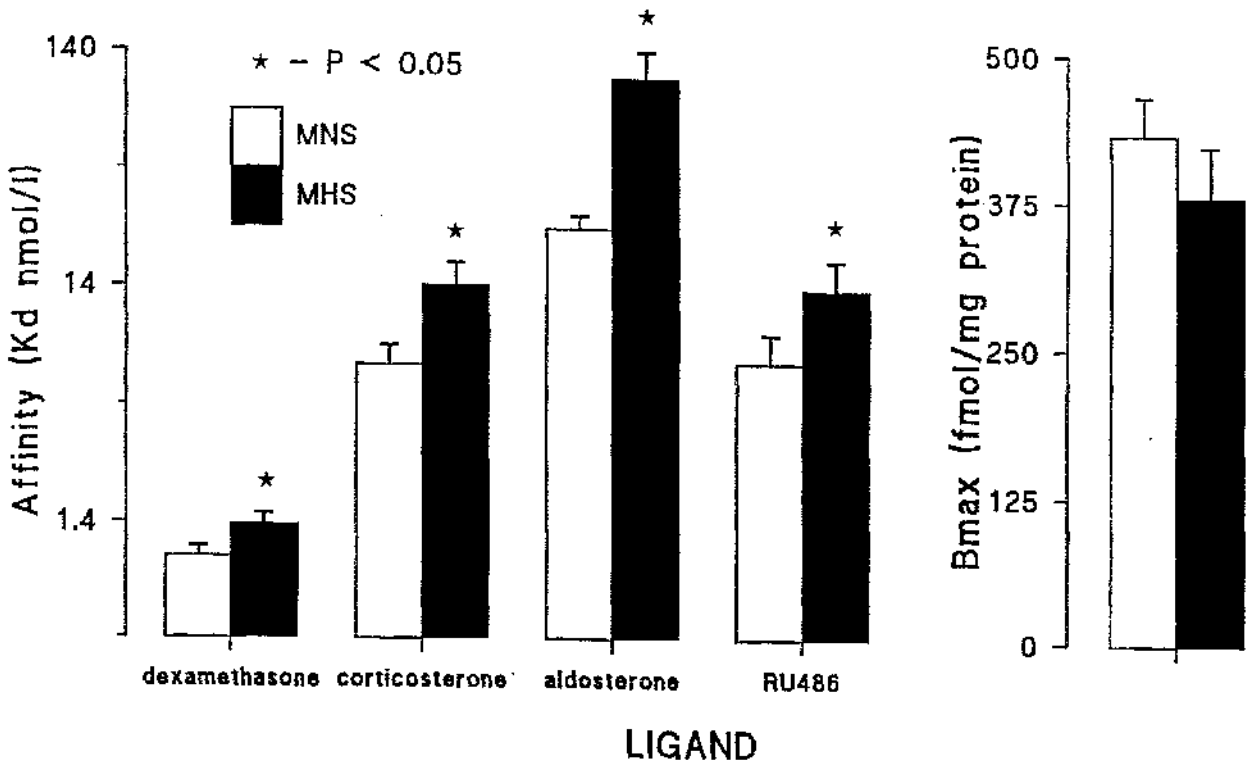

Fig. 33: Comparison of affinity constants for various ligands and binding capacities of hepatic glucocorticoid receptors in paired Milan hypertensive (MHS) and normotensive (MNS) rats (values are means ±SE; n=7)

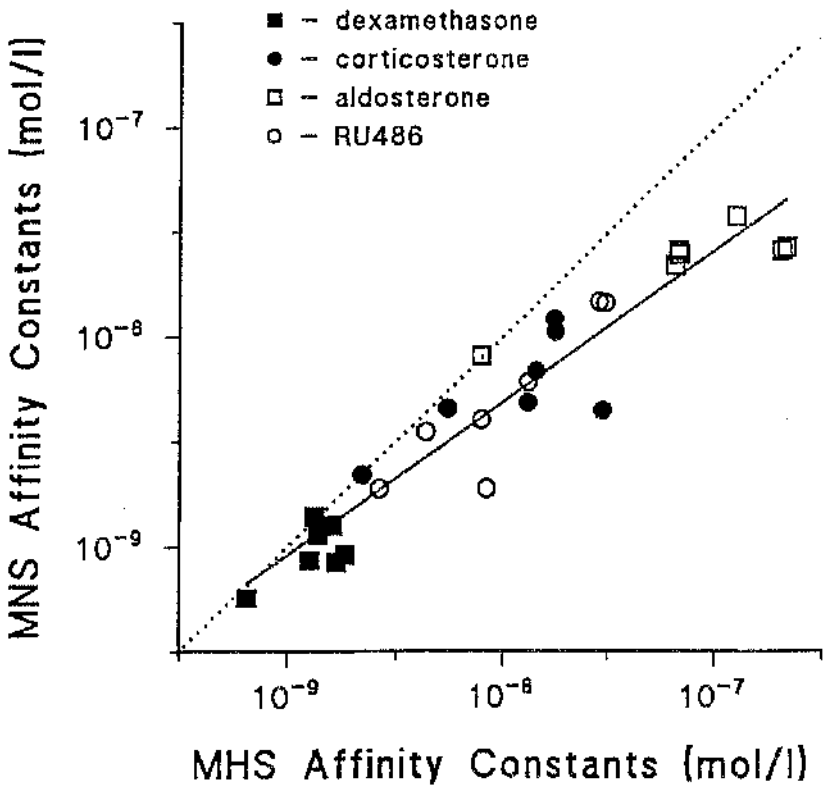

Fig. 34: Comparison of MHS liver cytosol glucocorticoid receptor affinity constants for various ligands with MNS cytosol.

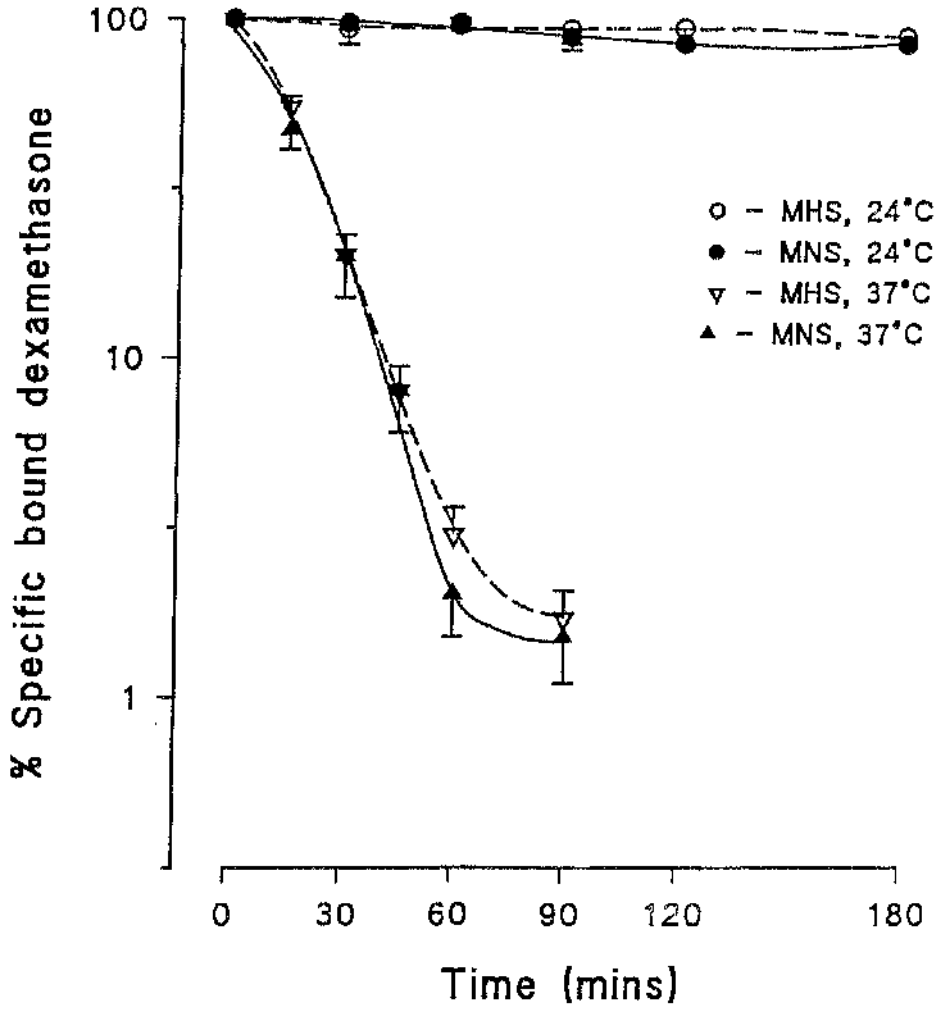

Fig. 35: Thermostability of glucocorticoid receptor complex in liver cytosol from MHS and MNS (values are means ±SE; n=5).

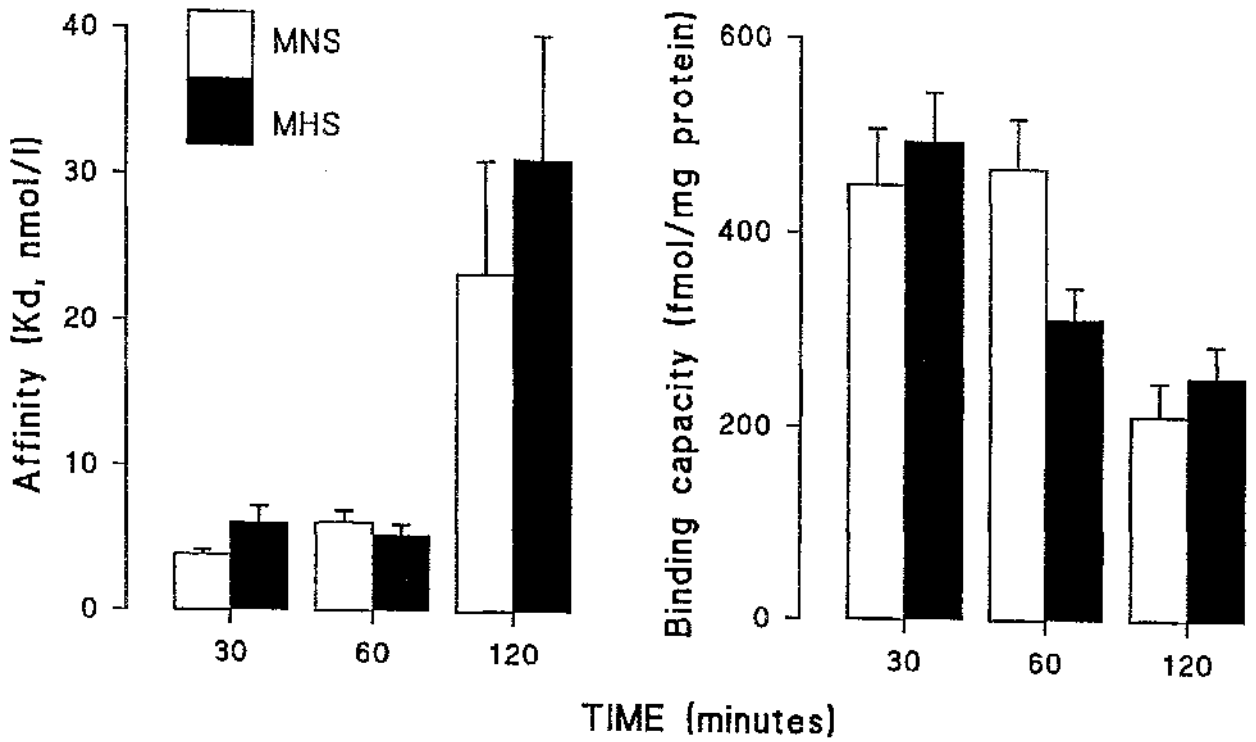

Fig. 36: Effect of prolonged incubation at 37^0 on glucocorticoid receptor binding characteristics in liver cytosol from MHS and MNS rats (values are means ±SE; n=7).

3.3.4 Discussion

Previous studies have shown that MHS rats have a high body sodium content with increased extracellular fluid volume and plasma ANP levels characteristic of hypermineralocorticoidism (Fraser et al., 1994). However, concentrations of the mineralocorticoid-type steroids, DOC and aldosterone, are not elevated in MHS during developmental stages although they may be raised in older rats.

Under normal circumstances, 11β-HSD analysis activity might be expected to protect Type I adrenocorticoid receptors from endogenous corticosterone. Stewart et al. (1993) considered the possibility that 11-βHSD might also be affected in MHS. They noted that expression of 11-βHSD activity in the liver was reduced in MHS but not in the kidney. Moreover, these studies were carried out more than 5 years ago at a time when it was not appreciated that there are two types of 11β-HSD. In the liver, the type 1 enzyme predominates and facilitates the net conversion of 11-dehydrocorticosterone to corticosterone. In the kidney, the type 2 enzyme, with different co-factor requirements, has a marked preference for corticosterone catalysing its conversion to 11-dehydrocorticosterone. The level of expression of this latter enzyme in MHS kidney has not been assessed. If it were reduced, as the type 1 in the liver, this could contribute to the mineralocorticoid activity of corticosterone in MHS.

It is theoretically possible that glucocorticoid binding could be affected by 11-βHSD activity. Reduced activity of the MHS liver type 1 enzyme would favour the inactive 11-dehydrogenated product. However, the hierarchy of difference in ligand binding between strains (dexamethasone < corticosterone ≤ RU 486 < aldosterone) does not correspond to the specificity of the enzyme. Also, binding studies were carried out in cytosol whereas the liver enzyme is microsomal. Binding efficiency for dexamethasone and corticosterone in rat liver cytosol is not affected by carbenoxolone, an inhibitor of 11-βHSD (Kenyon et al., personal comunication). Since the activity of the enzyme is regulated by

glucocorticoids, reduced glucocorticoid receptor binding affinity might explain the lower enzyme activity in the MHS liver. However, corticosterone secretion rate and plasma concentration have frequently been shown unequivocally to be raised in MHS rats throughout life. These data are compatible with the suggestion of glucocorticoid excess combined with impaired glucocorticoid receptor binding which in turn is responsible for the mineralococoticoid-type hypertension seen in these animals. More recent cosegregation studies (Kenyon et al., 1994) are contradictory whilst confirming glucocorticoid impairment. Genetic analysis of the GR gene suggests that the MHS GR genotype is associated with low blood pressure. The nature of the impairment is unlike other human examples. It is not associated with thermolability or differences in temperature-sensitive binding of ligands nor is there evidence of mutations in the hormone-binding domain (Heeley et al., personal comunication). A polymorphism in the N-terminal region in exon-2 of the GR-gene could account for the subtle differences in binding observed.

Impaired receptor binding requires raised agonist levels for normal activity. In human subjects with the syndrome of glucocorticoid resistance, high circulating cortisol levels are achieved by increased ACTH drive which also stimulates DOC secretion (Arai & Chrousos, 1994). Thus, patients develop hypertension with a hypokalaemic alkalosis. High cortisol levels may also contribute by interacting directly with Type I (mineralocorticoid) receptors. No comparison of ACTH concentrations has been made in Milan rats but DOC levels are not different between strains. However, the level of 11β-hydroxylase activity converting DOC to corticosterone appears to be higher in MHS rats (Fraser et al., 1994).

This study showed that the MHS liver cytosolic receptor has a lower affinity for the synthetic glucocorticoid, dexamethasone, than that of the MNS rat liver at 5^0. The difference was greater for the natural ligand, corticosterone. Receptor concentration was not different between

strains. Human glucocorticoid resistance has been shown to be caused by a variety of genetic defects in the receptor complex. These include point mutations at or near the steroid-binding domain. The guinea pig also exhibits glucocorticoid resistance due to mutations in this region (Keightley & Fuller, 1994) but the blood pressure implications of this are not known. Moreover, the obese Zucker rat, genetic model of juvenile onset obesity, has a decreased GR affinity in the anterior pituitary (Kd of obese rats was 50% greater than that in lean rats) suggesting a reduced feedback inhibition of corticosterone at the anterior pituitary. A similar trend was observed in liver cytosol (White & Martin, 1990). Although there was no report on blood pressure, this may suggest a possible relationship between glucocorticoid receptor and obesity (Weaver et al., 1992) and between obesity and hypertension.

Werner et al. (1992) also described human cases of increased receptor complex thermolability resulting in high turnover rates. In the current study, no evidence of differences in thermolability between MHS and MNS preparations has been found. This contrasts with the previous study of the SHR receptor where affinity was lower than that of the WKY rat receptor at 37^0 but not at 5^0 (section 3.2.). The detailed structure of the Milan rat glucocorticoid receptor gene has not yet been elucidated; although polymorphic differences between the MHS and MNS gene have been identified (Kenyon et al., 1994), their functional significance remains to be tested.

In summary, the results of this study suggest that the increased secretion rate of corticosterone in the MHS rat compared with the MNS rat is due to impaired glucocorticoid receptor binding due not to structural differences of or interaction with heat shock proteins but more probably to a modified ligand binding domain. The markedly raised corticosterone levels may then contribute to hypertension by interacting with Type I receptors to cause sodium retention. In terms of blood pressure regulation, GR impairment appears to have a negative effect on blood pressure

because of reduced glucocorticoid activity.

Chapter 4 GLUCOCORTICOID RECEPTOR AND CARDIOVASCULAR VARIABLES IN NORMOTENSIVE MALE VOLUNTEERS

4.1 Are glucocorticoid receptor genotypes associated with differences in binding characteristics and/or glucocorticoid responses?

4.1.1 Introduction

Glucocorticoid hormones (i.e. cortisol in man) have widespread effects on cardiovascular function and they probably raise blood pressure directly by a Type II receptor-dependent mechanism. In the earlier sections, the evidence has been reviewed that excess adrenal steroid secretion (e.g. Cushing syndrome) can cause hypertension and accelerated development of arterial disease. Glucocorticoid receptors are present in the cells of tissues involved in cardiovascular homeostasis (e.g. brain, kidney, blood vessels, heart). Following binding of glucocorticoid to the Type II receptor, the receptor-steroid complex binds to genetic control elements and alters gene expression (Miesfeld, 1990; Allan et al., 1991).

In the case of mineralocorticoid excess, there are a number of interrelated causes in the pathogenesis of hypertension including volume expansion, vascular hypertrophy, sensitisation to vasoconstrictors and increased contractility of vascular smooth muscle due to changes in membrane cation permeability. Glucocorticoid activity is less well understood. Using an animal model of glucocorticoid-induced hypertension created by low dose infusion of dexamethasone (Tonolo et al., 1988), a number of possible mechanisms have been investigated: altered G-protein regulation, reduced synthesis of vasorelaxants such as prostacyclin, atrial natriuretic peptide and nitric oxide (refer to table II, section 1.4.2), increased vascular reactivity to catecholamine (Russo et al., 1990), altered cation transport (Bastl et al., 1991; Berk et al., 1988; Freiberg et al., 1982), and activation of the renin-angiotensin system.

Although all of these factor are regulated by glucocorticoids, it is not clear that any one mechanism is of overriding importance in blood pressure control. However, some of these influences are exerted at concentrations of glucocorticoid hormones which are less than those required to produce symptoms of Cushing's syndrome. This apparent increase in sensitivity to glucocorticoids could be due to an abnormality at the receptor level. Support for this hypothesis derives from both *in vitro* studies (alteration of the glucocorticoid receptor binding by modification of the GR gene in the binding domain) and clinical observations (i.e. primary cortisol resistance syndrome).

Watt et al. (1992) reported that polymorphisms of the glucocorticoid receptor gene appear to be associated with a tendency to develop hypertension. When genomic DNA is digested with the restriction enzyme Bcl I, a cDNA probe to the glucocorticoid receptor identifies two alleles ("a" and "A"). Young adults with a familial predisposition to hypertension showed a small but significant increase in plasma cortisol concentration and a higher frequency of the genotype "AA" than in controls although this only achieved a statistical probability of 10%. However, when subjects with the genotype AA and aa were compared, those with AA had significantly ($p<0.05$) higher blood pressure scores. It could be argued of course, that a raised cortisol concentration is a consequence of a less effective receptor subtype leaving total glucocorticoid activity unchanged but one of the many factors which respond to cortisol, renin substrate concentration, is also raised. Similarly, it has been reported that plasma corticosterone concentrations and renin substrate levels are elevated in SHR compared to WKY rats. Differences in affinity and number of glucocorticoid receptors in SHR leucocytes compared with those of WHY rats has been noted by others and in the initial experimental part of this thesis (refer to section 3.1.).

In a separate study of the same polymorphism , Weaver et al., (1992) reported that inheritance of the A allele was associated with

hyperinsulinaemic obesity; blood pressure in this study was not reported. However, this condition frequently coexists with essential hypertension and is a risk for cardiovascular disease. Thus, both studies support the notion that genetic variability of the glucocorticoid receptor is associated with phenotypic changes implicated in the development of high blood pressure and atherosclerosis.

It is appropriate therefore to investigate more thoroughly the possible physiological and pathophysiological links between genotypic differences in glucocorticoid receptors, plasma glucocorticoid concentrations, glucocorticoid responsiveness and blood pressure regulation.

4.1.2 Methods

a) Subjects

Sixty-four Caucasian normotensive males aged 18-40 years were sampled. All subject were admitted at 08.30 h and blood samples were collected after the subjects had been lying at least 30 min. Family history of hypertension, diabetes mellitus and other cardiovascular diseases was collected. Blood pressure was measured (by random zero sphygmomanometer) twice on the same occasion in each individual, supine and after standing for at least 5 min. The mean of the two readings was used for analysis. Mean arterial blood pressure was calculated from the formula MAP=DBP+1/3 (SBP-DBP), where DBP and SBP were the diastolic and systolic blood pressure respectively. Height and weight were measured to calculate body mass index (BMI) from the formula BMI= weight/height^2. Body fat determinations were carried out by skinfold caliper, measuring representative sites throughout the body. These measurements were then put into a mathematical equation (which considers adjustments for age and sex of the subjects) to estimate the body's density and then this was converted to percent of fat. Venous blood was then drawn for routine biochemical and hormonal measurement,

DNA extraction and HML separation. A 24 h urine specimen was obtained prior the visit to the clinic. Finally, a skin vasoconstriction test was carried out in each individual. If the volunteer was a smoker, he was requested to abstain for at least 24 h before and during the test.

b) Procedures

Hormonal and routine biochemical measurements, DNA extraction and glucocorticoid receptor genotypical characterization were as described in the sections 2.1.6 - 2.1.9. HML preparation, glucocorticoid receptor binding measurement in mononuclear leucocytes and *in vitro* responsiveness to glucocorticoid in terms of lysozyme inhibition assay were as described in the sections 2.2.1, 2.2.2 and 2.2.3. The *in vivo* responsiveness to glucocorticoid as skin vasoconstriction test was as described in the section 2.1.10.

c) Statistics

The means±SEM for each variable were calculated for the three glucocorticoid receptor genotypes and compared by Student's t test.

4.1.3 Results (for overall summary refer to tables VII,VIII, IX, X and XI)

a) RFLP analysis

RFLP phenotypes after Bcl 1 digestion and Southern blotting are shown in Fig. 37. The RFLP analysis of GR identified 6 subjects homozygous for the allele "A", 7 homozygous for "a" and 51 were heterozygous. This distribution is comparable to findings in other studies. The expectation was that the number in each of the two homozygous groups would be nearer 15.

b) Clinical measurements

Thirteen subjects were smokers: 1 was in the group of subjects homozygos for the allele "A", 2 were in the group of homozygous for "a" and 10 were in the group of heterozygous.

Twenty eight subjects had a family history of cardiovascular

disease of which 2 were AA, 3 aa and 21 were Aa. The remaining 36 subjects had no family history for cardiovascular disease: 4 subjects were AA, 4 aa and 27 were heterozygous.

Systolic, diastolic and mean blood pressure, body mass index and fat distribution from skin-fold thickness measurements were not different between the three GR genotypes.

c) Whole-cell GR binding

Steroid binding studies were carried out in suspensions of mononuclear cells by homologous competition for ^{3}H-dexamethasone specific sites. In the overall group of 64 male volunteers, the mean±SEM number of receptor sites was 5537±209/cell (range 2322-8879); the affinity was 6.2±0.35 nmol/l (range 3.9-14 nmol/l).

Although a lower apparent affinity for dexamethasone was measured in the group AA, there was no significant statistical difference between genotypes.

d) In vitro responsiveness to glucocorticoid: lysozyme inhibition test

Inhibition of lysozyme synthesis by dexamethasone in mononuclear leucocytes (section 2.2.3) was used to assess *in vitro* responsiveness to glucocorticoids.

When comparing GR genotypes, IC_{50} values tended to be higher in subjects with the AA genotype compared to aa but again this was not statistically significant.

e) In vivo responsiveness to glucocorticoid: skin vasoconstriction test

The skin vasoconstriction test was used to investigate responsiveness to glucocorticoid *in vivo*. Various doses of budesonide were applied to the skin of the flexor aspect of the forearm and the degree of blanching was assessed after 18 h (see Fig. 38 and section 2.1.10). For each volunteer, a response curve was plotted and an ED_{50} value for budesonide calculated. The ED_{50} values for budesonide in the 64 male volunteers ranged from 2-90 ng (mean±SEM 2.8±3).

The AA group showed a statistically significant higher sensitivity

to budesonide compared to aa (p=0.02).

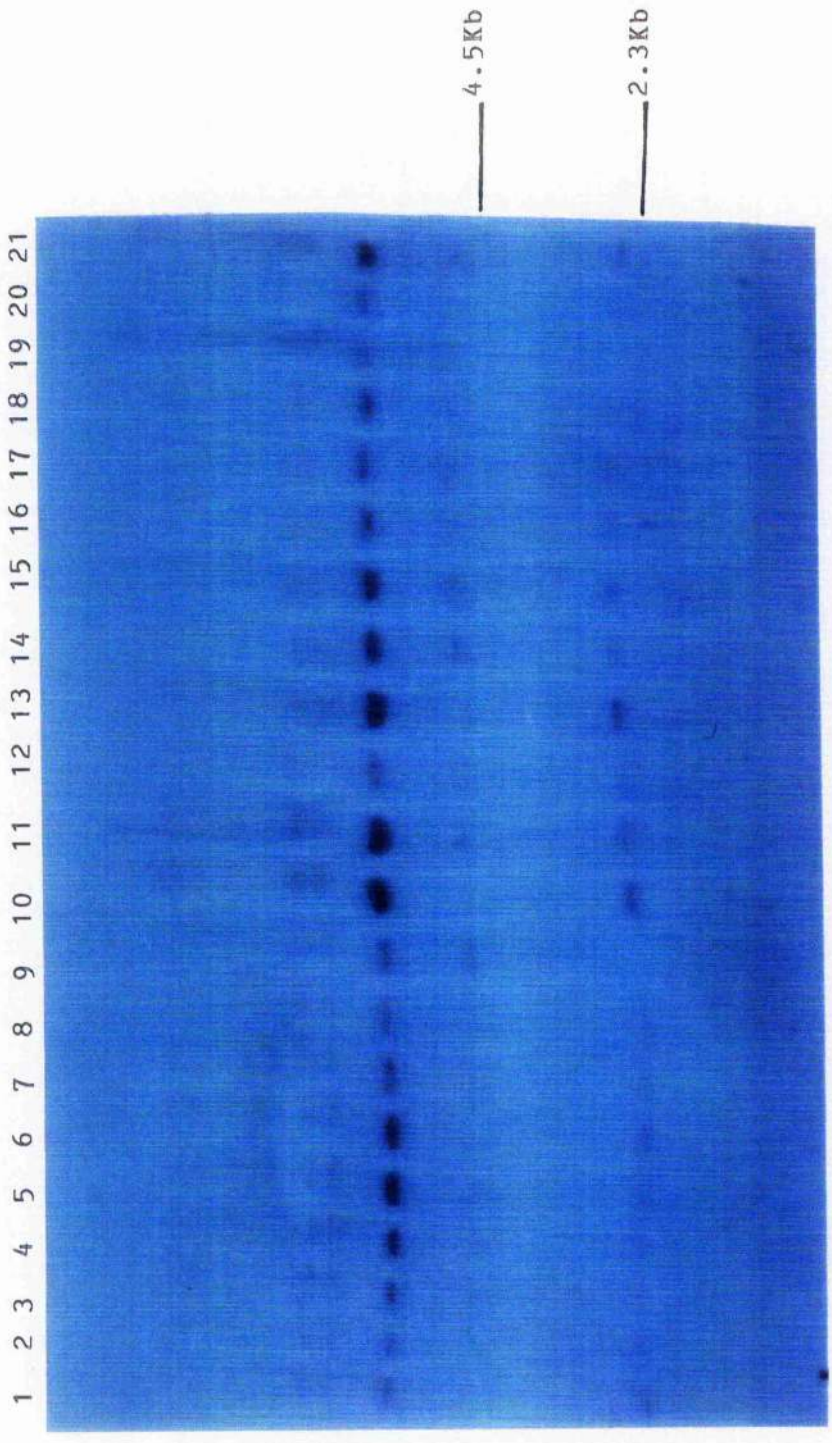

Fig. 37: Example of Southern blotting. This is an autoradiograph of a Southern blot probed with glucocorticoid receptor cDNA (OB7). Band sizes are in kilobases and 3 phenotypes are shown, 4.5:4.5 (AA), 4.5:2.3 (Aa) and 2.3:2.3 (aa). Lane 9 and 19 show 4.5:4.5, lane 1, 10 and 13 show 2.3:2.3.

Fig. 38: Skin vasoconstriction test. Representative blanching response to various concentrations of budesonide applied topically.

Table VII: Summary of clinical measurements (values are means ±SE)

Receptor genotype	Age	Smokers	Fam. hyst. cardiovasc	BMI	%Fat	CLISBP	CLIDBP	ORTSBP	ORTDBP	MAPCLI	MAPORT
AA n=6	27 ±1.6	1	2	24.4 ±1.1	17.2 ±2.4	111 ±2.9	71 ±4.5	109 ±4.6	78 ±3.6	84 ±3.8	88 ±4.5
Aa n=51	29 ±0.7	10	21	24.9 ±0.4	19.3 ±0.8	112 ±1.3	72 ±1.3	109 ±1.3	76 ±1.3	85 ±1.1	87 ±1.2
aa n=7	30 ±2.5	2	3	23.9 ±0.8	17.7 ±1.6	111 ±3.1	71 ±1.8	113 ±3.7	79 ±2.3	89 ±1.6	90 ±2.7
All n=64	29 ±0.70	13	28	24.7 ±0.35	18.9 ±0.68	112 ±1.13	72 ±1.11	110 ±1.2	76 ±1.13	86 ±0.9	88 ±1.1

Note: CLISBP and CLIDBP= Systolic and diastolic blood pressure measured in supine position; ORTSBP and ORTDBP= systolic and diastolic blood pressure measurements in upright position; MAP= mean arterial blood pressure.

Table VIII: Summary of biochemical results (values are means ±SE)

Rec. Genot.	Chol. mmol/l	Trig. mmol/l	Plasma Na^+ mmol/l	Plasma K^+ mmol/l	Plas. Ca^{++} mmol/l	Urinary Na^+ mM/24h	Urinary K^+ mM/24h	Urinay Ca^{++} mM/24h	UNa/K ratio	Urinary vol. l	Serum Creat. µmol/l	Urinary Creat. mM/24h	Creat. Clear. ml/24h	GFR ml/24h
AA n=6	4.9 ±0.24	1.35 ±0.27	139 ±0.67	3.7 ±0.15	2.36 ±0.04	165 ±39.3	60.6 ±4.4	5.3 ±3.1	2.9 ±0.76	1.64 ±0.5	88.3 ±5.6	16.9 ±1.6	136.2 ±13.3	100.5 ±9.2
Aa n=51	4.8 ±0.12	1.25 ±0.1	141 ±0.3	4.0 ±0.03	2.32 ±0.01	174 ±10.8	69.7 ±2.89	4.7 ±0.04	2.6 ±0.15	1.45 ±0.08	88.9 ±1.9	16.1 ±0.5	131.6 ±4.32	99.4 ±2.8
aa n=7	5.0 ±0.35	1.18 ±0.28	142 ±0.96	4.0 ±0.1	2.32 ±0.02	149 ±23.6	55.6 ±6.6	4.2 ±1.32	2.8 ±0.35	1.1 ±0.09	92.4 ±3.7	15.1 ±0.9	111.3 ±7.81	91.3 ±7.2
All n=64	4.8 ±0.11	1.25 ±0.08	141 ±0.27	3.9 ±0.03	2.3 ±0.009	170 ±9.6	67.3 ±2.5	4.7 ±0.3	2.6 ±0.14	1.43 ±0.08	89.2 ±1.69	16.1 ±0.45	129.8 ±3.8	98.6 ±2.5

Table IX: Summary of haematological measurements (values are means ±SE)

Receptor genotype	WBC x 10^9/L	Neutrophiles x 10^9/L	Lymphocytes x 10^9/L	Monocytes x 10^9/L	PLT x 10^9/L	Hgb gr/dl	Haematocrit %
AA n=6	6.73 ±0.61	3.67 ±0.421	2.37 ±0.199	0.502 ±0.068	240 ±30.5	15.1 ±0.26	43 ±0.6
Aa n=51	5.73 ±0.51	3.31 ±0.149	1.85 ±0.092	0.397 ±0.023	231 ±6.7	14.6 ±0.15	42 ±0.3
aa n=7	6.06 ±0.26	3.7 ±0.527	1.9 ±0.137	0.477 ±0.107	249 ±24.5	14.5 ±0.34	42 ±0.7
All n=64	5.87 ±0.2	3.38 ±0.136	1.91 ±0.078	0.415 ±0.023	240 ±6.49	14.6 ±0.127	42 ±0.3

Table X: Summary of hormonal measurements (values are means ±SE)

Receptor genotype	Plasma F nmol/l	Plasma Aldo nmol/l	Urinary F nM/24h	Urinary Aldo nM/24h	THE µg/24 h	THF µg/24 h	Allo THF µg/24 h	Metabolites Ratio	Renin ngAII/ml/h
AA n=6	551.6 ±67.2	0.362 ±0.05	104.3 ±15.7	30.9 ±4.9	1061 ±191	397 ±80.4	283 ±31.4	0.69 ±0.08	38.7 ±11.7
Aa n=51	417 ±16.8	0.385 ±0.03	100.2 ±8.6	27.9 ±1.9	878 ±68.9	334 ±32.6	311 ±21.4	0.77 ±0.03	29.7 ±2.34
aa n=7	464.8 ±33.6	0.310 ±0.064	91.8 ±16.2	19.9 ±3.9	735 ±158	351 ±60.1	396 ±81.4	0.87 ±0.102	34.1 ±7.12
All n=64	434 ±16.8	0.365 ±0.026	99.6 ±7.2	27.1 ±1.6	879 ±60.2	342 ±19.5	317 ±19.5	0.78 ±0.03	30.86 ±2.2

Table XI: Summary of glucocorticoid receptor responsiveness *in vitro* and *in vivo* measurements (values are means ±SE; * p = 0.02 AA vs. aa)

Receptor Genotype	**Glucocorticoid receptor affinity for dexamethasone (Kd) nM**	**Glucocorticoid receptor capacity (Bmax) sites/cell**	**Lysozyme inhibition to dexamethasone IC_{50} nM**	**Skin vasoconstriction EC_{50} ng**
AA n=6	7.7 ±1.56	6050 ±753	3.7 ±1.4	13* ±0.4
Aa n=51	6.0 ±0.4	5537 ±228	2.5 ±0.2	28 ±0.3
aa n=7	5.9 ±0.82	5103 ±747	2.0 ±0.25	41 ±0.9
All n=64	6.1 ±0.3	5537 ±209	2.6 ±0.21	28 ±0.3

4.1.4 Discussion

Endogenous glucocorticoids (i.e., cortisol in man and corticosterone in rats) can bind to two types of receptor. The Type I or mineralocorticoid receptor evokes effects on salt and water metabolism, the Type II or glucocorticoid receptor is conventionally linked to effects on intermediary metabolism (for details refer to section 1.2.1). However, Type II receptor responses can involve changes in electrolyte metabolism too (Berk et al., 1988; Bastl et al., 1991; Freiberg et al., 1982). Normally the access of cortisol and corticosterone to Type I receptors in the kidney and other tissues is prevented by the activity of the 11β-hydroxysteroid dehydrogenase converting cortisol to cortisone and corticosterone to 11-dehydrocorticosterone (Edwards et al., 1988).

In the literature review sections, it was emphasized that glucocorticoids, either via Type I or Type II receptors, can cause an increase in blood pressure. Moreover, two separate studies associated a particular RFLP for GR with hypertension (Watt et al., 1992) and hyperinsulinaemic obesity (Weaver et al., 1992). Because no abnormality of GR function had been identified, the main purpose of the current study was to investigate whether genotype variations of Type II receptor are associated with altered receptor function which in turn might lead to variations in blood pressure.

The GR genotypes in this study were based on a RFLP after digestion of genomic DNA with the enzyme Bcl I. The restriction site for this enzyme appears to be a non-coding region at the 5' end of the gene (Hollenberg et al., 1985). It is by no means certain that the amino acid sequence of the receptor is different in the three genotypes. So far there are no data on the effect of mutations within the flanking region of GR gene on receptor function. It could be that in studies where the association of the AA genotype with hypertension and obesity have been demonstrated, the polymorphism may merely have been a marker of a quite different genetic abnormality of metabolism/cardiovascular function

(i.e. linkage disequilibrium).

The whole-cell binding assay did not show differences in GR binding characteristics between genotypes nor was the lysozyme inhibition test able to distinguish differences although it is a particular sensitive index of glucocorticoid activity (refer to section 2.2.3). In contrast to the *in vitro* tests, greater sensitivity to topical budesonide was observed *in vivo* in subjects homozygous for the larger allele, A. Vasoconstriction, evaluated as degree of blanching, was obtained with lower doses of steroid in those with the AA compared with the aa genotype. There are three possible reasons for the discrepancy between *in vitro* and *in vivo* results:

a) The number of homozygous volunteers were fewer than expected. Trends showing lower Kd values and greater steroid-dependent inhibition of lysozyme release in volunteers with the A allele might have been confirmed with higher numbers in each group.

b) The synthetic glucocorticoid budesonide, which was used *in vivo*, may be better than dexamethasone, which was used *in vitro*, in demonstrating phenotypic differences. A precedent for this steroid selectivity was showed in the Milan hypertensive strain of rats (refer to section 3.3) where the phenotypic difference was more apparent in binding studies using corticosterone and aldosterone than those using dexamethasone. Unfortunately, the results of that study were not available when the protocol for the present study in volunteers was designed.

c) The polymorphism described may mark a genetic variable involved in skin vasoconstriction which is influenced by, but distinct from, the receptor gene (see above). It should be remembered that glucocorticoid hormones probably cause vasoconstriction, not by a direct effect on vascular tone but by potentiating the action of vasoconstrictors and/or by inhibiting the action of vasodilators (Russo et al., 1990). It follows that variability of any one of a large number of genes with a more direct influence on vasoconstriction could explain differences in steroid

responsiveness to steroids providing that the other candidate gene is closely linked to the glucocorticoid receptor locus.

In addition to characterising receptor genes, this study measured several other variables concerned with electrolyte homeostasis and the control of intermediary metabolism. These results are presented and discussed separately in section 4.2.

In summary, this study provides evidence that glucocorticoid-dependent vasoconstriction is affected by glucocorticoid receptor RFLP genotype. It also supports the previous observation that individuals predisposed to develop hypertension are more likely to have AA genotype (Watt et al., 1992).

4.2 Relationships of glucocorticoid receptor and phenotypes with cardiovascular and other metabolic variables

4.2.1 Introduction

The interrelationships of glucocorticoid receptor affinity and concentration with aspects of *in vitro* and *in vivo* phenotype and with RFLP genotype have been described and discussed. Cortisol also has predictable effects on many aspects of intermediary and electrolyte metabolism and indices of these were measured in the human population group. It was of interest to relate the values found for these to the principal determinants of glucocortiocid activity, cortisol levels and receptor affinity and capacity. As a possible control analysis, the relationship of mineralocorticoid-dependent changes to aldosterone levels was also examined.

4.2.2 Method

The same variables described in section 4.1.2 were considered. Correlation analysis between variables was carried out. A correlation coefficient *r* of 0.25 was critical to define a significance level of $p = 0.05$ with a sample of 64 subjects.

A full list of correlations is provided in appendix A.

4.2.3 Results (for more details see table in appendix A)

a) Cortisol

Urinary free cortisol excretion rate correlated positively with sodium and potassium excretion rate supporting the knowledge that glucocorticoid at physiological levels promote Na^+ and K^+ excretion. Cortisol did not correlate with Na^+/K^+ ratio which is usually taken as an index of mineralocorticoid activity. However, plasma cortisol concentration, an inferior index of secretion rate, correlated negatively with Na^+ excretion rate and Na^+/K^+ index. Neither plasma concentration

nor urinary excretion rate of cortisol correlated with plasma Na^+ and K^+ concentration. Urinary cortisol excretion rate correlated positively with white cell count, a known glucocorticoid-sensitive variable (Shoenfeld et al., 1981) and to creatinine clearance rate which is used as an index of GFR, known to increase during glucocorticoid administration (Bastl & Sebastian, 1987). However, the estimated GFR itself did not correlate with cortisol levels. There was a negative correlation between urinary cortisol excretion rate and age. Plasma and urinary cortisol levels did not correlate with one another.

b)Aldosterone

Urinary aldosterone excretion rate and plasma aldosterone concentration showed significant positive correlation; neither correlated with GFR. As the principal mineralocorticoid, aldosterone would be expected to cause Na^+ retention and K^+ loss. While urinary free aldosterone did correlate negatively with Na^+ excretion rate, as did Na^+/K^+ index, there was no correlation with urinary K^+. Plasma K^+ and urinary aldosterone correlated negatively. A negative correlation between plasma aldosterone and Na^+ excretion rate and a positive correlation with haematocrit were as expected. Plasma renin levels are a determinant of aldosterone secretion and these should therefore be positively correlated. However, renin and aldosterone did not correlate in this study, even though there was a negative correlation between renin and Na^+ excretion rate.

c) Blood pressure and metabolic indices

Obesity is frequently associated with hypertension. The two measurements relating to this were skinfold thickness and body mass index (BMI). Both correlated positively with blood pressure, urinary Na^+ excretion rate and GFR. Skinfold thickness also correlated with age and with serum cholesterol concentration; BMI correlated with triglyceride

levels.

d) Calcium levels

Glucocorticoids are known to influence Ca^{++} metabolism (Findling et al., 1982). In this study, the urinary calcium excretion rate correlated positively with urinary cortisol excretion rate but not with plasma cortisol concentration, while plasma calcium concentration correlated with neither plasma cortisol concentration nor urinary free cortisol excretion rate. Urinary calcium excretion rate correlated with THE, THF and alloTHF but did not correlate with THF+alloTHF/THE ratio. However, none of these metabolites correlated with plasma calcium concentration. Urinary calcium excretion rate correlated positively with urinary Na^+ and K^+ excretion rate and urinary Na^+/K^+ ratio, while it correlated negatively with haemoglobin, haematocrit, renin and plasma aldosterone concentrations. Plasma calcium concentration correlated negatively only with diastolic blood pressure measured in supine position, while urinary calcium excretion rate correlated with none of the blood pressure measurements.

e) Cortisol, cholesterol and blood pressure

Although cortisol is a key controlling factor in lipid metabolism, there were no correlations between cortisol levels and skinfold thickness, BMI, serum cholesterol or triglyceride in this study. However, there were strong correlations between glucocorticoid receptor affinity (Kd) and cholesterol and between cholesterol and systolic blood pressure measured in the lying position. Both systolic blood pressure and serum cholesterol are risk factors for cardiovascular disease.

4.2.4 Discussion

Urinary free cortisol excretion was a better index of cortisol activity than plasma level as is shown by its correlations with glucocorticoid-related variables. Although a blood sample for plasma cortisol concentration was always withdrawn at the same time of the day in each subject, this proved to be an inferior index of secretion rate. Indeed, in this study there was no correlation between plasma and urinary cortisol. As expected, urinary cortisol excretion rate correlated positively with urinary sodium and potassium excretion rate, white blood and polymorphonuclear cells count, and creatinine clearance. Although glucocorticoids usually do not change Na^+ excretion, in large amounts they may cause natriuresis. In fact glucocorticoids, by increasing GFR, enhance the amount of filtered Na^+ with consequent increase of salt and water excretion (Bastl & Sebastian, 1987). Glucocorticoids at physiologic doses markedly increase K^+ excretion by both increasing urine flow through increases in GFR and, possibly, by increasing protein catabolism with release of intracellular K^+ (Stanton et al., 1985).

As already mentioned, glucocorticoids increase GFR. In whole-kidney experiments, glucocorticoids decrease renal vascular resistance mediated by large decreases in afferent arteriolar resistance and smaller decreases in efferent resistance (Baylis & Brenner, 1978). It appears then that glucocorticoids are renal vasodilators, probably attenuating the effect of vasoconstrictors such as epinephrine, norepinephrine, angiotensin II and ADH in a dose dependent manner. It has been suggested that ANP production is regulated by glucocorticoid (Gardner et al., 1986). Since ANP appears to produce its physiological effect by renal vasodilation and the resultant increase in GFR, it has been speculated that it is by this mechanism that glucocorticoids enhance GFR. In this study, there was only a positive correlation between urinary cortisol secretion rate and creatinine clearance, a commonly used index of GFR. However, neither plasma nor urinary cortisol correlated with the calculated GFR. As

reported in the method section (refer to section 2.1.7), individual GFRs were estimated using a formula which depend on serum creatinine concentration alone. This calculation is based on the assumption that under normal circumstances, serum creatinine remains fairly constant unless renal function, i.e. GFR, changes. If endogenous production of creatinine remains constant then serum creatinine will be inversely proportional to GFR. However, one of the estimation problems is that the precision of serum creatinine determinations increases as the creatinine concentration rises. Since all the serum creatinine concentrations were within the reference range it is likely that the measurements were subject to variations. Indeed, the calculation of the creatinine clearance, by applying Fick's principle, gives better results in the estimation of GFR in healthy subjects.

Glucocorticoid administration to human subjects causes a circulating lymphocytopenia, monocytopenia and eosinopenia but increases the blood polymorphonuclear leucocyte concentration (Shoenfeld et al., 1981). Glucocorticoids accelerate the release of neutrophils from the bone marrow, increase the half-life of circulating neutrophils and neutrophils egress from the blood. In this study, only a positive correlation between urinary cortisol excretion rate and white blood cells and polymorphonucleate leucocytes (which largely influence the total white blood cells count) was observed, while neither plasma nor urinary cortisol correlated with lymphocytes and monocytes.

Urinary aldosterone excretion rate was a better index of aldosterone secretion rate than plasma aldosterone concentration. In this instance the effect of posture is important in plasma aldosterone measurement. However, the samples were withdrawn in each subject after at least 30 min of recumbent position. Contrary to what was expected, there was no correlation between urinary K^+ excretion and either plasma or urinary aldosterone. However, both plasma and urinary aldosterone correlated negatively with Na^+ excretion rate and urinary Na^+/K^+ ratio

where the first indicates the sodium retentive property of mineralocorticoids and the second is an index of mineralocorticoid activity. A limitation of this study in the evaluation of mineralocorticoid effects is that the subjects took an unrestricted salt diet. This may have affected correlations between plasma and urinary aldosterone and some of the mineralocortiocid-dependent variables and between renin and aldosterone in this study.

Findling et al. (1982) reported that although urinary calcium excretion was directly correlated to urinary free cortisol in Cushing's syndrome, there was no correlation during eucortisolemia. In this study a positive correlation between urinary calcium excretion rate and urinary free cortisol excretion rate was observed, even though all the subjects had urinary cortisol levels within the reference range. Interestingly, this correlation was also present when the excretion rate of urinary metabolites of cortisol (THE, THF and allo THF) was used as an index of adrenal activity. Glucocorticoid hormones are known to regulate bone and mineral homeostasis, excess leading to osteopenia by an imbalance in bone remodelling (Reid, 1993). They act on osteoblasts to decrease collagen synthesis and diminish conversion of precursor cells to functioning osteoblasts.It has also been proposed that glucocorticoids reduce intestinal calcium absorption and tubular reabsorption of phosphate which leads to increased urinary phosphate and calcium excretion and decreased serum calcium concentrations. However, in this study there was no relationship between either plasma or urinary glucocorticoid on serum calcium concentrations. Moreover, a positive correlation between urinary calcium excretion rate and urinary Na^+ and K^+ excretion rate and urinary Na^+/K^+ ratio was observed. This may indicate an expected relation between urinary calcium excretion and mineralocorticoids. Indeed it has been reported that inhibitory effects of NaCl-induced volume expansion and mineralocorticoid excess on calcium reabsorption by the collecting ducts results in increased urinary calcium

excretion (Marx & Bourdeau, 1987). However, in this study urinary calcium excretion correlated negatively with plasma aldosterone concentrations while it did not correlate with urinary aldosterone secretion rate.

Despite the described influence of glucocorticoids and mineralocorticoids on blood pressure control and of glucocorticoids on lipid metabolism, this study showed no correlation between these variables. In glucocorticoid excess, there is an increase in VLDL, LDL and HDL with consequent elevations of total triglyceride and cholesterol levels. In this study, there was no correlation between cortisol and cholesterol or triglyceride, even though there was a positive correlatation between glucocorticoid receptor affinity and total cholesterol concentrations. Although glucocorticoids are reported to inhibit the synthesis of cholesterol, at present the significance of the positive correlation between cholesterol and glucocorticoid receptor affinity needs further assessment.

In summary, in this study plasma total cholesterol concentrations correlated with systolic blood pressure and Kd values for dexamethasone binding. The same glucocorticoid variables also interacted with plasma and urinary electrolyte concentrations (sodium, potassium and calcium). These results, from a group of normal volunteers, may suggest a complex interaction of all these variables in the pathogenesis of hypertension.

Chapter 5 GENERAL CONCLUSIONS

The aetiology of essential hypertension has been discussed in relation to corticosteroid hormone in general and glucocorticoids in particular (Chapter 1). A series of experiments designed to investigate the importance of genetically-determined variations in the glucocorticoid receptor mechanism in the inheritance of hypertension in man and in hypertension-prone strains of rat has been carried out (Chapter 3). These investigations required the adaptation, validation and use of a number of biochemical and cell biology techniques and the development of a new method of measuring glucocorticoid potency (refer to section 2.3). My principal aims have been to measure glucocorticoid receptor activity accurately and specifically, to study this activity in man and well-established relevant rat models of genetic hypertension and to attempt to relate activity to receptor genotype and to cell and whole organism physiology.

Preliminary results obtained measuring the GR binding in mononuclear leucocytes from SHR (refer to section 3.1) lead to the development of a protocol to study in details the receptor binding characteristics in rat liver cytosol preparations. This considered not only sensitivity and specificity of ligand binding but also thermolability and thermostability of the GR-ligand complex.

In SHR liver cytosol, the difference in binding affinity observed using mononuclear leucocytes were not initially confirmed. One possible explanation of this discrepancy was the difference in the method of incubation. The binding in ML was carried out at 24^0 for 3 h whereas the liver cytosol was incubated overnight at 5^0. A temperature factor seemed likely. The review of the mechanism of action of the glucocorticoid receptor helped to interpret the results. The affinity of GR is influenced by the association with the Hsp; the GR-Hsp complex has high affinity for the ligands and can be dissociated not only by the binding with the ligand

itself but also by high temperatures and sodium concentrations (Nemoto et al., 1990a). When GR is not associated with Hsp, the binding affinity is reduced by approximately 100-fold. Indeed, the results of liver cytosol binding studies at higher temperature showed a lower affinity for dexamethasone for both SHR and WKY but the binding affinity in the SHR liver cytosol preparation was consistently higher than in WKY preparations (refer to section 3.2). A possible explanation of these results is that the relationship of the Hsp to the receptor protein in the ligand-free cytosolic receptor complex is different between strains. This abnormality may be due to either a mutation in the receptor protein or in the Hsp. However, both influence the stability of the GR-Hsp complex which in turn affects the affinity for the ligand. At present there is some evidence of a genetic difference in Hsp70 between SHR and WKY (Hamet et al., 1992), even though there are contradictory data on its cosegregation with blood pressure (Lodwick et al., 1993). The important point highlighted from this set of experiments is that although differences in receptor binding affinities have been identified, these, at least in SHR, do not reside in abnormalities in the GR binding domain but in Hsp or in the formation of the GR-Hsp complex.

The hypothesis of decreased glucocorticoid receptor activity was also tested. Impaired Type II receptor affinity can lead to excessively high plasma cortisol concentrations which although unable to bind to Type II receptors can bind to Type I sites, resulting in excess mineralocorticoid activity. A number of rare cases of primary cortisol resistance have been identified (Chrousos et al., 1982; Arai & Chrousos, 1994). These patients have hypertension caused by a mutation in the Type II receptor. A number of phenotypic differences including raised plasma cortisosterone suggested that MHS rats might also have a mineralocorticoid-like hypertension (refer to section 4.3) due to Type II receptor impairement. Binding studies with liver cytosol confirmed that affinities for a variety of ligands was lower in MHS compared with MNS. Unlike SHR,

differences in binding were not due to thermoinstability. Subsequent genetic studies have shown that the MHS GR gene has a negative effect on blood pressure, presumably due to reduced glucocorticoid activity. The suggestion that raised plasma corticosterone concentration leads to excess mineralocorticoid activity has been neither confirmed nor denied.

The last experimental section of this thesis investigated whether the polymorphism of the glucocorticoid receptor gene identified in the Ladywell study (Watt et al., 1992) has any physiological significance. This part of the project aimed to investigate links between polymorphism of the gene for GR and differences in GR characteristics and glucocorticoid responsiveness in a group of normal volunteers. Blood pressure, plasma and urinary steroid concentrations, GR binding characteristics, responsiveness to glucocorticoids (*in vivo* and *in vitro*) and other routine biochemical variables have been compared. The results did not show differences in GR binding or *in vitro* sensitivity to glucocorticoid between genotypes. As discussed the reason why no difference in *in vitro* GR properties was observed may be due to the small numbers of homozygous individuals that were tested or that the correct biochemical test was not applied. In tests in SHR and MHS, abnormalities in ligand binding were seen when considering thermolability and ligand specificity respectively and not necessarily in homologous competition studies carried out at 24^0.

To study human mononuclear leucocytes GR binding characteristics in the same detail, as reported for the animal studies, a very large amount of blood from each individual would be required. To overcome this methodological problem, two solutions can be found: use of i) fibroblasts in culture, which requires biopsy or ii) leucocytes transformed by Eptein-Barr virus (EBV). Tomita et al. (1986) reported that the GR in immortalized leucocytes maintains the same binding characteristics as in normal cells. Only the number of GR sites per cells varies since there is an enhanced expression of receptor induced by EBV.

This cell model may provide a valid alternative for GR binding studies in cells from subjects of known receptor genotype using a small amount of blood.

Another factor to consider is which population to sample since ethnic origin is important. Barley et al (1992) investigated the relationship between blood pressure and GR RFLP in a group of hypertensive patients. They detected no significant difference in RFLP frequency in the hypertensive and control group but a difference in GR allele frequency between Afro-Caribbean and white populations. Therefore it is necessary to take particular care in selecting ethnic groups in studies of hypertension and its association with gene markers. However, this was not the case in the present thesis because the sample selected was all Caucasian.

The Bcl I restriction site is not known. This could be either in the non-coding region, possibly at the 5' end of the GR gene, or intronic. Because there are no data on the effect of mutations within flanking regions of the GR gene on receptor function, to study the binding characteristics is only a preliminary approach. Indeed, if the mutation affects the coding region this may not affect the binding domain but might affect DNA or modulatory regions. As reported in asthmatic patients resistant to glucocorticoid therapy, the phenotypical abnormality could reside in the binding of the GR-ligand complex to the chromatin (Adcok et al., 1993). Unfortunately Adcok et al. (1993) did not carry out RFLP analysis for GR in those subjects. In this study, the absence of a significant statistical difference in GR affinity between genotypes combined with differences in vasoconstriction may support the idea of a defective binding of the GR-ligand complex to the chromatin which could be studied by gel-shift assay (Nemoto et al., 1990b). However, this hypothesis was not supported from the results obtained by inhibition of lysozyme synthesis by dexamethasone in mononuclear leucocyte since differences in IC_{50} for dexamethasone would be expected. Since receptor polymorphism did not significantly influence *in vitro* sensitivity of HML

to dexamethasone, the discrepancy between *in vivo* and *in vitro* tests of responsiveness might also be explained by tissue specificity for the effects of glucocorticoids, by differences in sensitivity to budesonide and dexamethasone (refer to section 4.1) or by close linkage disequilibrium with a second non-receptor blood pressure sensitive gene.

From the correlation studies between variables measured in the group of normal volunteers, glucocorticoid activity did not appear to be a major determinant of blood pressure or body fat distribution. However, the data confirm a complex interrelationship between body fat and blood pressure control (refer to section 4.2).

In conclusion, it is reasonable to state that glucocorticoid sensitivity may be a contributory factor in essential hypertension. If this is indeed the case, then hypertension - or predisposition to hypertension - should be associated with a constellation of glucocorticoid sensitivity-related changes. If sensitivity is impaired, mineralocorticoid-like changes may result. If sensitivity is increased, glucocorticoid-dependent variables should be apparent. Although neither hypothesis has been proven in the human population, this thesis has provided important rationale for future studies.

Appendix A: Correlation table of variables measured in a group of normal volunteers (refer to section 4.2)

	AGE	BMI	%FAT	CLISBP	CLIDBP	ORTSBP	ORTDBP
AGE	1.000						
BMI	0.187	1.000					
%FAT	0.275	0.840	1.000				
CLISBP	0.273	0.202	0.266	1.000			
CLIDBP	0.065	0.267	0.282	0.493	1.000		
ORTSBP	0.081	0.450	0.391	0.631	0.430	1.000	
ORTDBP	-0.032	0.309	0.236	0.513	0.590	0.728	1.000
MAPCLI	0.154	0.278	0.315	0.756	0.943	0.566	0.641
MAPORT	0.010	0.389	0.322	0.595	0.586	0.878	0.961
CHOL	0.511	0.281	0.276	0.346	0.131	0.125	0.122
TRIG	0.146	0.300	0.182	0.033	-0.017	0.035	0.074
PLASMA Na	0.189	0.007	0.065	-0.076	-0.152	-0.063	-0.191
PLASMA K	0.118	0.092	0.083	0.079	0.013	0.095	0.120
PLASMA Ca	-0.037	-0.225	-0.281	-0.136	-0.260	-0.152	0.091
URIN. Na	-0.198	0.182	0.251	0.073	0.118	0.065	0.002
URIN.K	-0.034	0.216	0.176	-0.053	-0.044	0.037	0.001
URIN.Ca	0.005	0.037	0.002	-0.030	-0.023	0.004	-0.122
UNa/UK	-0.181	0.066	0.144	0.080	0.144	0.024	-0.037
UNa/CREAT.	-0.117	0.157	0.322	0.155	0.163	0.061	0.026
UK/CREAT.	0.131	0.145	0.213	-0.014	0.045	0.052	0.090
UCa/CREAT.	0.104	-0.017	0.007	-0.017	0.026	-0.035	-0.104
URIN.VOL.	0.004	0.186	0.032	0.033	0.007	0.031	0.023
SERUM.CREAT.	0.098	0.105	-0.001	-0.011	-0.184	0.333	0.073
URIN.CREAT.	-0.199	0.100	-0.032	-0.099	-0.103	-0.017	-0.112
CREAT. CLEAR.	-0.200	0.138	0.101	-0.092	0.057	-0.186	-0.146
GFR	-0.240	0.361	0.329	0.047	0.298	-0.047	0.137
WBC	-0.146	0.335	0.236	0.069	0.122	0.116	0.200
Hgb	-0.241	0.149	0.069	-0.048	0.056	0.044	0.186
HCT	-0.155	0.180	0.068	0.036	0.074	0.097	0.217
NEUTROPH.	-0.163	0.334	0.212	0.053	0.070	0.132	0.115
LYMPH.	0.018	0.272	0.171	0.002	0.036	0.125	0.144
MONOS.	0.037	0.120	0.024	0.192	0.062	0.325	0.448
PLT	0.071	0.087	0.105	0.174	0.062	0.034	0.032
PLASMA F	0.013	-0.140	-0.110	-0.050	-0.048	0.050	0.159
PLASMA ALDO.	-0.028	0.084	-0.037	-0.033	0.104	0.198	0.378
RENIN	-0.161	0.047	0.043	-0.088	-0.208	-0.124	0.031
URIN.F	0.321	-0.009	0.033	-0.110	0.075	-0.102	0.105
URIN.ALDO	0.190	-0.052	-0.062	-0.123	-0.146	-0.115	-0.203
UF/CREAT.	-0.259	-0.082	0.037	-0.121	0.087	-0.146	-0.131
UALDO/CREAT.	0.298	-0.043	0.008	-0.094	-0.076	-0.083	-0.100
THE	0.071	-0.049	-0.042	-0.089	-0.027	-0.148	-0.222
THF	-0.095	0.006	0.076	-0.133	0.025	0.021	-0.103
ALLOTHF	-0.054	0.014	0.054	-0.294	-0.139	-0.070	-0.232
RATIO	-0.132	0.013	0.111	-0.225	-0.104	0.052	-0.025
Kd	0.050	0.092	0.180	0.192	0.159	0.162	0.185
Bmax	0.025	-0.009	-0.064	0.028	0.099	0.196	0.147
IC50	0.073	-0.043	-0.160	-0.052	-0.189	-0.068	0.014
SKIN	0.121	-0.049	0.103	0.009	0.007	-0.009	-0.008

	MAPCLI	MAPORT	CHOL	TRIG	PLASMA Na	PLASMA K	PLASMA Ca
AGE							
BMI							
%FAT							
CLISBP							
CLIDBP							
ORTSBP							
ORTDBP							
MAPCLI	1.000						
MAPORT	0.670	1.000					
CHOL	0.233	0.133	1.000				
TRIG	-0.000	0.083	0.479	1.000			
PLASMA Na	-0.143	-0.153	0.069	0.028	1.000		
PLASMA K	0.040	0.085	0.230	0.076	-0.175	1.000	
PLASMA Ca	-0.248	-0.124	0.181	0.034	0.081	-0.106	1.000
URIN. Na	0.117	0.026	-0.102	0.041	-0.171	0.046	-0.246
URIN.K	-0.054	0.006	0.066	0.182	0.029	0.096	0.061
URIN.Ca	-0.029	-0.076	-0.021	0.063	-0.127	-0.088	0.071
UNa/UK	0.139	-0.009	-0.165	-0.096	-0.227	-0.066	-0.277
UNa/CREAT.	0.182	0.038	-0.028	-0.005	-0.164	0.077	-0.273
UK/CREAT.	0.029	0.069	0.209	0.110	0.088	0.150	0.077
UCa/CREAT.	0.013	-0.079	0.042	0.058	-0.098	-0.088	0.101
URIN.VOL.	0.018	0.018	0.124	0.159	-0.300	0.170	0.084
SERUM.CREAT.	-0.142	0.153	0.146	0.086	0.112	0.128	0.056
URIN.CREAT.	-0.116	-0.077	-0.131	0.086	-0.087	-0.050	-0.010
CREAT. CLEAR.	0.008	-0.148	-0.072	0.042	-0.187	-0.077	-0.045
GFR	0.243	0.102	-0.107	0.107	-0.152	-0.142	-0.201
WBC	0.118	0.190	0.009	0.206	-0.110	-0.104	-0.182
Hgb	0.024	0.157	0.028	0.186	0.103	-0.006	0.052
HCT	0.070	0.195	0.119	0.159	0.103	0.059	0.069
NEUTROPH.	0.073	0.134	-0.064	0.096	-0.031	0.020	-0.291
LYMPH.	0.028	0.155	-0.028	0.140	0.133	-0.250	-0.008
MONOS.	0.122	0.430	0.080	0.034	-0.199	0.088	0.220
PLT	0.113	0.039	0.250	-0.001	-0.083	0.232	-0.001
PLASMA F	-0.055	0.130	0.154	0.076	-0.108	-0.181	0.227
PLASMA ALDO.	0.066	0.325	0.114	0.080	0.088	-0.068	0.157
RENIN	-0.192	-0.034	-0.202	-0.189	0.115	0.107	-0.079
URIN.F	0.014	-0.109	-0.227	-0.091	-0.204	-0.133	-0.061
URIN.ALDO	-0.158	-0.170	0.089	0.126	0.084	-0.257	0.098
UF/CREAT.	0.019	-0.145	-0.169	-0.126	-0.170	-0.142	-0.052
UALDO/CREAT.	-0.093	-0.094	0.217	0.079	0.133	-0.191	0.107
THE	-0.055	-0.198	-0.041	0.069	-0.088	-0.081	0.054
THF	-0.032	-0.045	-0.161	-0.030	-0.075	-0.192	-0.132
ALLOTHF	-0.217	-0.186	-0.215	0.014	-0.011	-0.277	-0.121
RATIO	-0.165	0.009	-0.199	-0.039	0.015	-0.141	-0.332
Kd	0.193	0.220	0.285	0.237	0.089	-0.006	-0.090
Bmax	0.086	0.182	0.069	0.099	-0.361	0.212	0.032
IC50	-0.163	-0.025	0.104	0.156	-0.064	-0.027	0.316
SKIN	0.009	-0.005	0.108	-0.121	0.049	0.140	-0.099

	URIN.Na	URIN.K	URIN.Ca	UNa/UK	UNa/CREAT.	UK/CREAT.	UCa/CREAT.
AGE							
BMI							
%FAT							
CLISBP							
CLIDBP							
ORTSBP							
ORTDBP							
MAPCLI							
MAPORT							
CHOL							
TRIG							
PLASMA Na							
PLASMA K							
PLASMA Ca							
URIN. Na	1.000						
URIN.K	0.374	1.000					
URIN.Ca	0.545	0.320	1.000				
UNa/UK	0.731	-0.303	0.381	1.000			
UNa/CREAT.	0.859	0.226	0.313	0.715	1.000		
UK/CREAT.	-0.048	0.677	-0.119	-0.491	0.172	1.000	
UCa/CREAT.	0.377	0.184	0.882	0.327	0.361	0.073	1.000
URIN.VOL.	0.082	0.410	0.362	-0.205	-0.068	0.190	0.236
SERUM.CREAT.	-0.013	0.124	0.272	-0.114	-0.114	0.000	0.160
URIN.CREAT.	0.515	0.395	0.571	0.247	0.041	-0.383	0.156
CREAT. CLEAR.	0.445	0.281	0.272	0.266	0.147	-0.205	0.005
GFR	0.194	0.033	-0.219	0.175	0.181	-0.019	-0.273
WBC	0.099	0.245	0.064	-0.068	0.075	0.183	0.066
Hgb	-0.149	0.068	-0.319	-0.211	-0.132	0.059	-0.342
HCT	-0.212	0.039	-0.285	-0.260	-0.190	0.047	-0.293
NEUTROPH.	-0.336	0.274	0.161	0.138	0.216	0.043	0.073
LYMPH.	-0.122	0.205	-0.001	-0.221	-0.140	0.205	0.021
MONOS.	-0.172	0.200	0.002	-0.322	-0.173	0.234	-0.003
PLT	-0.018	-0.010	-0.108	0.007	-0.028	0.008	-0.111
PLASMA F	-0.338	0.010	-0.248	-0.371	-0.257	0.204	-0.175
PLASMA ALDO.	-0.387	0.022	-0.342	-0.455	-0.278	0.305	-0.241
RENIN	-0.236	-0.049	-0.357	-0.237	-0.253	-0.052	-0.393
URIN.F	0.513	0.360	0.397	0.226	0.329	-0.004	0.271
URIN.ALDO	-0.139	0.405	0.108	-0.422	-0.256	0.293	0.058
UF/CREAT.	0.335	0.221	0.224	0.150	0.366	0.182	0.287
UALDO/CREAT.	-0.365	0.152	-0.170	-0.494	-0.230	0.495	-0.008
THE	0.435	0.266	0.384	0.216	0.198	-0.123	0.218
THF	0.433	0.273	0.290	0.213	0.191	-0.076	0.127
ALLOTHF	0.233	0.168	0.344	0.236	0.044	-0.161	0.184
RATIO	0.001	-0.015	-0.001	0.128	0.020	0.029	0.009
Kd	0.037	0.181	-0.013	-0.137	-0.008	0.132	-0.051
Bmax	0.070	0.137	0.033	-0.032	-0.033	0.093	-0.048
IC50	-0.243	-0.036	0.107	-0.143	-0.280	-0.018	0.164
SKIN	-0.082	-0.199	-0.135	0.009	0.051	0.016	-0.067

	URIN.VOL.	SERUM CREAT.	URIN.CREAT.	CREAT.CLEAR.	GFR	WBC
AGE						
BMI						
%FAT						
CLISBP						
CLIDBP						
ORTSBP						
ORTDBP						
MAPCLI						
MAPORT						
CHOL						
TRIG						
PLASMA Na						
PLASMA K						
PLASMA Ca						
URIN. Na						
URIN.K						
URIN.Ca						
UNa/UK						
UNa/CREAT.						
UK/CREAT.						
UCa/CREAT.						
URIN.VOL.	1.000					
SERUM.CREAT.	0.211	1.000				
URIN.CREAT.	0.307	0.199	1.000			
CREAT. CLEAR.	0.247	-0.463	0.654	1.000		
GFR	-0.064	-0.770	0.050	0.625	1.000	
WBC	0.126	-0.103	0.032	0.121	0.295	1.000
Hgb	-0.260	-0.337	-0.047	0.124	0.433	0.295
HCT	-0.190	-0.311	-0.072	0.095	0.378	0.282
NEUTROPH.	0.190	-0.079	0.266	0.293	0.290	0.844
LYMPH.	0.099	0.077	-0.033	-0.010	0.133	0.632
MONOS.	0.283	0.217	-0.096	-0.213	-0.164	0.504
PLT	-0.002	-0.067	-0.015	0.004	-0.063	0.304
PLASMA F	0.026	0.106	-0.227	-0.181	-0.202	0.208
PLASMA ALDO.	0.025	0.221	-0.385	-0.348	-0.119	0.163
RENIN	-0.267	-0.219	-0.029	0.091	0.262	0.412
URIN.F	0.290	-0.095	0.421	0.473	0.147	0.333
URIN.ALDO	0.317	-0.030	0.172	0.271	0.005	0.130
UF/CREAT.	0.166	-0.136	0.021	0.221	0.084	0.332
UALDO/CREAT.	0.199	-0.066	-0.371	-0.063	-0.021	0.075
THE	0.179	-0.145	0.477	0.549	0.149	0.045
THF	0.067	-0.071	0.451	0.451	0.145	0.136
ALLOTHF	0.098	0.040	0.456	0.398	0.054	-0.132
RATIO	-0.190	0.144	-0.000	-0.108	-0.119	0.031
Kd	-0.060	-0.016	0.068	0.003	0.046	0.001
Bmax	0.154	0.109	0.123	0.094	-0.075	0.087
IC50	0.108	0.051	0.016	-0.082	0.161	0.097
SKIN	-0.140	-0.287	-0.272	0.061	0.243	-0.098

	Hgb	HCT	NEUTROPH.	LYMPH.	MONS.	PLT	PLASMA F
AGE							
BMI							
%FAT							
CLISBP							
CLIDBP							
ORTSBP							
ORTDBP							
MAPCLI							
MAPORT							
CHOL							
TRIG							
PLASMA Na							
PLASMA K							
PLASMA Ca							
URIN. Na							
URIN.K							
URIN.Ca							
UNa/UK							
UNa/CREAT.							
UK/CREAT.							
UCa/CREAT.							
URIN.VOL.							
SERUM.CREAT.							
URIN.CREAT.							
CREAT. CLEAR.							
GFR							
WBC							
Hgb	1.000						
HCT	0.947	1.000					
NEUTROPH.	0.165	0.136	1.000				
LYMPH.	0.286	0.245	0.274	1.000			
MONOS.	0.141	0.172	0.220	0.552	1.000		
PLT	0.135	0.154	0.350	0.127	0.366	1.000	
PLASMA F	0.227	0.205	0.006	0.246	0.401	0.276	1.000
PLASMA ALDO.	0.269	0.281	-0.127	0.381	0.458	-0.003	0.517
RENIN	0.365	0.347	0.297	0.330	0.148	0.213	0.164
URIN.F	0.043	0.016	0.441	0.023	0.069	0.148	0.111
URIN.ALDO	0.085	0.097	0.057	0.221	0.062	-0.050	0.292
UF/CREAT.	0.046	0.029	0.352	0.016	0.062	0.110	0.242
UALDO/CREAT.	0.019	0.045	-0.090	0.217	0.098	-0.084	0.376
THE	-0.067	-0.120	0.168	0.003	-0.165	0.068	0.047
THF	-0.068	-0.158	0.305	-0.044	-0.122	0.153	0.062
ALLOTHF	-0.155	-0.246	-0.034	-0.015	-0.307	-0.181	-0.009
RATIO	-0.127	-0.189	0.054	0.008	-0.059	-0.012	-0.018
Kd	0.230	0.184	-0.058	0.069	-0.065	0.190	-0.007
Bmax	-0.132	-0.091	-0.086	0.159	0.214	0.165	0.037
IC50	-0.001	0.021	-0.030	0.221	0.140	0.323	0.206
SKIN	0.067	0.067	-0.101	-0.136	0.051	-0.085	-0.075

	PLASMA ALDO	RENIN	URIN.F	URIN.ALDO	UF/CREAT.	UALDO/CREAT.
AGE						
BMI						
%FAT						
CLISBP						
CLIDBP						
ORTSBP						
ORTDBP						
MAPCLI						
MAPORT						
CHOL						
TRIG						
PLASMA Na						
PLASMA K						
PLASMA Ca						
URIN. Na						
URIN.K						
URIN.Ca						
UNa/UK						
UNa/CREAT.						
UK/CREAT.						
UCa/CREA'T.						
URIN.VOL.						
SERUM.CREAT.						
URIN.CREAT.						
CREAT. CLEAR.						
GFR						
WBC						
Hgb						
HCT						
NEUTROPH.						
LYMPH.						
MONOS.						
PLT						
PLASMA F						
PLASMA ALDO.	1.000					
RENIN	0.209	1.000				
URIN.F	-0.099	-0.032	1.000			
URIN.ALDO	0.269	0.097	0.321	1.000		
UF/CREAT.	0.072	-0.042	0.891	0.292	1.000	
UALDO/CREAT.	0.504	0.067	0.037	0.798	0.239	1.000
THE	-0.285	-0.067	0.610	0.430	0.410	0.107
THF	-0.207	-0.113	0.691	0.366	0.499	0.068
ALLOTHF	-0.315	-0.182	0.312	0.241	0.164	-0.039
RATIO	0.126	-0.142	-0.003	-0.111	0.014	-0.131
Kd	0.060	-0.042	-0.077	0.074	-0.134	0.042
Bmax	0.167	-0.155	0.006	0.127	-0.044	0.100
IC50	0.054	0.187	-0.063	0.055	-0.077	0.018
SKIN	0.081	-0.058	-0.257	-0.105	-0.115	0.115

	THE	THF	ALLOTHF	RATIO	Kd	Bmax	IC50	SKIN
AGE								
BMI								
%FAT								
CLISBP								
CLIDBP								
ORTSBP								
ORTDBP								
MAPCLI								
MAPORT								
CHOL								
TRIG								
PLASMA Na								
PLASMA K								
PLASMA Ca								
URIN. Na								
URIN.K								
URIN.Ca								
UNa/UK								
UNa/CREAT.								
UK/CREAT.								
UCa/CREAT.								
URIN.VOL.								
SERUM.CREAT.								
URIN.CREAT.								
CREAT. CLEAR.								
GFR								
WBC								
Hgb								
HCT								
NEUTROPH.								
LYMPH.								
MONOS.								
PLT								
PLASMA F								
PLASMA ALDO.								
RENIN								
URIN.F								
URIN.ALDO								
UF/CREAT.								
UALDO/CREAT.								
THE	1.000							
THF	0.769	1.000						
ALLOTHF	0.357	0.473	1.000					
RATIO	-0.323	0.134	0.536	1.000				
Kd	0.081	0.165	-0.104	-0.076	1.000			
Bmax	0.065	0.044	-0.057	-0.001	0.212	1.000		
IC50	0.029	-0.061	-0.032	-0.056	0.305	0.230	1.000	
SKIN	-0.104	-0.088	-0.054	-0.027	0.014	-0.006	-0.207	1.000

Abbreviations used in Appendix A

- BMI: body mass index
- %Fat: percentage of body fat
- CLIDBP and CLISBP: diastolic and systolic blood pressure measure in supine position
- ORTDBP and ORTSBP: diastolic and systolic blood pressure measured in upright position
- MAP: mean arterial blood pressure
- CHOL: cholesterol
- TRIG: triglyceride
- UNA/UK: urinary sodium/potassium ratio
- CREAT.: creatinine
- URIN.VOL.: urinary volume
- CREAT.CLEAR.: creatinine clearance
- GFR: glomerular filtration rate
- WBC: white blood cells
- Hgb: haemoglobin
- HCT: haematocrit
- NEUTROPH.: neutrophiles cells
- LYMPH.: lymphocytes
- MONOS.: monocytes
- PLT: platelet

REFERENCES

Adcock IM, Brown CR, Peters MJ, Lane S, Gelder CM, Virdee H, Lee T, Barnes PJ. Target sequence binding of glucocorticoid receptor from peripheral blood monocytes of steroid sensitive and resistant patients. *J Endocrinol* **137** (Suppl.):RC2; 1993

Adragna NC, Canessa ML, Solomon H, Slater E, Tosteson DC. Red cell lithium-sodium countertransport and sodium-potassium cotransport in patients with essential hypertension. *Hypertension* **4**: 795-804; 1982

Allan GF, Tsai SY, O'Malley BW, Tsai M-J. Steroid hormone receptors and *in vitro* transcription. *BioEssays* **13**: 73-78; 1991

Anderson EA, Mahoney LT, Lauer RM, Clarke WR. Enhanced forearm blood flow during mental stress in children of hypertensive parents. *Hypertension* **10**: 544-549; 1987

Annest JL, Sing CF, Biron P, Mongeau JH. Familial aggregation of blood pressure and weight in adoptive families, I. Comparison of blood pressure and weight statistics among families with adopted, natural or both natural and adopted children. *Am J Epidemiol* **110**: 479-491; 1979a

Annest JL, Sing CF, Biron P, Mongeau. Familial aggregation of blood pressure and weight in adoptive families, II. Estimation of relative contribution of genetic and common environmental factors to blood pressure correlations between family members. *Am J Epidemiol* **110**: 492-503; 1979b

Antakly T, Raquidan D, O'Donnell D, Katnick L. Regulation of glucocorticoid receptor expression. I. Use of a specific radioimmunoassay and antiserum to a synthetic peptide of the N-terminal domain. *Endocrinology* **126**: 1821-1828, 1990

Arai K., Chrousos GP. Glucocorticoid resistance. *Bailliere's Clin Endocrinol Metab* **8**: 317-331; 1994

Armanini D, Strasser T, Weber PC. Characterization of aldosterone binding sites in circulating human mononuclear leukocytes. *Am J Physiol (Endocrinol Metab)* **248**: E388-E390; 1985

Arriza JL, Weinberg C, Cerelli G, Glaser TM, Handelin BL, Housman DE, Evans RM. Cloning of human mineralocorticoid receptor complementary DNA: structural and functional kinship with the glucocorticoid receptor. *Science* **237**: 268-275; 1987

Arteaga E, Biglieri EG, Kater CE, Lopez JM, Schambelan M. Aldosterone-producing adrenocortical carcinoma. *Ann Intern Med* **101**: 316-321; 1984

Aqel MB, Sharma RV, Bhalla RC. Increased norepinephrine sensitive intracellular Ca^{2+} pool in the caudal artery of spontaneously hypertensive rats. *J Hypertension* **5**: 249-253; 1987

Bader M, Kaling M, Metzger R, Peters J, Wagner J, Ganten D. Basic methodology in the molecular characterization of genes. *J Hypertension* **10**: 9-16; 1992

Baer PG, Bianchi G. Renal micropuncture study of normotensive and Milan hypertensive rats before and after development of hypertension. *Kidney Internat* **13**: 452-466; 1978

Bailey JM. New mechanisms for the effects of anti-inflammatory glucocorticoids. *BioFactors* **3**: 97-102; 1991

Barker D, Bull A, Osmond C, Simmonds S. Fetal and placental size and risk of hypertension in adult life. *Br Med J* **301**: 259-262; 1990

Barley J, Carter ND, Cruickshank JK, Jeffery S, Smith A, Charlett A, Webb DJ. Renin and atrial natriuretic peptide restriction fragment length polymorphisms: association with ethnicity and blood pressure. *J Hypertension* **9**: 993-996; 1991

Barley J, Sagnella GA, Cruickshank JK, Webb DJ, Carter ND. Glucocorticoid receptor restriction fragment length polymorphisms and blood pressure. *Eur J Int Med* **2**: 263-265; 1992

Bastl CP and Sebastian A. Physiologic effects of the endocrine system and gastrointestinal tract. Adrenal hormones. In: Clinical Disorder of Fluid and Electrolyte Metabolism, edited by Maxwell MH, Kleeman CR, Narins RG. McGraw-Hill Inc., London 317-341; 1987

Bastl CP, Bressler L, Schulman G, Mendez M, Cragoe EJ. Low-dose glucocorticoids maintain Na-H exchange in distal colon of adrenalectomized rats. *Am J Physiol (Renal Fluid Electrolyte Physiol)* **261**: F545-F553; 1991

Baxter JD, Funder JW. Hormones receptors. *N Engl J Med* **301**: 1149-1161; 1979

Baylis C, Brenner BM. Mechanism of the glucocorticoid-induced increase in glomerular filtration rate. *Am J Physiol* **234**: F166-F170; 1978

Beaglehole R, Eyles E, Salmond CE. Blood pressure in Tokelauans children in two contrasting environments. *Am J Epidemiol* **108**: 283-288; 1978

Beaglehole R. International trend in coronary heart disease mortality, morbidity, and risk factors. *Epidemiol Rev* **12**: 1-15; 1990

Beato M. Gene regulation by steroid hormones. *Cell* **56**: 335-344; 1989

Bell PA, Greaves MF, Sloman JC, Thompson EN, Whittaker JA. Significance of glucocorticoid receptors and responses in leukaemia. *J Steroid Biochem* **19**: 851-855; 1983

Ben-Ari ET, Lynch KR, Garrison JC. Glucocorticoids induce the accumulation of novel angiotensinogen gene transcripts. J Biol Chem 264: 13074-13079; 1989

Ben-Ishay D, Saliternick R, Winer A. Separation of two strains of rats with inbred dissimilar sensitivity to DOCA-salt hypertension. *Experientia* **28**: 1321-1322; 1972

Bennet NE. Hypertension in the elderly. *Lancet* **344**: 447-449; 1994

Berk BC, Vallega G, Griendling KK, Gordon JB, Cragoe EJ, Canessa M, Alexander RW. Effects of glucocorticoids on Na/H exchange and growth in cultured vascular smooth muscle cells. *J Cell Physiol* **137**: 391-401; 1988

Bianchi G, Fox U, Imbasciati E. The development of a new strain of spontaneously hypertensive rats. *Life Sci* **14**: 339-347; 1974

Bianchi G, Cusi D, Gatti M, Lupi GP, Ferrari P, Barlasina C, Picotti GB, Bracchi G, Colombo G, Gori D, Velis O, Mazzei D. A renal abnormality as a possible cause of 'essential' hypertension. *Lancet* **i**: 173-177: 1979

Bianchi G, Cusi D, Barlasina C, Lupi GP, Ferrari P, Picotti GB, Gatti M, Polli E. Renal dysfunction as a possible cause of essential hypertension in predisposed subjects. *Kidney Int* **23**: 870-875; 1983

Bianchi G, Ferrari P. Animal models for arterial hypertension. In: Hypertension. Eds. Genest J, Kuchel O, Hamet P, Cantin M. New York: McGraw-Hill; 534-554; 1983

Bianchi G, Ferrari P, Baker BR. The Milan hypertensive strain. In: Handbook of Hypertension edited by de Jong W. Elsevier publisher, Amsterdam 328-349; 1984

Biglieri EG, Herron MA, Brust N. 17-Hydroxylation deficiency in man. *J Clin Invest* **45**: 1946-1954; 1966

Bingham AH, Atkinson T, Sciaky D, Roberts RJ. A specific endonuclease from Bacillus caldolyticus. *Nucl Acid Res* **5**: 3457-3467; 1978

Blaustein MP. Sodium transport and hypertension. Where are we going? *Hypertension* **6**: 445-453; 1984

Bodine PV, Litwack G. Purification and structural analysis of the modulator of the glucocorticoid receptor complex. Evidence that the modulator is a novel phosphoglyceride. *J Biol Chem* **263**: 6501-6512; 1988

Bodine PV, Litwack G. Purification and characterization of two novel phoshoglycerides that modulate the glucocorticoid-receptor complex. Evidence for two modulator binding sites in occupied, unactivated steroid hormone receptor. *J Biol Chem* **265**: 9544-9554; 1990

Boggaram V, Smith ME, Mendelson CR. Post-transcriptional regulation of surfactant protein-A messenger RNA in human fetal lung *in vitro* by glucocorticoids. *Mol Endocrinol* **5**: 414-423; 1991

Boyum A. Isolation of mononuclear cells and granulocytes from human blood. Isolation of mononuclear cells by one centrifugation step and of granulocytes by combining centrifugation and sedimentation at 1G. *Scand J Clin Lab Invest* **21** (Suppl): 77-89; 1968

Borghi C, Costa FV, Boschi S, Ambrosioni E. Impaired vasodilator capacity and exaggerated pressor response to isometric exercise in subjects with family history of hypertension. *Am J Hypertens* **1**: 106s-109s; 1988

Brandon DD, Markwick AJ, Flores M, Dixon K, Albertson BD, Loriaux DL. Genetic variation of the glucocorticoid receptor from a steroid-resistant primate. *J Mol Endocrinol* **7**: 89-96; 1991

Braunsberg CJ, James VHT. The determination of cortisol and corticosterone in blood: a review. *J Clin Endocrinol Metab* **21**: 1146-1188; 1961

Bronnegard M, Werner S, Gustafsson J-A. Primary cortisol resistance associated with a thermolabile glucocorticoid receptor in a patient with fatigue as the only symptom. *J Clin Invest* **78**: 1270-1278; 1986

Brown JJ, Davies DL, Lever AF, Robertson JIS. Plasma renin activity in human hypertension II: renin in relation to the aetiology. *Br Med J* **2**: 1215-1219; 1965

Brownie AC, Alfano J, Gallant S. Rat models of experimental hypertension: their adrenocortical components. In: Biglieri EG, Melby JC, eds. Endocrine Hypertension. New York:Raven Press, 29-69: 1990

Bruner CA. Vascular responsiveness in rats resistant to aldosterone-salt hypertension. *Hypertension* **20**: 59-66; 1992

Bruns RF, Lawson-Wendling K, Pugsley TA. A rapid filtration assay for soluble receptors using polyethylenimine-treated filters. *Anal Biochem* **132**: 74-81; 1983

Burnstein KL, Bellingham DL, Jewell CM, Powell-Oliver FE, Cidlowski JA. Autoregulation of glucocorticoid receptor gene expression. *Steroids* **56**: 52-58; 1991

Calogero AE, Fornito MC, Aliffi A, Vicari E, Moncada ML, Mantero F, Polosa P, D'Agata R. Role of peripherally infused angiotensin II on the human hypothalamus-pituitary-adrenal axis. *Clin Endocrinol* (Oxf.) **34**: 183-186; 1991

Cambien F, Poirier O, Lecerf L, Evans A, Cambou J-P, Arveiler D, Luc G, Bard J-M, Bara L, Ricard S, Tiret L, Amouyel P, Alhenc-Gelac F, Soubrier F. Deletion polymorphism in the gene for angiotensin-converting enzyme is a potent risk factor for myocardial infarction. *Nature* 641-644; 1992

Camussi A. Genetics: quantitative versus qualitative characters. *J Cardiovasc Pharmacol* **12** (Suppl. 3): S1-S6; 1988

Carlstedt-Duke J, Stromstedt P-E, Persson B, Cerlund E, Gustafsson J-A, Jornvall H. Identification of hormone interacting amino acid residues within the steroid-binding domain of the glucocorticoid receptor in relation to other steroid hormone receptors. *J Biol Chem* **263**; 6842-6846; 1988

Carson-Jurica MA, Schrader WT, O'Malley BW. Steroid receptor family: structure and functions. *Endocr Rev* **11**: 201-220; 1990

Caulfield M, Lavander P, Farral M, Munroe P, Lawson M, Turner P, Clark AJL. Linkage of the angiotensinogen gene to essential hypertension. *N Engl J Med* **330**: 1629-1633; 1994

Castro-Magana M, Maddaiah VT, Collip PJ, Angula M. Synergistic effects of growth hormone therapy on plasma levels of 11-deoxycortisol and cortisol in growth hormone-deficient children. *J Clin Endocrinol Metab* **56**: 662-667, 1983

Chakraborti PK, Garabedian MJ, Yamamoto KR, Simons SS. Creation of "super" glucocorticoid receptors by point mutation in the steroid binding domain. *J Biol Chem* **33**: 22075-22078; 1991

Chrousos GP, Vingerhoeds A, Brandon DD, Eil C, Pugeat M, DeVroede M, Loriaux DL, Lipsett MB. Primary cortisol resistance in man. A glucocorticoid receptor-mediated disease. *J Clin Invest* **69**: 1261-1269; 1982

Chrousos GP, Karl M. The molecular mechanisms of glucocorticoid resistance. In: Cellular and Molecular Biology of the adrenal Cortex, edited by Saez JM, Brownie AC, Capponi A, Chambaz EM, Mantero F. Colloque INSERM 227-232; 1992

Clauser E, Gaillard I, Corvol P. Regulation of angiotensinogen gene. *Am J Hypertens* **2**: 403-410; 1989

Coghlan JP, Denton DA, Dan JSK, Dougall JG, Scoggins BA. Hypertensive effect of 17α,20α-dihydroxyprogesterone and 17α-hydroxyprogesterone in sheep. *Nature* **263**: 608-609; 1976

Coghlan JP, Reid AF, Spence CD, Scoggins BA, Whitworth JA. Receptor and steroid-dependent hypertension. *Clin Exp Pharmacol Physiol* **15**: 163-172; 1988

Connell JMC, Kenyon CJ, Corrie JET, Fraser R, Watt R, Lever AF. Dexamethasone-suppressible hyperaldosteronism. Adrenal transition cell hyperplasia? *Hypertension* **8**: 669-676; 1986

Connell JMC, Whitworth JA, Davies DL, Lever AF, Richards AM, Fraser R. Effects of ACTH and cortisol administration on blood pressure, electrolyte metabolism, atrial natriuretic peptide and renal function in normal man. *J Hypertension* **5**: 425-433; 1987

Connell JMC, Whitworth JA, Davies DL, Richards AM, Fraser R. Haemodynamic, hormonal and renal effects of adrenocorticotrophic hormone in sodium-restricted man. *J Hypertension* **6**: 17-23; 1988

Cooper R, LeGrady D, Nanas S, Trevisan M, Mansour M, Histand P, Ostrow D, Stamler J. Increased sodium-lithium countertransport in college students with elevated blood pressure. *JAMA* **249**:1030-1034; 1983

Cornoni-Huntley J, La Croix AZ, Havlik RJ,. Race and sex differentials in the impact of hypertension in the United States. *Arch Intern Med* **149**:780-788, 1989

Corrigan CJ, Brown PH, Barnes NC, Tsai J-J, Frew AJ, Kay AB. Glucocorticoid resistance in chronic asthma. Glucocorticoid pharmacokinetics, Glucocorticoid receptor characteristics, and inhibition of peripheral blood T cell proliferation by glucocorticoids *in vitro*. *Am Rev Respir Dis* **144**: 1016-1025; 1991a

Corrigan CJ, Brown PH, Barnes K, Szefler SJ, Tsai J-J, Frew AJ, Kay AB. Glucocorticoid resistance in chronic asthma. Peripheral blood T lymphocyte activation and comparison of the T-Lymphocyte inhibitor effects of glucocorticoids and cyclosporin. *Am Rev Respir Dis* **144**: 1026-1032; 1991b

Cugini P, Manconi, R, Serdoz R, Mancini A, Meucci T. Influence of propranolol on circadian rhythms of plasma renin, aldosterone, and cortisol in healthy supine man. *Boll Soc Ital Biol Sper* **53**: 263-269; 1977

Dahl LK, Heine M, Tassinari L. Effects of excess salt ingestion. Evidence that genetic factors play an important role in susceptibility to experimental hypertension. *J Exp Med* **115**: 1173-1190; 1962

Dale SL, Holbrook MM, Komanicky P, Melby JC. Urinary 19-nor-deoxycorticosteone excretion in the spontaneously hypertensive rat. *Endocrinology* **110**: 1989-1993; 1982

Dallman MF, Akana SF, Cascio CS, Darlington DN, Jacobson L, Levin N. Regulation of ACTH secretion: variation on a theme of B. *Recent Progress Horm Res* **43**: 113-173; 1987

Dal Palu' C, Pessina ASC. Genetic and acquired factors in essential hypertension. *Medicographia* **71**: 12-13; 1985

DeForrest JM, Davis JO, Freeman RH, Stephens GA, Watkins BE. Circadian changes in plasma renin activity and plasma aldosterone concentration in two-kidney hypertensive rats. *Hypertension* **1**: 142-149; 1979

Denton RR, Eisen LP, Elsasser, MS, Harmon JM. Differential autoregulation of glucocorticoid receptor expression in human T- and B-cell lines. *Endocrinology* **133**: 248-256, 1993

De Simone G, Tommaselli AP, Rossi R, Valentino R, Lauria R, Scopacasa F, Lombardi G. Partial deficiency of adrenal 11-hydroxylase - a possible cause of primary hypertension. *Hypertension* 7: 204-210; 1985

De Souza EB, Van Loon GR. D-Ala^2-met-enkephalinamide, a potent opioid peptide, alters pituitary-adrenocortical secretion in rats. *Endocrinology* **111**: 1483-1490, 1982

Distler A, Philip T, Luth B, Wucherer G. Studies on the mechanism of the mineralocorticoid-induced blood pressure in man. *Clin Sci* **57**: 3035-3059; 1979

Dluhy RG, Bavli SZ, Leung FK, Solomon HS, Moore TJ, Hollenberg NK, Williams GH. Abnormal adrenal responsiveness and angiotensin II dependency in high renin essential hypertension. *J Clin Invest* **64**: 1270-1276; 1979

Dong Y, Poellinger L, Gustafsson J-A, Okret S. Regulation of glucocorticoid receptor expression: evidence for transcriptional and post-translational mechanisms. *Mol Endocrinol* **2**: 1256-1264; 1988

Dzau VJ. Molecular and physiological aspects of tissue renin-angiotensin system: emphasis on cardiovascular control. *J Hypertension* **6** (Suppl 3): S7-S12; 1988a

Dzau VJ, Burt DW, Pratt RE. Molecular biology of the renin-angiotensin system. *Am J Physiol (Renal Fluid Electrolyte Physiol)* **255**: F563-F573; 1988b

Dzau VJ, Paul M, Nakamura N, Pratt RE, Ingelfinger JR. Role of molecular biology in hypertension research. State of the art lecture. *Hypertension* **13**: 731-740; 1989

Edwards CRW, Burt D, McIntyre MA, de Kloet ER, Stewart PM, Brett LP, Sutanto WS, Monder C. Localization of 11beta-hydroxysteroid dehydrogenase - tissue specific protector of the mineralocorticoid receptor. *Lancet* **ii**: 986-989; 1988

Edwards CRW, Stewart PM. The cortisol-cortisone shuttle and the apparent specificity of glucocorticoid and mineralocorticoid receptors. *J Steroid Biochem Molec Biol* **39**: 859-865; 1991

Edwards CRW, Benediktsson R, Lindsay RS, Seckl JR. Disfunction of placental glucocorticoid barrier: link between fetal environment and adult hypertension? *Lancet* **341**: 355-357; 1993

Elford J, Phillips A, Thomson AG, Shaper AG. Migration and geographic variations in blood pressure in Britain. *Br Med J* **300**:291-295; 1990

Encio IJ, Detera-Wadleigh SD. The genomic structure of the human glucocorticoid receptor. *J Biol Chem* **266**: 7182-7188; 1991

Eriksson P, Daneholt B, Wrange O. The glucocorticoid receptor in homodimeric and monomeric form visualised by electron microscopy. *J Structural Biol* **107**: 48-55; 1991

Evans VG, Rose GA. Hypertension. *Br Med Bull* **27**: 32-42, 1971

Feldman D, Funder J, Loose D. Is the glucocorticoid receptor identical in various target organs? *J Steroid Biochem* **9**: 141-145; 1978

Feldmer M, Kaling M, Takahashi S, Mullins JJ, Ganten D. Glucocorticoid- and estrogen-responsive elements in the 5'-flanking region of the rat angiotensinogen gene. *J Hypertension* **9**: 1005-1012; 1991

Ferranini E., Haffner SM, Stern MP. Essential hypertension: an insulin-resistant state. *J Cardiovasc Pharmacol* **15** (Suppl. 5): S18-S25; 1990

Ferrari P, Mantero F, Bianchi G. Role of adrenal steroids in the rat genetic models of hypertension. In: Mantero F, Biglieri EG, Funder JW, Scoggins BA, eds. The Adrenal Gland and Hypertension. Serono Symposia 27: 149-158; 1985

Ferriss JB, Beevers DG, Brown JJ, Davies DL, Fraser R, Lever AF, Mason P, Neville AM, Robertson JIS. Clinical, biochemical and pathological features of low-renin 'primary' hyperaldosteronism. *Am Heart J* **95**: 375-388; 1978

Findling JW, Adams ND, Lemann Jjr, Gray RW, Carol JT, Tyrrell JB. Vitamin D metabolites and parathyroid hormone in Cushing's syndrome: relationship to calcium and phosphorus homeostasis. *J Clin Endocrinol Metab* **54**: 1033-1044; 1982

Fraser R. Inborn errors of corticosteroid biosynthesis and metabolism: their effects on electrolyte metabolism and blood pressure. In: Clinical Aspects of Secondary Hypertension, edited by Robertson JIS. Amsterdam: Elsevier, 162-188; 1983

Fraser R, Padfield PL. Role of mineralocorticoids. In: Essential Hypertension as an Endocrine Disease, edited by Edwards CRW, Carey RM. London: Butterworths 158-183; 1985

Fraser R, Brown JJ, Mason PA, Morton JJ Lever AF, Robertson JIS, Lee HA, Miller H. Severe hypertension with absent secondary sex characteristics due to partial deficiency of steroid 17α-hydroxilase activity. *J Human Hypertension* **1**: 53-58; 1987

Fraser R, Davies DL, Connell JMC. Hormones and hypertension. *Clin Endocrinol* **31**: 701-746; 1989

Fraser R. Peripheral metabolism of corticosteroids as a mechanism of potency modulation. *J Endocrinol* **125**: 1-2; 1990

Fraser R, Ancil AK, Brown WB, Ingram MC, Holloway CD, Henderson IW, Kenyon CJ. Evidence of abnormalities in corticosteroid secretion leading to volume-dependent hypertension in Milan rats. *Hypertension* **24**: 512-515; 1994

Freedman LP. Anatomy of the steroid receptor zinc finger region. *Endocrine Rev* **13**: 129- 145; 1992

Freedman LP, Luisi BF. On the mechanism of DNA binding by nuclear hormone receptors: a structural and functional perspective. *J Cell Biochem* **51**: 140-150; 1993

Freiberg JM, Kinsella J, Sacktor B. Glucocorticoids increase the Na-H exchange and decrease the Na gradient-dependent phosphate-uptake systems in renal brush border membrane vesicles. *Proc Natl Acad Sci* **79**: 4932-4936; 1982

Fuller PJ. The steroid receptor superfamily: mechanisms of diversity. *FASEB J* **5**: 3092-3099; 1991

Funder JW, Feldman D, Edelman IS. The roles of plasma binding and recepor specificity in the mineralocorticoid action of aldosterone. *Endocrinology* **92**: 994-1004; 1973

Funder JW, Pearce PT, Smith R, Smith AT. Mineralocorticoid action:target tissue specificity is enzyme, not receptor mediated. *Science* **242**: 583-585; 1988

Ganguly A, Winberger MH, Guthrie GP, Finerberg NS. Adrenal steroid responses to ACTH in glucocorticoid-suppressible aldosteronism. *Hypertension* **6**: 563-567; 1984

Garay RP, Dagher G, Meyer P. An inherited sodium ion-potassium ion cotransport defect in essential hypertension. *Clin Sci* **59**: 191s-193s; 1982

Gardner DG, Hane S, Trachewsky D, Schenk D, Baxter JD. Atrial natriuretic peptide mRNA is regulated by glucocorticoids in vivo. *Biochem Biophys Res Comm* **139**: 1047-1054; 1986

Garty H. Mechanism of aldosterone action in tight epithelia. *J Membr Biol* **90**: 193-205; 1986

Girardin E, Garoscio-Cholet M, Dechaud H, Lejeune H, Carrier E, Tourniaire J, Pugeat M. Glucocorticoid receptors in lymphocytes in anorexia nervosa. *Clin Endocrinol* **35**: 79-84; 1991

Glorioso N, Troffa C, Tonolo G, Melis MG, Manunta P, Soro A, Madeddu P, Pazzola A, Pala F. Stimulation of plasma inactive renin by dexamethasone. *Am J Physiol (Endocrinol Metab)* **257**: E879-E884; 1989

Godfrey KM, Redman CWG, Baker DJP, Osmond C. The effect of maternal anaemia and iron deficiency on the ratio of fetal weight to placental weight. *Br J Obstet Gynecol* **98**: 886-891; 1991

Goldsmith DJA, Tribe RM, Poston L, Cappuccio FP, Markandu ND, MacGregor GA, Hilton PJ. Leukocyte intracellular pH and Na-H exchange activity in essential hypertension: an *in vitro* study under physiological conditions. *J Hypertension* **9**: 645-653; 1991

Gomez-Sanchez C, Montgomery M, Ganguly A, Holland O, Gomez-Sanchez E, Grim C, Weinberger M. Elevated urinary excretion of 18-oxocortisol in glucocorticoid-suppressible aldosteronism. *J Clin Endocrinol Metab* **59**: 1022-1024; 1984

Gomez-Sanchez C. 18 Hydroxycortisol and 18 oxocortisol steroids from the transition zone. *Endocr Res* **10**: 609-615; 1985

Gomez-Sanchez CE, Holland OB, Upcavage R. Urinary free 19-nor-deoxycorticosterone and deoxycorticosterone in human hypertension. *J Clin Endocrinol Metab* **60**: 234-238, 1985

Gomez-Sanchez EP. Intracerebroventricular infusion of aldosterone induces hypertension in rats. *Endocrinology* **8**: 819-823; 1986

Gomez-Sanchez EP, Fort C, Thwaits D. Central mineralocorticoid receptor antagonism blocks hypertension in Dahl S/JR rats. *Am J Physiol* **262**: E96-E99; 1992

Gomez-Sanchez EP. Role of the central nervous system in mineralocorticoid hypertension. Proceding, 75th Annual Meeting, Endocrine Society, Las Vegas. Bethesda, MD: Endocrine Society Press, 1993

Gordon RD, Gomez-Sanchez CE, Hamlet SM, Tunny TJ, Klemm SA. Angiotensin-responsive aldosterone-producing adenoma masquerades as idiopathic hyperaldosteronism (IHA: adrenal hyperplasia) or low-renin essential hypertension. *J Hypertension* **5** (Suppl. 5): 103-106; 1987

Granger JP, Burnett JC, Romero JC, Opgernorth TJ, Salazar J, Joyce M. Elevated levels of natriuretic peptide during aldosterone escape. *Am J Physiol* **252**: R878-R882; 1987

Grekin RJ, Sider RS. Aldosterone receptor assay in rat kidney cytosol. *J Steroid Biochem* **13**: 835-837; 1980

Griffing GT, Dale SL, Holbrook MM, Melby JC. The regulation of urinary free 19-nor-deoxycorticosteone and its relation to systemic arterial blood pressure in normotensive and hypertensive subjects. *J Clin Endocrinol Metab* **56**: 99-105; 1985

Grunfeld J-R, Eloy L, Araujo A, Russo-Marie F. Effect of gluco- and antiglucocorticoids on renal and aortic prostaglandin synthesis. *Am J Physiol* **251**: F810-F816; 1986

Gurwitz ET, Jones AW. Altered ion transport and its reversal in aldosterone hypertensive rat. *Am J Physiol* **243** : H927-H933; 1982

Guthrie GP, Kotchen TA, Kotchen JM. Suppression of adrenal mineralocorticoid production in prehypertensive young adult men. *J Clin Endocrinol Metab* **56**: 87-94; 1983

Haack D, Engel R, Vecsei P. The effect of chronic ACTH treatment on blood pressure and urinary excretion of steroids in the rats. *Klin Wochenschr* **56** (Suppl 1): 183-186; 1978

Haigh RM, Jones CT. Effet of glucocorticoids on alpha1-adrenergic receptor binding in rat vascular smooth muscle. *J Mol Endocrinol* **5**: 41-48; 1990

Hall JE, Ganger JP, Smith MJ Jr, Premen AJ. Role of renal haemodynamics and arterial pressure in aldosterone escape. *Hypertension* **6** (Suppl., I): I183-I192; 1984

Hamet P, Kong D, Pravenec M, Kunes J, Kren V, Klir P, Sun Y-L, Tremblay J. Restriction fragment length polymorphism of hsp70 gene, localized in the RT1 complex, is associated with hypertension in spontaneously hypertensive rats. *Hypertension* **19**: 611-614; 1992

Hamet P. Abnormal hsp70 gene expression: its potential key role in metabolic defects in hypertension. *Clin Exp Pharmacol Physiol* **19** (Suppl. 20): 53-59; 1992

Hamilton BP, Zadick Z, Edwin CM, Hamilton JH, Kowarski AA. Effect of adrenal suppression with dexamethasone in essential hypertension. *J Clin Endocrinol Metab* **48**: 848-853; 1979

Hasegawa H, Nasjletti A, Rice K, Masson CMG. Role of pituitary and adrenal in the regualtion of plasma angiotensinogen. *Am J Physiol* **225**: 1-6; 1973

Hendrick JP, Hartl F-U. Molecular chaperone functions of heat-shock proteins. *Annu Rev Biochem* **62**: 349-384; 1993

Hers HG. Effects of glucocorticoids on carbohydrate metabolism. *Agents Act* **17**: 248-262; 1985

Hilbert P, Lindpaintner K, Beckmann JS, Serikawa T, Soubrier F, Dubay C, Cartwright P, De Gouyon B, Julier C, Takahasi S, Vincent M, Ganten D, Georges M, Lathrop GM. Chromosomal mapping of two genetic loci associated with blood-pressure regulation in hereditary hypertensive rats. *Nature* **353**: 521-529; 1991

Hollenberg SM, Weinberg C, Ong ES, Cerelli G, Oro A, Lebo R, Thompson EB, Rosenfeld MG, Evans RM. Primary structure and expression of a functional human glucocorticoid receptor cDNA. *Nature* **318**: 635-641; 1985

Honda M, Nowaczynski W, Guthrie GP Jr., Messerli FH, Tolis G, Kuchel O, Genest J. Response of several adrenal steroids to ACTH stimulation in essential hypertension. *J Clin Endocrinol Metab* **44**: 264-272; 1977

Howell GM, Gustafsson J-A, Lefebvre Y. Glucocorticoid receptor identified on nuclear envelopes of male rat livers by affinity labeling and immunochemistry. *Endocrinology* **127**: 1087-1096; 1990

Howell GM, Lefebvre Y. Characterization of high affinity and low affinity dexamethasone binding sites on male rat liver nuclear envelopes. *J Steroid Biochem* **33**: 977-986; 1989

Hunt SC, Williams RR, Barlow GK. A comparison of positive family history definitions for defining risk for future disease. *J Chron Dis* **39**: 809-821; 1986

Hyatt PJ. Functional significance of the adrenal zones. In: Recent Advances in Adrenal regulation and Function, edited by D'Agata R, Chrousos GP. Serono Publication, Raven Press, New York 40: 35-47; 1987

Ichinose M, Miki K, Tatematsu M, Furihata C, Matsushima M, Ichihara Y, Tanji M, Konishi T, Obara M, Inoue H, Kurokawa K, Takahashi T, Kageyama T, Takahashi K. Hydrocortisone-induced enhancement of expression and changes in methylation of pepsinogen genes in stomach mucosa of the developing rat. *Biochem Biophys Res Comm* **172**: 1086-1093; 1990

Iida S, Nakamura Y, Fujii H, Nishmura J-I, Tsugawa M, Gomi M, Fikata J, Tarui S, Morikawa K, Kitani T. A patient with hypocortisolism and Cushing's syndrome-like manifestations: cortisol hyperreactive syndrome. *J Clin Endocrinol Metab* **70**: 729-737; 1990

Ikeda K, Nara Y, Nabica T, Fujimoto A, Endo J, Ishino H, Yamori Y. Genetic factors regulate the rise in blood pressure in F2 generation crossed between stroke-prone spontaneously hypertensive rats and Wistar-Kyoto rats. *Clin Exp Pharmac Physiol* **18**: 593-597; 1991

Isles CG, Macdougall IC, Lever AF, Fraser R. Hypermineralocorticoidism due to adrenal carcinoma: plasma corticosteroids an thei response to ACTH and angiotensin II. *Clin Endocrinol* **26**: 253-272; 1987

Intersalt Cooperative Research Group. Intersalt: an international study of electrolyte excretion and blood pressure. Results for 24 hour urinary sodium and potassium excretion. *Br Med J* **297**: 319-328; 1988

Irony I, Biglieri EG, Kater CE. The adrenocortical hormone in hypertensive disorders. In; Endocrine Mechanism in Hypertension, edited by Laragh JH, Brenner BM, Kaplan NM. Raven Press, New York 1-18; 1989

Jacob HW, Lindpaintner K, Lincoln SE, Kusumi K, Bunker RK, Mao Y-P, Ganten D, Dzau V, Lauder S. Genetic mapping of a gene causing hypertension in the stroke-prone spontaneously hypertensive rat. *Cell* **67**: 213-224; 1991

Jacobsen L, Sapolsky R. The role of hippocampus in feedback regualtion of the hypothalamic-pituitary-adrenal axis. *Endocrine Rev* **12**: 118-134; 1991

Jamieson A, Connell JMC, Fraser R. Glucocorticoid-suppressible hyperaldosteronism: from confusion to conclusion? *J Mol Endocrinol* **10**: 3-5; 1993

Jazayeri A, Meyer III WJ. Glucocorticoid modulation of beta-adrenergic receptors of cultured rat arterial smooth muscle cells. *Hypertension* **12**: 396-398; 1988

Jeunemaitre X, Lifton RP, Hunt SC, Williams RR, Lalouel J-M. Absence of linkage between the angiotensin converting enzyme locus and human essential hypertension. *Nature Genet* **1**: 72-75; 1992a

Jeunemaitre X, Soubrier F, Kotelevtsev YV, Lifton RP, Williams CS, Charru A, Hunt RR, Hopkins PN, Williams RR, Lalouel J-M, Corvol P. Molecular basis of human hypertension: role of angiotensinogen. *Cell* **71**: 169-180; 1992b

Joint National Committee on Detection, Evaluation and Treatment of High Blood Pressure. The fifth report of the Joint National Committee on the Detection, Evaluation, And Treatment of High Blood Pressure (JNC V). *Arch Intern Med* **153**: 154-183, 1993

Joab I, Radanyi C, Renoir M, Buchou T, Catelli M-G, Binart N, Mester J, Baulieu E-E. Common non-hormone binding component in non-transformed chick oviduct receptors of four steroid hormones. *Nature* **308**: 850-853; 1984

Julius S, Conway J. Hemodynamic studies in patients with borderline blood pressure elevation. *Circulation* **38**: 282-288; 1968

Junker K. Glucocorticoid receptors of human mononuclear leukocytes in vitro. *J Clin Endocrinol Metab* **57**: 506-512; 1983

Kalinyak JE, Dorin RI, Hoffman AR, Perlman AJ. Tissue-specific regulation of glucocorticoid receptor mRNA by dexamethasone. *J Biol Chem* **262**: 10441-10444; 1987

Kalsner S. Mechanism of hydrocortisone potentiation of response to epinephrine and norepinephrine in rabbit aorta. *Circ Res* **24**: 383-395; 1969

Kannel WB, Wolf PA, McGee DL, Dawber TR, McNamara PM, Castelli WP. Systolic blood pressure, arterial rigidity and risk of stroke. The Framingham study. *JAMA* **245**: 1225-1228; 1981

Kannel WB, Dannenberg AL, Abbott RD. Unrecognized myocardial infarction and hypertension: the Framingham study. *JAMA* **109**: 581-585; 1985

Karl M, Lamberts SWJ, Detera-wadleigh SD, Encio IJ, Stratakis CA, Hurley DM, Acili D, Chrousos GP. Familial glucocorticoid resistance caused by a splice site deletion in human glucocortiocid receptor gene. *J Clin Endocrinol Metab* **76**: 683-689; 1993

Katz FH, Romfh P, Smith JA. Diurnal variation of plasma aldosterone, cortisol and renin activity in supine man. *J Clin Endocrinol Metab* **40**: 125-131; 1975

Keating M. Linkage analysis and long QT syndrome. *Circulation* **85**: 1973-1986; 1992

Keightley M-C, Fuller PJ. Unique sequences in the guinea pig glucocorticoid receptor induce constitutive transactivation and decrease sensitivity. *Mol Endocrinol* **8**: 431-439; 1994

Kenyon CJ, Saccoccio NA, Morris DJ. Glucocorticoid inhibition of mineralocorticoid action in the rat. *Clin Sci* **67**: 329-335; 1984

Kenyon CJ, Panarelli M, Holloway CD, Dunlop D, Morton JJ, Connell JMC, Fraser R. The role of glucocorticoid activity in the inheritance of hypertension: studies in the rat. *J Steroid Biochem Molec Biol* **45**: 7-11; 1993

Kenyon CJ, Casari G, Zagato L, Inglis GC, Heeley R, Torielli L, Connell JMC, Szpirer C, Klinga K, Sutcliffe RG, Bianchi G. Glucocorticoid receptor and 11β-hydroxylase genes interact epistatically with adducin in blood pressure control of Milan rats. *J Endocrinol* **141** (Suppl.): P77; 1994

Kern JA, Laumb RT, Reed JC, Daniele RP, Nowell PC. Dexamethasone inhibition of interleukin-1β production by human monocytes. Post transcriptional mechanism. *J Clin Invest* **81**: 237-244; 1988

King RJB. Effects of steroid hormones and related compounds on gene transcription. *Clin Endocrinol* **36**: 1-14; 1992

Kish ES, Dluhy RG, Williams GH. Enhanced aldosterone response to angiotensin II in human hypertension. *Circ Res* **38**: 502-505; 1976

Knox FG, Burnett JC, Kohan DE, Spielman WS, Strand JC. Escape from the sodium-retaining effects of mineralocorticoids. *Kidney Internat* **17**: 263-276; 1980

Kornel L, Margolis E. Evidence for overall deficiency of cortisol 11β-hydroxydehydrogenase in patients with essential hypertension. *Proc 9th Int Congr Endocrinol* **142** (abstract); 1992

Kornel L. The role of vascular steroid receptors in the control of vascular contractility and peripheral vascular resistance. *J Steroid Biochem Molec Biol* **45**: 195-203; 1993

Koutouzov S, Remmal A, Marche P, Meyer P. Hypersensitivity of phospholipase C and diglyceride lipase activity in spontaneously hypertensive rats. *Hypertension* **10**: 100-106, 1987

Krakoff LR. Measurement of plasma renin substrate by radioimmunoassay of angiotensin-I: concentrations in syndrome associated with steroid excess. *J Clin Endocrinol Metab* **37**: 110-117; 1973

Krakoff LR, Sevadurai R, Sutter E. Effect of methylprednisolone upon arterial pressure and the renin-angiotensin system in the rat. *Am J Physiol* **228**: 613-617; 1975

Kreutz R, Higuchi M, Ganten D. Molecular genetics of hypertension. *Clin Exper Hyper -Theory and Practice* **A14**(1&2): 15-34; 1992

Kroger H, Arias-Stella J. The placenta and the newborn infant at high altitude. *Am J Obstet Gynecol* **106**: 586-591; 1970

Kurtz TW, Morris RC Jr. Biological variability in Wistar-Kyoto rats. Implications for research with the spontaneously hypertensive rat. *Hypertension* **10**: 127-131; 1987

Kurtz TW, Simonet L, Kabra PM, Wolfe S, Hjelle BL. Cosegration of the renin allele in the spontaneously hypertensive rat with an increase in blood pressure. *J Clin Invest* **85**: 1328-1332; 1990

Kushi LH, Lew RA, Stave FJ, Ellison CR, eL Lozy M, Bourke G, Daly L, Graham I, Hickey N, Mulcahy R, Kevaney J. Diet and 20-year mortality from coronary heart disease. The Ireland-Boston heart study. *N Engl J Med* **312**: 811-818, 1985

Lamberts SW, Koper JW, Bemod P, den Holder FH, de Jong FH. Cortisol receptor resistance: the variability of its clinical presentation and response to treatment. *J Clin Endocrinol Metab* **74**: 313-321; 1992

Lan NC, Graham B, Bartter FC, Baxter JD. Binding of steroids to mineralocorticoid receptors: implication for *in vivo* occupancy by glucocorticoids. *J Clin Endocrinol Metab* 54: 332-342; 1982

Lappin DF, Whaley K. Modulation of complement gene expression by glucocorticoids. *Biochem J* **280**: 117-123; 1991

Larsen PJ, Jessop DS, Chowdrey HS, Lightman SL, Mikkelsen JD. Chronic administration of glucocorticoids directly urpegulates prepro-neuropeptide Y and Y1-receptor mRNA levels in the arcuate nucleus of the rat. *J Neuroendocrinol* **6**: 153-159; 1994

Laue L, Kawai S, Brandon DD, Brightwell D, Barnes K, Knazek RA, Loriaux DL, Chrousos GP. Receptor-mediated effects of glucocorticoids on inflammation: enhancement of the inflammatory response with a glucocorticoid antagonist. *J Steroid Biochem* **29**: 91-598; 1988

Laux M, Bauer H, Ganten U, Zimmerman F, Vecsei P. Glucocortiocid receptors and dissociation costant (Kd) are decreased in mononuclear leukocytes of spontaneously hypertensive rats (SHR-SP) as compared to normotensive Wistar-Kyoto rats (WKY). *J Steroid Biochem* **34**: 331-332; 1989

Law CM, de Swiet M, Osmond C, Fayers PM, Barker DJP, Cruddas AM, Fall CHD. Initiation of hypertension in utero and its amplification throughout life. *Br Med J* **306**: 24-27; 1993

Law PY, Edelman IS. Induction of citrate synthase by aldosterone in the rat kidney. *J Mol Biol* **41**: 41-64; 1978

Leach KL, Dahmer MK, Hammond ND, Sando JJ, Pratt WB. Molybdate inhibition of glucocorticoid receptor inactivation and transformation. *J Biol Chem* **254**: 11844-11890; 1979

Lebrethon MC, Jaillard C, Defayes G, Begeot M, Saez JM. Human cultured adrenal fasciculate-reticularis cells are targets for angiotensin-II: effects on cytochrome P450 cholesterol side-chain cleavage, cytochrome P450 17α-hydroxylase, and 3β-hydroxysteroid-dehydrogenase messenger ribonucleic acid and proteins and on steroidogenic responsiveness to corticotropin and angiotensin-II. *J Clin Endocrinol Metab* **78**: 1212-1219; 1994

Lever AF, Harrap SB. Essential hypertension: disorder of growth with origins in childhood? *J Hypertension* **10**: 101-120; 1992

Lifton RP, Hunt SC, Williams RR, Pouyssegur J, Lalouel J-M. Exclusion of the Na-H antiporter as a candidate gene in human essential hypertension. *Hypertension* **17**: 8-14; 1991

Lifton RP, Dluhy RG, Power M, Rich GM, Cook S, Ulick S, Lalouel J-M. A chimeric 11-beta-hydroxylase/aldosterone synthase gene causes glucocorticoid-remediable aldosteronism and human hypertension. *Nature* **355**: 262-265; 1992

Lifton RP, Jeunemaitre X. Finding genes that cause human hypertension. *J Hypertension* **11**: 231-236; 1993

Light KC, Obrist PA. Cardiovascular reactivity to behavioural stress in young males with and without marginally elevated casual systolic pressures. Comparison of clinic, home and laboratory measures. *Hypertension* **2**: 802-808; 1980

Lijnen P, M'Buyamba-Kabangu J, Fagard RH, Groeseneken DR, Staessen JA, Amery AK. Intracellular concentration and transmembrane fluxes of sodium and postassium in erythrocytes of white normal male subjects with and without a family history of hypertension. *J Hypertension* **2**: 25-30; 1984

Lindpaintner K, Takahashi S, Ganten D. Structural alterations of the renin gene in stroke-prone spontaneously hypertensive rats: examination of genotype-phenotype correlations. *J Hypertension* **8**: 763-773; 1990

Lindpaintner K. Genetic linkage analysis in hypertension: principles and practice. *J Hypertension* **10**: 121-124; 1992

Lindquist S, Craig EA. The heat-shock proteins. *Annu Rev Genet* **22**: 631-677; 1988

Lodwick D, Kaiser MA, Harris J, Privat P, Vincent M, Sassard J, Samani NJ. Failure of the heat shock protein 70 locus to cosegregate with blood pressure in spontaneously hypertensive rat x Wistar-Kyoto rat cross. *J Hypertension* **11**: 1047-1051; 1993

Lombes M, Kenouch S, Souque A, Farman N, Rafestin-Oblin M-E. The mineralocorticoid receptor discriminates aldosterone from glucocorticoids independently of the 11β-hydroxysteroid dehydrogenase. *Endocrinology* **135**: 834-840; 1994

Lopez S, Miyashita Y, Simons SS Jr. Structurally based, selective interaction of arsenite with steroid receptors. *J Biol Chem* **265**: 16039-16042; 1990

Lowry OH, Rosenbrough NJ, Farr AL, Randall RJ. Protein measurement with the folin phenol reagent. *J Biol Chem* **193**: 265-275; 1951

Lowy MT. Corticosterone regulation of brain and lymphoid corticosteroid receptors. *J Steroid Biochem Molec Biol* **39**: 147-154, 1991.

MacGregor GA. Sodium is more important than calcium in essential hypertension. *Hypertension* 7: 628-637; 1985

MacMahon S, Peto R, Cutler J, Collins R, Sorlie P, Neaton J, Abbott R, Goodwin J, Dyer A, Stamler J. Blood pressure, stroke, and coronary heart disease. Part I, prolonged differences in blood pressure: prospective observational studies corrected for the regression dilution bias. *Lancet* **335**: 765-774; 1990

Malo D, Pang SC, Schlager G, Tremblay J, Hamet P. Decrease of blood pressure in spontaneously hypertensive mice by heat tratment. *Am J Hypertension* **3**: 400-404; 1989

Mann SJ. Systolic hypertension in the elderly. *Arch Intern Med* **152**: 1977-84; 1992

Mantero F, Armanini D, Boscaro M. Plasma renin activity and urinary aldosterone in Cushing's syndrome. *Horm Metab Res* **10**: 65-71; 1978

Mantero F, Nussdorfer GG, Robba C, Opocher G, Ferrari P, Bianchi G. Evidence for mineralocorticoid hyperactivity in the Milan hypertensive strain of rats. *J Hypertension* **1** (suppl. 2): 150-152; 1983

Marver D, Goodman D, Edelman IS. Relationships between renal cytoplasmic and nuclear aldosterone-receptors. *Kidney Int* **1**: 210-223; 1972

Marx SJ, Bourdeau JE. Calcium metabolism. In: Clinical Disorder of Fluid and Electrolyte Metabolism, edited by Maxwell MH, Kleeman CR, Narins RG. McGraw-Hill Inc., London 222-224; 1987

Mason PA, Fraser R, Semple PF, Morton JJ. The interaction of ACTH and angiotensin in the control of corticosteroid plasma concentration in man. *J Steroid Biochem* **10**: 235-239; 1979

Mathers JA, Osborne RH, de George FV. Studies of blood pressure, rate and electrocardiogram in adult twins. *Am Heart J* **62**: 634-642; 1961

Matsukawa N, Nonaka Y, Higaki J, Nagano M, Mikami H, Ogihara T, Okamoto M. Dahl's salt-resistant normotensive rat has mutations in cytochrome P450(11beta), but salt-sensitive hypertensive rat does not. *J Biol Chem* **268**: 9117-9121; 1993

McDonald AR, Goldfine ID. Glucocorticoid regulation of insulin receptor gene transcription in IM-9 cultured lymphocytes. *J Clin Invest* **81**:499-504; 1988

McGimsey WC, Cidlowski JA, Stumpf WE, Sar M. Immunocytochemical localization of the glucocorticoid receptor in rat brain, pituitary, liver, and thymus with two new polyclonal antipeptide antibodies. *Endocrinology* **129**: 3064-3072; 1991

McKenzie AW, Soughton RB. Methods for comparing percutaneous absorption of steroids. *Arch Dermatol* **98**: 209-215; 1962

Mendelson FAO, Lloyd CJ, Kachel C, Funder JW. Induction by glucocorticoids of angiotensin converting enzyme production from bovine endothelial cells in culture and rat lung in vivo. *J Clin Invest* **70**: 684-692; 1982

Metzler CH, Gardner DG, Keel LC, Baxter JD, Ramsay DR. Increased synthesis and release of atrial peptide during DOCA escape in conscious dogs. *Am J Physiol* **252**: R188-R192; 1987

Meyer III WJ, Nichols NR. Mineralocorticoid binding in cultured smooth muscle cells and fibroblasts from rat aorta. *J Steroid Biochem* **14**: 1157-1168; 1981

Miall WE. Heredity and hypertension. *Practitioner* **207**: 2027-2030; 1971

Miesfield RL. Molecular genetics of corticosteroid action. *Am Rev Respir Dis* **141**: S11-S17; 1990

Miller SA, Dykes DD, Polesky HF. A simple salting out procedure for extracting DNA from human nucleated cells. *Nucleic Acids Res* **16**: 1215.; 1988

Mitchell KA, Romen RJ. The renin-angiotensin-aldosterone system in volume control. *Bailliere Clin Endocrinol Metab* **3**: 393-430; 1989

Monder C. Corticosteroids, receptors, and the organ-specific functions of 11 beta-hydroxysteroid dehydrogenase. *FASEB J* **5**: 3047-3054; 1991

Mongeau J-G, Biron P, Sing CF. The influence of genetics and household environments upon the variability of blood pressure: the Montreal adoption study. *Clin Exp Hypertens* [A] **8**: 653-660; 1986

Moreland RS, Land FS, Webb RC, Bohr DF. Functional evidence for increased sodium permeability in aortae from DOCA hypertensive rats. *Hypertension* **6** (Suppl. I): I88- I94; 1984

Morris BJ, Catanzaro DF, Hardman J, Mesterovic N, Tellam J, Hort Y, Shine J. Human renin gene sequence, gene regulation and prorenin processing. *J Hypertension* **2** (suppl. 3): 231-233; 1984

Morris DJ, Kenyon CJ. Aldosterone and its metabolism in spontaneously hypertensive rats (SHR). *Clin Exp Hypert* [A] **5**: 11-19; 1983

Morris DJ, Souness GW. Protective and specificity-conferring mechanisms of mineralocorticoid action. *Am J Physiol (Renal Fluid Electrolyte Physiol)* **263**: F759-F768; 1992

Morton JJ, Kenyon CJ, Beatti EC. Hormone and electrolyte changes in post-deoxycorticosterone salt hypertension. *J Hypertension* **8**: 1021-1026; 1990

Muller M, Renkawitz R. The glucocorticoid receptor. *Bioch Bioph Acta* **1088**: 171-182; 1991

Munck A, Wira C, Young DA, Mosher KM, Hallahan C, Bell PA. Glucorticoid-receptor complexes and the earliest steps in the action of glucocorticoid on thymus cell. *J Steroid Biochem* **3**: 567-578; 1972

Munck A, Mendel DB, Smith LI, Orti E. Glucocorticoid receptors and actions. *Am Rev Respir Dis* **141**: S2-S10; 1990

Munson PJ, Rodbard D. Ligand: versatile computerized approach for characterisation of ligand-binding systems. *Anal Biochem* **107**: 220-239; 1980

Murakami K, Kojima S-I, Kimura G, Sanai T, Yoshida K, Imanishi M, Abe H, Kawamura M, Kawano Y, Ashida T, Yoshimi H, Kuramochi M, Omae T. The association between salt sensitivity of blood pressure and family history of hypertension. *Clin Exp Pharmacol Physiol* **19** (Suppl.20): 61-63; 1992

Murakami T, Brandon DD, Rodbard D, Loriaux DL, Lipsett MB. Glucocorticoid receptor in polymorphonuclear leukocytes: a simple method for leukocytes glucocorticoid receptor characterization. *J Steroid Biochem* **10**: 475-481; 1978

Murakami T, Brandon DD, Rodbard D, Loriaux DL, Lipsett MB. Glucocorticoid receptor in circulating mononuclear leukocytes. *Endocrinology* **104**: 500-505; 1979

Murray JC, Smith RF, Ardinger HA, Weinberger C. RFLP for the glucocorticoid receptor (GRL) located at 5q11-5q13. *Nucl Acid Res* **15**: 6765; 1987

Nadeau K, Das A, Walsh CT. Hsp90 chaperonins possesses ATPase activity and bind heat shock transcription factors and peptidyl prolyl isomerases. *J Biol Chem* **268**: 1479-1487; 1993

Neilfield JP, Lippman M, Tormey DC. Steroid hormone receptors in normal human lymphocytes. Induction of glucocorticoid receptor activity by phytohemagglutinin. *J Biol Chem* **252**: 2972-2977; 1977

Nemoto T, Ohara-Nemoto Y, Denis M, Gustafsson J-A. The transformed glucocorticoid receptor has a lower steroid-binding affinity than the nontransformed receptor. *Biochem* **29**: 1880-1886; 1990a

Nemoto T, Mason GGF, Wilhelmsson A, Cuthill S, Hapgood J, Gustafsson J-A, Pollinger L. Activation of the dioxin and glucocorticoid receptors to a DNA binding state under cell-free conditions. *J Biol Chem* **265**: 2269-2277; 1990b

Neville AM, O'Hare MJ. Histopathology of the human adrenal cortex. *Clinics Endocrinol Metab* **14**: 791-820; 1985

Nussdorfer GG. Cytophysiology of the adrenal cortex. *Int Rev Cytol* **98**: 1-405; 1986

O'Malley BW. The steroid receptor superfamily: more exitement predicted for the future. *Mol Endocrinol* **4**: 363-369; 1990

Ouali R, Poulette S, Penhoat A, Saez JM. Characterization and coupling of angiotensin-II receptor subtypes in cultured bovine adrenal fasciculata cells. *J Steroid Biochem Mol Biol* **43**: 271-280; 1992

Pardes EM, de Yampey JW, Soto RJ, Moses DF, De Nicola AF. A correlative study between glucocorticoid receptor levels in human mononuclear leukocytes and biochemical data in Cushing's disease. *Acta Endocrinol (Copenh)* **120**: 55-61; 1989

Pardridge WM. Plasma protein-mediated transport of steroid and thyroid hormones. *Am J Physiol (Endocrinol Metab)* **252**: E157-E164; 1987

Parfrey PS, Wright P, Ledingham JM. Prolonged isometric exercice, part 2: effect on circulation and on renal excretion of sodium and potassium in young males genetically predisposed to hypertension. *Hypertension* **3**: 188-191; 1981

Parker LN, Lifrak ET, Kawahara CK, Geduld SI, Kozbur XM. Angiotensin II potentiates ACTH-stimulated adrenal androgen secretion. *J Steroid Biochem* **18**: 205-208; 1983

Pearce D. A mechanistic basis for distinct mineralocorticoid and glucocorticoid receptor transcriptional specificities. *Steroids* **59**: 153-159; 1994

Pedersen SB, Jonler M, Richelsen B. Characterization of regional and gender differences in glucocorticoid receptors and lipoprotein lipase activity in human adipose tissue. *J Clin Endocrinol Metab* **78**: 1354-1359; 1994

Peiffer A, Lapointe B, Barden N. Hormonal regulation of type II glucocorticoid receptor messenger ribonucleic acid in rat brain. *Endocrinology* **129**: 2166-2174; 1991

Pham-Huu-Trung MT, Bogyo A, Leneuve P, Girard F. Compared effects of ACTH, angiotensin II and POMC peptides on isolated human adrenal cells. *J Steroid Biochem* **24**: 345-348; 1986

Picard D, Yamamoto KR. Two signals mediate hormone-dependent nuclear localization of the glucocorticoid receptor. *EMBO J* **6**: 3333-3340; 1987

Pickering TG. Blood pressure measurement and detection of hypertension. *Lancet* **344**: 31-35, 1994

Pietinen PI, Wong O, Altschul AM. Electrolyte output, blood pressure, and family history of hypertension. *Am J Clin Nutr* **32**: 997-1005; 1979

Place VA, Velasquez JG, Burdick KH. Precise evaluation of topically applied corticoisteroid potency. Modification of the Stoughton-Mckenzie assay. *Arch Dermatol* **101**: 531-537; 1970

Pratt WB. Transformation of glucocorticoid and progesterone receptors to the DNA-binding state. *J Cell Biochem* **35**: 51-68; 1987

Pratt WB, Jolly DJ, Pratt DV, Hollenberg SM, Giguere V, Cadepond FM, Schweizer-Groyer G, Catelli M-G, Evans RE, Baulieu E-E. A region in the steroid binding domain determines formation of the non-DNA binding, 9S glucocorticoid receptor complex. *J Biol Chem* **263**: 267-273; 1988

Pratt WB. Interaction of hsp90 with steroid receptors: organising some diverse observations and presenting the newest concepts. *Mol Cell Endocrinol* **74**: c69-c76; 1990

Pratt WB. The role of heat shock proteins in regulating the function, folding, and trafficking of the glucocorticoid receptor. *J Biol Chem* **268**: 21455-21458; 1993

Quinn SJ, Williams GH. Regulation of aldosterone secretion. *Annu Rev Physiol* **50**: 409-426; 1988

Quirk SG, Gannel JE, Funder JW. Aldosterone binding sites in pregnant and lactating mammary gland. *Endocrinology* **113**: 1812-1817; 1983

Radomski MW, Palmer MJ, Moncada S. Glucocorticoids inhibit the expression of an inducible, but not the constitutive, nitric oxide synthase in vascular endothelial cells. *Proc Natl Acad Sci* **87**: 10043-10047; 1990

Rapp JP, Dahl LK. Mendelian inheritance of 18 and 11 beta steroid hydroxylase activities in the adrenals of rats genetically susceptible or resistant to hypertension. *Endocrinology* **90**: 1435-1446; 1972

Rapp JP. Dahl salt-susceptible and salt-resistant rats. A review. *Hypertension* **4**: 753-763; 1982

Rapp JP, McPartland RP, Sustarsie DL. Anomalous response of urinary kallikrein to deoxycorticosteone in Dahl-salt sensitive rats. *Hypertension* **4**: 20-26; 1982

Rapp JP, Wang SM, Dene H. A genetic polymorphism in the renin gene of Dahl rat cosegregates with blood presure. *Science* **243**: 542-544; 1989

Rayyis SS, Horton R. Effect of angiotensin II on adrenal and pitutary function in man. *J Clin Endocrinol Metab* **32**: 539-546; 1979

Reid IR. Effects of glucocorticoids on bone mineral metabolism, growth and fracture rates. In: Christiansen C and Krane SM, eds. Advances in corticosteroids. A seminar in print. Addis International 27-38; 1993

Reul JHM, de Kloet ER. Two receptor systems for corticosterone in rat brain: microdistribution and differential occupation. *Endocrinology* **117**: 2505-2511; 1985

Reul JMH, Pearce PT, Funder JW, Krozowski ZS. Type I and type II corticosteroid receptor gene expresson in the rat: effect of adrenalectomy and dexamethasone administration. *Mol Endocrinol* **3**: 1674-1680; 1989

Reul JHM, de Kloet ER, van Sluijs FJ, Rijnberk A, Rothuizen M. Binding characteristics of mineralocorticoid and glucocorticoid receptors in dog brain and pituitary. *Endocrinology* **127**: 907-915; 1990

Rexin M, Bush W, Gehring U. Protein components of the nonactivated glucocorticoid receptor. *J Biol Chem* **266**: 24601-24605; 1991

Rexin M, Bush W, Segnitz B, Gehring U. Structure of the glucocorticoid receptor in intact cells in the absence of hormone. *J Biol Chem* **267**: 9619-9621; 1992

Ritchie CM, Sheridan B, Fraser R, Hadden DR, Kennedy AL, Riddell J, Atkinson AB. Studies on the pathogenesis of hypertension in Cushing's disease and acromegaly. *Q J Med* **76**: 855-867; 1990

Robertson JIS. Salt, volume, and hypertension: causation or correlation? *Kidney Int* **32**: 590-602; 1987

Robertson TL, Kato H, Gordon T, Kagan A, Rhoads GG, Land CE, Worth RM, Belsky JL, Docks DS, Miyanishi M, Kawamoto S. Epidemiologic studies of coronary heart disease and stroke in Japanese men living in Japan, Hawai and California. Coronary heart disease risk factors in Japan and Hawai. *Am J Cardiol* **39**: 244-249; 1977

Rolland P, Roland AM, Bankoel L, Toga M. Prostaglandins and steroidogenesis in isolated bovine adrenal cells. Effects of ACTH, prostaglandin synthesis inhibitors, prostaglandins and prostaglandins analogs. *Mol Cell Endocrinol* **22**: 179-193; 1981

Ross EJ, Linch DC. Cushing's syndrome - killing disease: Discriminatory value of signs and symptoms aiding early diagnosis. *Lancet* **2**: 646-649; 1982

Ruch W, Baumann JB, Hausler A, Otten VH, Siegl H, Girard J. Importance of the adrenal cortex for developement and maintenance of hypertension in spontaneously hypertensive rats. *Acta Endocrinol* **105**: 417-424; 1984

Russo D, Fraser R, Kenyon CJ. Increased sensitivity to noradrenaline in glucocorticoid-treated rats: the effects of indomethacin and desipramine. *J Hypertension* **8**: 827-833; 1990

Salmond CE, Prior IAM, Wessen AF. Blood pressure patterns and migration: a 14 year cohort study of adult Tokelauans. *Am J Epidemiol* **130**: 37-52; 1989

Samani NJ, Brammar WJ, Swales JD. A major structural abnormality in the renin gene of the spontaneously hypertensive rat. *J Hypertension* **7**: 249-254; 1989a

Samani NJ, Swales JD, Brammar WJ. A widespread abnormality of renin gene expression in the spontaneously hypertensive rat: modulation in some tissues with the development of hypertension. *Clin Sci* **77**: 629-636; 1989b

Sanchez ER, Toft DO, Schleinger MJ, Pratt WB. Evidence that the 90 kDa phosphoprotein associated with the untransformed L-cell glucocorticoid receptor is a murine heat shock protein. *J Biol Chem* **260**: 12398-123401; 1985

Saruta T, Suzuky H, Handa M, Igarashi Y, Kondo K, Senba S. Multiple factors contribute to the pathogenesis of hypertension in Cushing's syndrome. *J Clin Endocrinol Metab* **62**: 275-279; 1986

Sato A, Suzuki H, Murakami M, Nakazato U, Iwaita Y, Saruta T. Glucocorticoid increases angiotensin II type 1 receptor and its gene expression. *Hypertension* **23**: 25-30; 1994

Scherrer LC, Dalman FD, Massa E, Meshinchi S, Pratt WB. Structural and functional reconstruction of the glucocorticoid receptor-Hsp90 complex. *J Biol Chem* **265**: 24397-21400; 1990

Schlechte JA, Ginsberg BH, Sherman BM. Regulation of the glucocorticoid receptor in human lymphocytes. *J Steroid Biochem* **16**: 69-74; 1982

Schlechte JA, Sherman BM. Lymphocytes glucocorticoid receptor binding in depressed patients with hypercortisolemia. *Psyconeuroendocrinol* 10: 469-474; 1985

Schlechte JA, Simons JrSS, Lewis DA, Thompson EB. [^{3}H]Cortivazol: a unique high affinity ligand for the glucocorticoid receptor. *Endocrinology* **117**: 1355-1362; 1985

Schuchard M, Landers JP, Sandhu NP, Spelsberg TC. Steroid hormone regualtion of nuclear proto-oncogenes. *Endocrine Rev* **14**: 659-669; 1993

Schule R, Muller M, Kaltschmidt C, Tenkawits R. Many transcription factors interact synergistically with steroid receptors. *Science* **242**: 1418-1420; 1988

Scoggins BA, Denton DA, Whitworth JA, Coghlan JP. ACTH-dependent hypertension. *Clin Exp Hypertension [A]* **6**: 599-646; 1984

Seckl JR, Brown RW. 11-Beta-hydroxysteroid dehydrogenase: on several roads to hypertension. *J Hypertension* **12**: 105-112; 1994

Seidman CE, Bloch KD, Klein KA, Seidman JG. Nucleotide sequences of the human and mouse atrial natriuretic factor genes. *Science* **226**: 1206-1209; 1984

Sessa, WC, Lin L, Nasjletti A. Reciprocal effects of dexamethasone on vasodilatory response to arachidonic acid and prostanoids in the isolated perfused rabbit kidney. *Hypertension* **15**: I93-I96; 1990

Shackleton CHL, Rodriguez J, Arteaga E, Lopez JM, Winter JSD. Congenital 11β-hydroxisteroid dehydrogenase deficiency associated with juvenile hypertension: corticosteroid metabolite profiles in four patients and their parents. *Clin Endocrinol* **22**: 701-712; 1985

Sheppard K, Funder JW. Mineralocorticoid specificity of renal Type I receptors: *in vivo* binding studies. *Am J Physiol (Endocrinol)* **252**: E224-E229; 1987a

Sheppard K, Funder JW. Type I receptors in parotid, colon, and pituitary are aldosterone selective *in vivo*. *Am J Physiol (Endocrinol)* **253**: E467-E471; 1987b

Shipman GF, Bloomfield CD, Gajl-Peczalska KJ, Munck AU, Smith KA. Glucocorticoid and lymphocytes III. Effects of glucocorticoid administration on lymphocyte glucocorticoid receptors. *Blood* **61**: 1086-1090; 1983

Shoenfeld Y, Gurewich Y, Gallant LA, Pinkhas J. Prednisone-induced leukocytosis: influence of dosage, method and duration of administration on the degree of leukocytosis. *Am J Med* **71**: 773- 778; 1981

Simmons PS, Miles JM, Gerich JE, Haymond MW. Increased proteolysis. An effect of increases in plasma cortisol within the physiologic range. *J Clin Invest* **73**: 412-420; 1984

Singer FR. Metabolic bone disease. In: Endocrinology and Metabolism 2nd ed., edited by Felig P, Baxter JD, Broadus AE, Frohman LA. New York, McGraw-Hill, 1454-1499; 1987

Smith DF, Toft DO. Steroid receptors and their associated proteins. *Mol Endocrinol* **7**: 4-11; 1993

Smith JB, Ash KO, Hunt SC, Hentschel WM, Sprowell W, Dadone MM, Williams RR. Three red cell sodium transport systems in hypertensive and normotensive Utah adults. *Hypertension* **6**: 159-166; 1984

Smith RG, Nag A, Syms AJ, Norris JS. Steroid regulation of receptor concentration and oncogene expression. *J Steroid Biochem* **24**: 51-55; 1986

Smith TW, Turner Cw, Ford MH, Hunt SC, Barlow GK, Stults BM, Williams RR. Blood pressure reactivity in adult male twins. *Health Psycology* **6**: 209-220; 1987

Smith WM. Epidemiology of hypertension. *Med Clin North Am* **61**: 467-486; 1977

Soro A, Ingram MC, Tonolo G, Glorioso N, Fraser R. Evidence of coexisting changes in 11β-hydroxysteroid dehydrogenase and 5β-reductase activity in patients with untreated essential hypertension. *Hypertension* **25**: 67-70, 1995

Soubrier F, Houot AM, Jeunemaitre X, Plouin PF, Corvol P. Molecular biology as a tool for genetic research in hypertension: application to the renin gene. *J Cardiovasc Pharmacol* **12** (Suppl. 3): S155-S159; 1988

Southern EM. Detection of specific sequences among DNA fragments separated by gel electrophoresis. *J Mol Biol* **98**: 503-517; 1975

Spira A, Spira N, Goujjrd J, Goujard J, Schwartz D. Smoking during pregnancy and placental weight: a multivariant analysis of 3759 cases. *J Perinat Med* **3**: 237-241; 1975

Stamler R, Stamler J, Riedlinger WF, Algera G, Roberts RH. Family (parental) history and prevalence of hypertension. Results of a nationwide screening program. *JAMA* **241**: 43-46; 1979

Stamler J, Stamler R, Neaton JD. Blood pressure, systolic and diastolic, and cardiovascular risks: US population data. *Arch Intern Med* **153**: 598-615, 1993

Stanton B, Giebisch G, Klein-Robbenhaar G, Wade J, DeFronzo RA. Effects of adrenalectomy and chronic adrenal corticosteroid replacement on potassium transport in rat kidney. *J Clin Invest* **75**: 1317-1326; 1985

Steiner C, Muller M, Baniahmad A, Renkawitz R. Lysozyme gene activity in chicken macrophages is controlled by positive and negative regulatory elements. *Nucleic Acids Res* **15**: 4163-4178; 1987

Stephenson G, Krozowski Z, Funder JW. Extravascular CGB-like sites in rat kidney and mineralocorticoid receptor specificity. *Am J Physiol* (Fluid and Electrolytes) **246**: F227-F233; 1984

Stewart PM, Edwards CRW. The cortisol-cortisone shuttle and hypertension. *J Steroid Biochem Molec Biol* **40**: 501-509; 1991

Stewart PM, Whorwood CB. Congenital and acquired deficiency of 11Beta-hydroxysteroid dehydrogenase. In: Saez JM, Brownie AC, Capponi A, Chambaz EM, Mantero F, eds. Cellular and Molecular Biology of the Adrenal Cortex. Colloque INSERM/John Libbey Eurotext Ltd., 175-187; 1992

Stewart PM, Whorwood CB, Valentino R, Burt D, Sheppard MC, Edwards CRW. 11β-hydroxisteroid dehydrogenase activity and gene expression in the hypertensive Bianchi-Milan rat. *J Hypertension* **11**: 349-354; 1993

Stewart PM. 11β-Hydroxisteroid dehydrogenase. *Bailliere's Clin Endocrinol Metab* **8**: 357-378; 1994

Strunk RC, Kunke KS, Musson RA. Lack of requirement for spreading for macrophages to synthesize complement. *J Reticuloendothel Soc* **28**: 483-493; 1980

Sumida C, Vallette G, Nunez EA. Interaction of unsaturated fatty acids with rat liver glucocorticoid receptors: studies to localize the site of interaction. *Acta Endocrinol (Copenh)* **129**: 348-355; 1993

Tai P-KK, Maeda Y, Nakao K, Wakim NG, Duhring JL, Faber LE. A 59-kilodalton protein associated with progestin, estrogen, androgen, and glucocorticoid receptors. *Biochemistry* **25**: 5269-5275; 1986

Tait JF, Tait SAS. The effect of plasma protein binding on the metabolism of steroid hormones. *J Endocrinol* **131**: 339-357; 1991

Tanaka H, Akama H, Ichikawa Y, Homma M, Oshima H. Glucocorticoid receptors in normal leukocytes: effects of age, gender, season and plasma cortisol concentration. *Clin Chem* **37**: 1715-1719; 1991

Tanaka H, Akama H, Ichikawa Y, Homma M, Makino I. Glucocorticoid receptor and inhibition of 3-O-methyl-D-glucose uptake by glucocorticoids in peripheral blood leukocytes from normal humans: correlation between receptor level and hormone effect in vitro. *Acta Endocrinol (Copenh)* **126**: 29-36; 1992

Tannin GM, Agarwal AK, Monder C, New MI, White PC. The human gene for 11β-hydroxysteroid dehydrogenase. *J Biol Chem* **266**: 16653-16658; 1991

Tiret L, Kee F, Poirier O, Nicaud V, Lecerf L, Evans A, Cambou J-P, Arveiler D, Luc G, Amouyel P, Cambien F. Deletion polymorphism in angiotensin-converting enzyme gene associated with parental history of myocardial infarction. *Lancet* **341**: 991-992; 1993

Tobian I, Binion JT. Tissue cations and water in arterial hypertension. *Circulation* **5**: 754-758; 1952

Tomita M, Brandon DD, Chrousos GP, Vingerhoeds ACM, Foster CM, Fowler D, Loriaux DL, Lipsett MB. Glucocorticoid receptors in Epstein-Barr virus-transformed lymphocytes from patients with glucocorticoid resistance and a glucocorticoid-resistant new world primate species. *J Clin Endocrinol Metab* **62**: 1145-1154; 1986

Tonolo G, Fraser R, Connell JMC, Kenyon CJ. Chronic low-dose of dexamethasone in rats: effects on blood pressure, body weight and plasma atrial natriuretic peptide. *J Hypertension* **6**: 25-31; 1988

Tully DB, Cidlowski JA. Affinity of interactions between human glucocorticoid receptor and DNA, at physiologic ionic strength, stable binding occurs only with DNAs containing partially symmetric glucocorticoid response elements. *Biochemistry* **29**: 6662-6670, 1990

Turla MB, Webb RC. Enhanced vascular activity to protein kinase C activators in genetically hypertensive rats. *J Hypertension* **9**: 150-154; 1987

van Rijen EAM, Harvey JG, Rose RN, Barton RN, Horan MA. Sensitivity to dexamethasone in elderly women after upper femur fracture. *J Endocrinol* **137** (Suppl.): P15; 1993

Van Voorhis BJ, Anderson DJ, Hill JA. The effects of RU 486 on immune function and steroid-induced immunosuppression *in vitro*. *J Clin Endocrinol Metab* **69**: 1195-1199; 1989

Vecsei P, Frank K, Haack D, Heinze V, Ho A-D, Honour JW, Lewicka S, Schoosch M, Ziegler R. Primary glucocorticoid receptor defect with likely familial involvement. *Cancer Res* **49**: 2220s-2221s; 1989

Veterans Administration Cooperative Study Group on Antihypertensive agents. Effects of treatment on morbidity in hypertension, II: results in patients with diastolic blood pressure averaging 90 through 114 mm Hg. *JAMA* **213**: 1143-1152; 1970

Vincent M, Gomez-Sanchez CE, Bataillard A, Sassard J. Steroid during development of genetic hypertension in rats of the Lyon strain. *Am J Physiol* **257**: H506-H510; 1989

Vinson GP, Whitehouse, B, Hinson J. Nature, biosynthesis and metabolism of the adrenocortical secretion. In: The Adrenal Cortex. Prentice Hall Endocrinology Series, 34-64; 1992

Walker BR, Yau JL, Brett LP, Seckl JR, Monder C, Williams BC, Edwards CRW. 11-Beta-hydroxisteroid dehydrogenase in vascular smooth muscle and heart: implications for cardiovascular responses to glucocorticoids. *Endocrinology* **129**: 3305-3312; 1991

Walker BR, Edwards CRW. Cushing's syndrome. In: James VHT, ed. The adrenal gland. New York:Raven Press, Ltd 289-318; 1992

Walker BR, Stewart PM, Shaskleton CHL, Padfield PL, Edwards CRW. Deficient inactivation of cortisol by 11β-hydroxysteroid dehydrogenase in essential hypertension. *Clin Endocrinol* **39**: 221-227; 1993

Walker BR. Defective enzyme-mediated receptor protection: novel mechanisms in the pathophysiology of hypertension. *Clin Sci* **85**: 257-263; 1993

Ward K, Hata A, Jeunemaitre X, Helin C, Nelson L, Namikawa C, Farrington PF, Ogasawara M, Suzumori K, Tomoda S, Berrebi S, Sasaki M, Corvol P, Lifton RP, Lalouel J-M. A molecular variant of angiotensinogen associated with pre-eclampsia. *Nature Genet* **4**: 59-61; 1993

Ward RH, Pearl GC, Prior IAM. Tokelau Island Migrant Study. Effect of migration on the familial aggregation of blood pressure. *Hypertension* **2** (suppl. I): I-43-I-54; 1980

Ward RH. Familial aggregation and genetic epidemiology of blood pressure. In: Laragh JH, Brenner BM, eds. Hypertension pathophysiology, diagnosis and mangement. New York: Raven, 81-100; 1990

Watkins PC. Restriction fragment length polymorphism (RFLP): applications in human chromosome mapping and genetic disease restriction. *Bio Techniques* **6**: 310-320; 1988

Watt GCM, Foy CJW. Dietary sodium and arterial pressure: problems of studies within a single population. *J Epidemiol Comm Health* **36**: 197-201; 1982

Watt GCM, Foy CJW, Hart JT, Bingham G, Edwards C, Hart M, Thomas E, Walton PAM. Dietary sodium and arterial blood pressure: evidence against genetic susceptibility. *Br Med J* **291**: 1525-1528; 1985

Watt GCM, Harrap SB, Foy CJW, Holtonm DW, Edwards HV, Davidson HR, Connor JM, Lever AF, Fraser R. Abnormalities of glucocorticoid metabolism and the renin-angiotensin system: a four-corners approach to the identification of genetic determinats of blood pressure. *J Hypertension* **10**: 473-482; 1992

Weaver JU, Hitman GA, Kopelman PG. An association between BclI restriction fragment length polymorphism of the glucocorticoid receptor locus and hyperinsulinaemia in obese women. *J Mol Endocrinol* **9**: 295-300; 1992

Webb RC. Increased vascular sensitivity to serotonin and methylsergide in hypertension in ras. *Clin Sci* **63**: 735-755; 1982

Wehling M, Christ M, Theisen K. High affinity aldosterone binding to plasma membrane rich fractions from mononuclear leukocytes: is there a membrane receptor for mineralocorticoids? *Biochem Biophys Res Comm* **181**: 1306-1312; 1991a

Wehling M, Kasmayr J, Theisen K. The Na-H exchanger is stimulated and cell volume increased in lymphocytes from patients with essential hypertension. *J Hypertension* **9**: 519-524; 1991b

Wehling M, Eisen C, Christ M. Aldosterone-specific membrane receptors and rapid non-genomic actions of mineralocorticoids. *Mol Cell Endocrinol* **90**: C5-C9; 1992a

Wehling M, Kasmayr J, Christ M, Sippell W, Theisen K, Muller O-A. The early mineralocorticoid effector mechanism, the sodium-proton exchanger, is sensitized in lymphocytes from patients with Cushing's syndrome. *Clin Endocrinol* **37**: 260-265; 1992b

Weinberger MH, Grim CE, Hollifield JW, Kem DC, Ganguly A, Kramer NJ, Yune HY, Wellman H, Donohue JP. Primary aldosteronism: diagnosis, localization and treatment. *Ann Intern Med* **90**: 386-395; 1979

Wenham PR. DNA-based techniques in clinical biochemistry: a beginner's guide to theory and practice. *Ann Clin Biochem* **29**: 598-624; 1992

Wenting GJ, Man in t'Veld AJ, Verhoeven RP, Derkx FA, Schalekamp MAP. Volume-pressure relationships during development of mineralocorticoid hypertension in man. *Circulation Res* **40** (Suppl. 1): 163-170; 1977

Werner S, Thoren M, Gustafsson J-A, Bronnegard M. Glucocorticoid receptor abnormalities in fibroblasts from patients with idiopathic resistance to dexamethasone diagnosed when evaluated for adrenocortical disorders. *J Clin Endocrinol Metab* **75**: 1005-1009; 1992

West MJ, Summers KM, Huggard PR. Polymorphisms of candidate genes in essential hypertension. *Clin Exp Pharmacol Physiol* **19**: 315-318; 1992

Whelton PK, He J, Klag MJ. Blood pressure in westernized populations. In: Swales JD, Ed. Textbook of hypertension. Oxford: Blackwell Scientific 11-21; 1994

White BD, Martin RJ. Alterations in the binding characteristics of glucocorticoid receptors from Zucker rats. *J Steroid Biochem* **36**: 681-686; 1990

Whitworth JA, Saines D, Thatcher R, Putkus A, Scoggins BA. Blood pressure and metabolic effects of ACTH in normotensive and hypertensive man. *Clin Exp Hypertension* **5**: 5501-522; 1983

Whitworth JA. Mechanism of glucocorticod-induced hypertension. *Kidney Int* **31**: S49-S51; 1987

Whitworth JA, Gordon D, McLachlan-Troup N, Scoggins BA, Moulds RWF. Dexamethasone suppression in essential hypertension: effects on cortisol and blood pressure. *Clin Exp Hypertension* [A] **11**: 323-335; 1989

Whitworth JA, Hewitson TD, Li M, Wilson RS, Scoggins BA, Wright RD, Kincaid Smith P. Adrenocorticotrophin-induced hypertension in the rat: haemodynamic, metabolic and morphological characteristics. *J Hypertension* **8**: 27-36; 1990

Whitworth JA, Scoggins BA. A hypertensinogenic class of steroid hormone activity in man? *Clin Exp Pharmacol Physiol* **17**: 163-166; 1990

Whitworth JA. Adrenocorticotrophin and steroid-induced hypertension in humans. *Kidney Int* **41** (Suppl.37): S34-S37; 1992

Whorwood CB, Sheppard MC, Stewart PM. Licorice inhibits 11β-hydroxysteroid dehydrogenase messenger ribonucleic acid levels and potentiates glucorticoid hormone action. *Endocrinology* **132**: 2287-2292; 1993

Wiech H, Buchner J, Zimmermann R, Jakob U. Hsp 90 chaperones protein folding in vitro. *Nature* **358**: 169-170; 1992

Williams B. Insulin resistance: the shape of things to come. *Lancet* **344**: 521-524; 1994

Williams GH, Hollenberg NK, Moore TS, Swarta SL, Duhly RG. The adrenal receptor for angiotensin II is altered in essential hypertension. *J Clin Invest* **63**: 419-427; 1979

Williams GR, Franklin JA. Physiology of the steroid-thyroid hormone nuclear receptor superfamily. *Bailliere's Clin.Endocrinol Metab* **8**: 241-266; 1994

Williams RR, Hunt SC, Hasstedt SJ, Berry TD, Wu LL, Barlow GK, Stults BM, Kuida H. Definition of genetic factors in hypertension: a search for major genes, polygenes, and homogeneous subtypes. *J Cardiovasc Pharmacol* **12** (Suppl. 3): S7-S20; 1988

Williams RR. Will gene markers predict hypertension? *Hypertension* **14**: 610-613; 1989

Wisgerhof M, Brown RD. Increased adrenal sensitivity to angiotensin II in low-renin essential hypertension. *J Clin Invest* **61**: 1456-1462; 1978

Wrange O, Eriksson P, Perlmann T. The purified activated glucocorticoid receptor is a homodimer. *J Biol Chem* **264**: 5253-5259; 1989

Yagisawa H, Emori Y, Nojima H. Phospholipase C genes display restriction fragment length polymorphisms between the genomes of normotensive and hypertensive rats. *J Hypertension* **9**: 303-307; 1991

Yasunari K, Kohono M, Murakawa K, Yokokawa K, Takeda T. Effect of glucocorticoid on prostaglandin E1 mediated cyclic AMP formation by vascular smooth muscle cells. *J Hypertension* **6**: 1023-1028; 1988

Zee RYL, Lou Y-K, Griffiths LR, Morris BJ. Association of a polymorphism of the angiotensin I-converting enzyme gene with essential hypertension. *Biochem Biophys Res Comm* **184**: 9-15; 1992

Zoccali C, Usherwood T, Brown JJ, Lever AF, Robertson JIS, Fraser R. A comparison of the effects of angiotensin II infusion and variations in salt intake on plasma aldosterone levels in normal subjects, patients with essential hypertension and patients with hyperaldosteronism. *J Steroid Biochem* **9**: 327-331; 1983

PUBLICATIONS

The following papers have been published as a result of the work carried out for this thesis:

Kenyon CJ, Panarelli M, Holloway CD, Dunlop D, Morton JJ, Connell JMC, Fraser R. The role of glucocorticoid activity in the inheritance of hypertension: studies in the rat. *J Steroid Biochem Molec Biol* **45**: 7-11; 1993

Panarelli M, Holloway CD, Mulatero P, Fraser R, Kenyon CJ. Inhibition of lysozyme release from human mononuclear leucocytes by dexamethasone: an index of glucocorticoid activity. *J Clin Endocrinol Metab* **78**: 872-877; 1994

Panarelli M and Fraser R. The glucocorticoid receptor and hypertension. *Endocrine Res* **20**: 101-116; 1994

Panarelli M, Holloway CD, Barr ABP, Fraser R, Kenyon CJ. Differences in temperature-sensitive receptor binding of glucocorticoid in spontaneously hypertensive (SHR) and normotensive Wistar Kyoto (WKY) rats. *Steroid* **60**: 73-75; 1995

The following paper has been submitted for publication:

Panarelli M, Holloway CD, Fraser R, Kenyon CJ. Evidence of an impaired glucocorticoid receptor in Milan hypertensive rats.

In addition, the following abstracts were presented to scientific meetings:

Panarelli M, Holloway CD, Fraser R, Connell JMC, Cook A, Kenyon CJ. Are glucocorticoid receptor (GR) genotypes associated with differences in binding characteristics and/or glucocorticoid responses? *J Endocrinol* **132** (suppl); 1992

Panarelli M, Holloway CD, Connell JMC, Fraser R, Kenyon CJ. Do variation in glucocorticoid receptor genotype affect dexamethasone binding or responsiveness? *Proc 9th Int Cong Endocrinol* Nice; 1992

Holloway CD, Panarelli M, Fraser R, Kenyon CJ. Inhibition of lysozyme release from monocytes by dexamethasone. *Proc 9th Int Cong Endocrinol* Nice; 1992

Panarelli M, Holloway CD, Mulatero P, Fraser R, Kenyon CJ. Inhibition of lysozyme release from human mononuclear leucocytes by dexamethasone: a sensitive and specific assay of glucocorticoid activity. *J Endocrinol* **135** (suppl.); 1992

Panarelli M, Holloway CD, Barr ABP, Fraser R, Kenyon CJ. Differences in temperature-sensitive receptor binding of glucocorticoid in spontaneously hypertensive (SHR) and normotensive Wistar Kyoto (WKY) rats. *J Endocrinol* **135** (suppl.); 1992

Panarelli M, Holloway CD, Fraser R, Kenyon CJ. Evidence of an impaired glucocorticoid receptor in Milan hypertensive rats. *J Endocrinol* **140** (suppl.); 1994

Panarelli M, Fraser R, Holloway CD, Connell JMC, Kenyon CJ. Glucocorticoid activity and cardiovascular risk factors. *J Endocrinol* **141** (suppl.); 1994