REGULATION OF NORADRENALINE RELEASE FROM RAT BRAIN TISSUE CHOPS BY α_2-ADRENOCEPTORS

by

ONG MEI LENG

Thesis submitted for the degree of

Doctor of Philosophy

Department of Biochemistry,

University of Glasgow,

Glasgow, G12 8QQ.

January, 1989

Dedicated to

my parents

and

all the lives of the male wistar rats
which had been sacrificed to make this
thesis possible

ACKNOWLEDGEMENTS

With utmost gratitude and sincere appreciation I would like to thank the following people to whom I am deeply indebted, for the assistance in my postgraduate research and in the preparation of this thesis:

The late Professor R.M.S. Smellie and Professor M.D. Houslay for the laboratory facilities in the Department of Biochemistry;

Dr. P.F.T. Vaughan for his supervision;

Dr. R.H.C. Strang for helpful and informal discussion;

Mrs. Janet Greenwood for typing this thesis;

Mr. Ian Ramsden and Mr. Allan Hughes of the Medical Illustration Unit for the graphic presentation;

The ORS Committee for granting me the ORS award in my 2nd and 3rd years of postgraduate studies;

The Medical Faculty for granting me a University Postgraduate award to pay for my final year university fees;

My colleagues, Mary Woods, Dr. Tony Balmforth and Dr. Douglas Hedley for their informative and constructive discussion;

Everyone at the Animal Unit for the regular supply of rats;

Foreign students, Advisors of the University of Glasgow and Department of Biochemistry, Miss A.M. McGregor and Dr. R.Y. Thomson respectively for their guidance and advice;

Mr. Tom Mathieson for his assistance in ordering radioactive materials and some drugs used in the thesis;

Dr. John Connell, Dr. Gordon Inglis and Dr. Fiona Lyall of the Blood Pressure Unit for extending their assistance and generosity by allowing me to stay on in the Goldberg Laboratory after the transfer of Dr. P.F.T. Vaughan to Leeds University in my final year of postgraduate study;

My parents for their support and encouragement throughout my postgraduate studies

and finally Dr. Piang Chik Chien for encouragement and occasional financial support.

ABBREVIATIONS

CNS	central nervous system
O.C.	occipital cortex
NA	noradrenaline
DA	dopamine
cAMP	3'-5' cyclic adenosine monophosphate
db-cAMP	N^6-2'-O-dibutyrladenosine 3'-5' cyclic monophosphate
8-Br-cAMP	8-bromo-cyclic adenosine monophosphate
ATP	adenosine-5'-triphosphate
PDE	phosphodiesterase
IBMX	3-isobutyl-1-methylxanthine
RO 20-1724	4-(3-butoxy-4-methoxybenzyl)-2-imidazolidinone
ZK 62711	4-(3-cyclopentyloxy-4-methoxyphenyl)-2-pyrrolidone
BHT-920	2-amino-6-allyl-5,6,7,8-tetrahydro-4H-thiazolo-[4,5-d]-azapine
UK 14304-18	5-bromo-6-[2-imidazolin-2-ylamino]-quinoxoline
CHA	N^6-cyclohexyl adenosine
CPDPX	8-cyclopentyl-1,3-dipropylxanthine
DMI	desipramine
TCA	tricholoacetic acid
PCA	perchloric acid
HEPES	N-2-hydroxyethylpiperazine-N-2-ethane sulphonic acid
EGTA	ethylene glycol-bis-(β-amino-ethyl-ether)N, N'-tetra acetic acid
HPLC-ED	high performance liquid chromatography with electrochemical detection
Pertussis-toxin (IAP)	exotoxin of Bordetella pertussis
Cholera toxin	exotoxin of Vibrio cholerae

TABLE OF CONTENTS

LIST OF FIGURES

Figure

Figure

LIST OF TABLES

SUMMARY

(1) Regulation of NA release from rat O.C. tissue chops is K^+ and Ca^{2+} dependent.

(2) K^+-stimulated release of [^{3}H]NA from rat O.C. and hypothalamic tissue chops is inhibited by α_2-adrenergic agonists and the inhibitory effect is reversed by α_2-adrenergic antagonists.

(3) In O.C. tissue chops, 20mM K^+-stimulated release of [^{3}H]NA is enhanced by db-cAMP, forskolin and IBMX but inhibited by RO 20-1724. In addition, db-cAMP or forskolin when combined with IBMX further enhanced the K^+-stimulated release of [^{3}H]NA while the inhibitory effect of RO 20-1724 on K^+-stimulated release of [^{3}H]NA is reversed by db-cAMP or forskolin. The α_2-adrenergic agonist inhibition of K^+-stimulated release of [^{3}H]NA is partially reversed by db-cAMP alone but not by forskolin.

(4) In hypothalamic tissue chops, 25mM, but not 30mM, K^+-stimulated release of [^{3}H]NA is enhanced by db-cAMP or forskolin combined with IBMX. However, the inhibitory effect of clonidine on K^+-stimulated release of [^{3}H]NA is not reversed by either db-cAMP or forskolin in the presence of IBMX.

(5) The selective adenosine A_1 agonist, N^6-cyclohexyl adenosine (CHA) inhibited 20mM K^+-stimulated release of [^{3}H]NA from O.C. tissue chops more potently than adenosine. The inhibitory effect of adenosine is reversed by IBMX but not by RO 20-1724.

Furthermore, IBMX (10^{-4} M) is not only able to reverse the adenosine inhibition of K^+-stimulated release of [^{3}H]NA, but also enhances the release of [^{3}H]NA to approximately 20%. Also, the selective adenosine A_1 antagonist 8-cyclopentyl-1,3-dopropylxanthine (CPDPX) (10^{-5} M) not only reverses the adenosine inhibition of K^+-stimulated release of [^{3}H]NA but further enhances the release of [^{3}H]NA by about 10%.

(6) Direct measurement of intracellular cAMP formation by the method of Shimizu et al. (1969) showed that the β-adrenergic agonist, isoprenaline,as well as NA, adenosine, forskolin and K^+ all stimulated cAMP formation maximally by about 1.5, 3.0, 3.5, 7 and 2.5 fold repesctively.

(7) Isoprenaline stimulation of cAMP formation is potentiated by the α_1-adrenergic agonist, phenylephrine, but not affected by the α_2-adrenergic agonist, UK 14304-18.

(8) NA stimulation of cAMP formation is not affected by phenylephrine, but partially inhibited by α_2-adrenergic agonists in a biphasic manner with the following order of potency (IC_{50} values in parenthesis) UK 14304-18 (6×10^{-9} M) > clonidine (10^{-8} M) > BHT-920 (5×10^{-7} M). However, there is no evidence that α_2-adrenergic agonists inhibit the isoprenaline, forskolin and K^+-stimulation of cAMP formation.

(9) α- and β-Adrenergic antagonists inhibit NA stimulation of cAMP formation with the following order of potency, propranolol (β) > prazosin (α_1) = yohimbine (α_2) and with IC_{50} values of 6×10^{-9} M, 3×10^{-8} M and 3×10^{-8} M respectively.

(10) Isoprenaline, NA, adenosine and 20mM K^+ stimulation of cAMP formation is blocked by IBMX, but not by RO 20-1724. In contrast, forskolin stimulation of cAMP formation is not affected by either IBMX or RO 20-1724.

(11) Adenosine stimulation of cAMP formation is potentiated by NA and UK 14304-18.

(12) These results are discussed in relation to current hypotheses on the mechanisms by which α_2-adrenergic agonists modulate NA release in the CNS.

1. INTRODUCTION

1.1 Neurotransmitters

Neurotransmitters are chemical compounds found in the nerve terminals of neurons which are released into the synapse in response to nerve impulses (Katz and Miledi, 1969) or membrane depolarization (Schoffelmeer et al., 1981). Examples of neurotransmitters identified in the brain include catecholamines (noradrenaline and dopamine), amino acids [glutamate, γ-aminobutyric acid (GABA) and aspartate], acetylcholine, histamine, serotonin (see Chesselet, 1984; Middlemiss, 1988, for review) and several neuropeptides including methionine or leucine eukephalin, endorphins, substance P, neurotensin, cholecystokinin (Bradford, 1986b).

Neurotransmitters play a very important role in neuroscience as they provide the basic link between neurons. Thus, many biological and physiological changes are affected by the release of neurotransmitter. A few examples are (a) high concentrations of NA cause hypertension (De Champlain et al., 1967; Przuntek et al., 1971), (b) dopamine deficiency leads to mental deterioration in Parkinson's disease (Lees and Smith, 1983) and (c) loss of cholinergic neurons in the brain lead to senile dementia, or Alzheimer's disease (Robbins, 1988). Furthermore, deficits in the noradrenergic system have been reported in postmortem Alzheimer brains. This involves the decrease of both noradrenaline (Aldolfsson et al., 1979) and dopamine-β-hydroxylase levels (Cross et al., 1981), particularly in the cortex and hypothalamus. Thus, the study of the regulation of neurotransmitter release may lead to more understanding on neuronal functions that may indirectly control our biological responses.

1.1.1 Criteria identification of a neurotransmitter

A number of criteria have been suggested which a compound has to fulfil before it can be classified as a neurotransmitter. When a chemical compound fulfils most but not all the requirements for a neurotransmitter, it is then known only as a putative neurotransmitter.

(1) The chemical compound must be stored in those neurons from which it is released.

(2) Precursors for the biosynthesis of the chemical compound must be present.

(3) Enzymes necessary for the synthesis of the chemical compound must be present in the same neurons.

(4) Ca^{2+} dependent release of the chemical compound to the extracellular fluids upon nerve stimulations.

(5) Mechanisms e.g. active uptake into nerve ending, must be present for the inactivation of the released chemical compound.

(6) Specific neuroreceptors for the chemical compound must be present at both pre- and post-synaptic sites.

(7) It must be able to recognise the specific pharmacological agents, such as agonists or antagonists at its receptor sites. Drugs used must be able to mimic the response of the chemical compounds at the synaptic sites.

The above statements can be depicted diagrammatically as in Figure 1.

1.2 Catecholamines

Catecholamines are compounds with amines attached to a benzene ring bearing two o-phenolic hydroxy groups (Catechol). Examples of naturally occurring catecholamines are dopamine [β(3,4-dihydroxyphenyl)

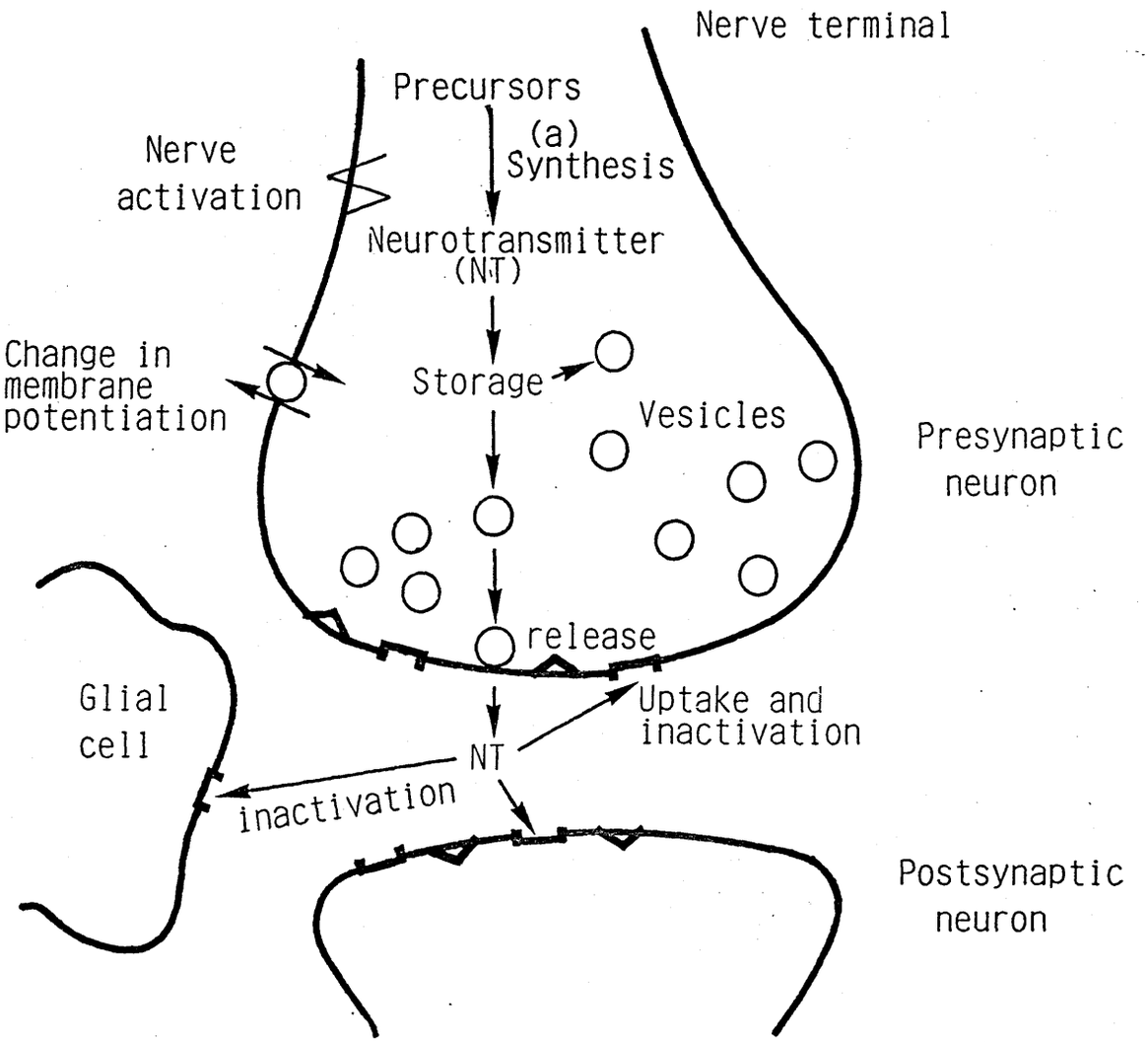


Figure 1 Basic principle of neurotransmitters at synaptic sites.

ethyl amine], Noradrenaline (Norepinephrine), and Adrenaline (Ephinephrine). Their structural formulae are depicted in Figure 2. The main sites of production of catecholamines are the brain, the sympathetic neurons and the chromaffin cells of the adrenal medulla. However, the most important catecholamine neurotransmitters produced in the brain are dopamine and noradrenaline, while epinephrine is mainly produced quantitatively by the chromaffin cells of the adrenal medulla.

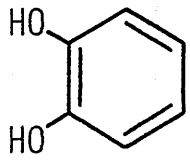
1.2.1 Noradrenergic pathways

The neuroautomical distribution of noradrenaline was detected by the use of formaldehyde histofluroescence technique (Falk et al., 1962; Fuxe, 1965). The noradrenergic pathways were distinguished by the fluroescence colours given by the aldehyde derivatives of noradrenaline. The use of high performance liquid chromatography with electrochemical detection (HPLC-ED), which is a highly selective and sensitive method of measuring catecholamines down to pico mole amounts helped to confirm the histochemical results as it allowed the same degree of precision in the localization of catecholaminergic pathways (Keller et al., 1976; Felice et al., 1978; Westerink and Mulder, 1981).

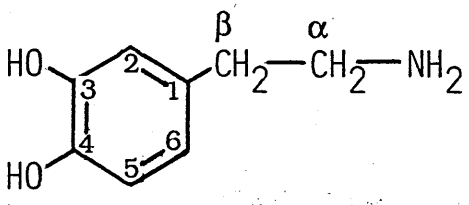
Noradrenaline nerve terminals in the cerebellum, hypothalamus, hippocampus and cerebral cortex arise from the perikarya in the lower brain stem, most notably the locus coeruleus as depicted in Figure 3.

1.2.2 Biosynthesis of catecholamines

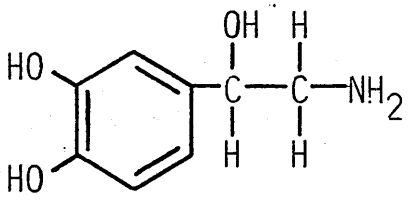
Tyrosine was shown to be the direct precursor for catecholamine biosynthesis in the in vivo and in vitro study using radiolabelled tyrosine (Clonet et al., 1970; Harris and Roth, 1970; Heffner et al., 1980; Bennett et al., 1981). The biosynthetic pathway for catecholamines is as shown in Figure 4.



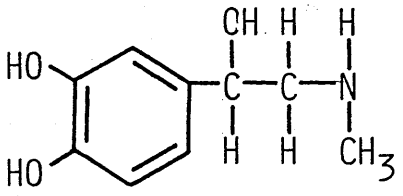
Catechol (dihydroxybenzene)



Dopamine [β(3,4-dihydroxypheynl) ethyl amine]



Noradrenaline (Norepinephine)



Adrenaline (Epinephine)

Figure 2 Structural formulae of catecholamines

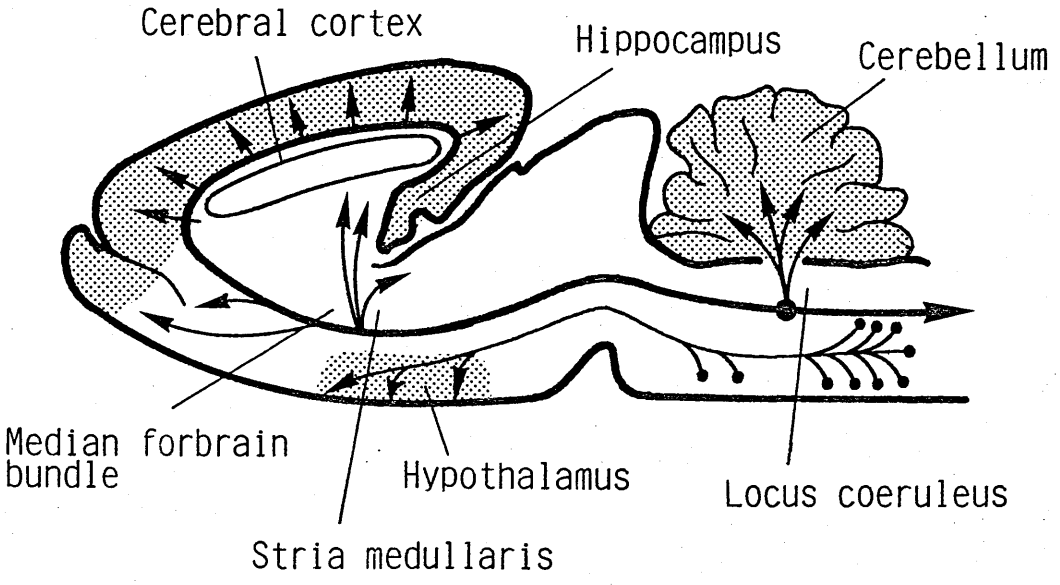


Figure 3 Noradrenaline pathways in rat brain shown in sagittal section

Hatched areas represent nerve terminal fields. Pathways descending to the cerebellum and brain stem nuclei are not shown (From Ungerstedt, U. (1971) Acta Physiol. Scand. Suppl. 367, 49-67).

Tyrosine is converted to 3,4-dihydroxyphenylalanine (dopa) by the enzyme Tyrosine hydroxylase (Tyrosine-3-mono-oxygenase, EC 1.14.6.2) which uses tetrahydrobiopterine, molecular O_2, Fe^{2+} and NADPH as its cofactors. Tyrosine hydroxylase is considered to be the rate-limiting step for dopamine and noradrenaline synthesis, since the enzyme reaction is the slowest of the sequence (Bradford, 1986). Catecholamine depletion is observed when tyrosine hydroxylase is inhibited by drugs like α-methyl para-tyrosine (α-MpT) (Iversen and Glowinski, 1966; Bennett et al., 1981). cAMP-mediated phosphorylation may lead to the activation of tyrosine hydroxylase, thereby increasing its afinity for the pteridine cofactor for tyrosine (Mestikawy and Glowinski, 1983). Increase in the conductance of Ca^{2+} either by K^{+}-depolarization of neuronal membrane potential (Harris and Roth, 1970) or via the regulation of cAMP dependent protein kinase may also affect the tyrosine hydroxylase activity (Harris and Roth, 1970). When the noradrenergic vesicles are filled with noradrenaline, further synthesis of noradrenaline is inhibited. This is because excess NA competes with pteridine cofactors for the tyrosine hydroxylase, thereby decreasing the enzyme activity (Harris and Roth, 1970). Feedback inhibition of dopamine, synthesis is also observed in dopaminergic neurons (McGeer et al., 1967).

In both the noradrenergic and dopaminergic neurons, dopa is then decarboxylated by the enzyme dopa-decarboxylase which uses pyridoxal phosphate as its cofactor to convert it to dopamine. However in noradrenergic neurons the enzyme dopamine-β-hydroxylase, using ascorbate, molecular O_2 and Ca^{2+} as its cofactors will then covert dopamine to noradrenaline.

For a small group of neurons in the brain stem and chromaffin cells of adrenal medulla, NA is further converted to adrenaline by the

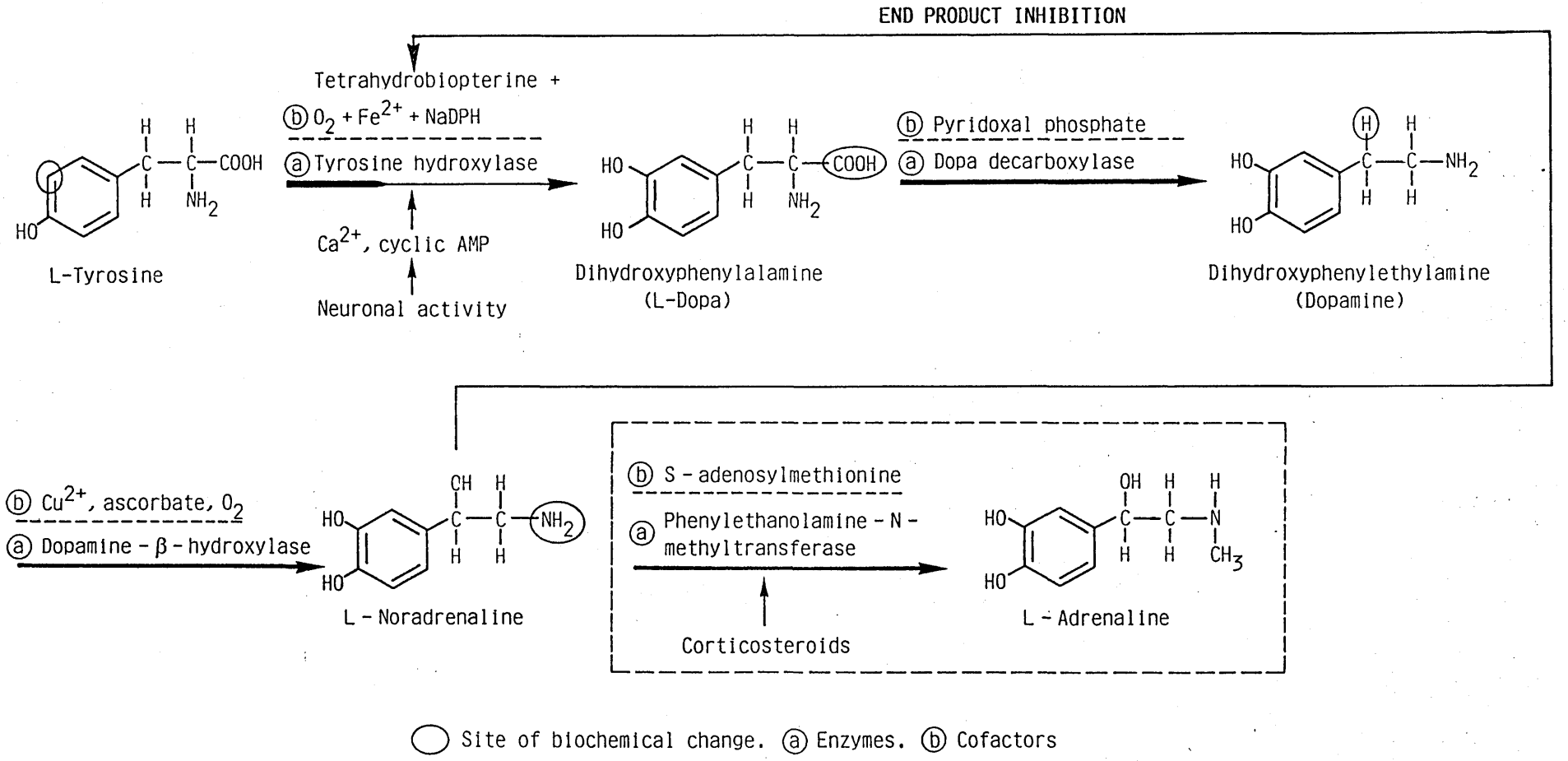


Figure 4 Biosynthesis of catecholamines

enzyme phenylethalamine N-methyl transferase (PNMT). The enzyme uses S-adenosylmethionine as its cofactor. Surprisingly, the PNMT exists in a soluble cytosol outside the catecholamine storage vesicles. The level of PNMT can also be regulated by corticoisteroids.

1.2.3 Receptors for Noradrenaline

Based on the various physiological studies on the relative potency of agonists, Alquist (1948) proposed that there were two types of catecholamine receptors, which he termed α - and β -adrenoceptors. This concept was further confirmed by Powell and Slater (1958) when they introduced dichloroisoproterenol (DCI), the first series of drugs capable of blocking β-adrenergic receptors response. The structural analogs of isoproterenol were then synthesised and made available by Biel and Lum (1966) and Moran (1967). It was thus possible to distinguish α - and β - adrenoceptors not only on the relative potency of agonists, but also on the types of antagonists which could prevent the response in question.

Phentolamine, phenoxybenzamine and dibenamine blocked the α-receptors response; while β-adrenoceptors blocking agents include DCI, propanolol, practolol and alprenolol(Bradford, 1986).

Depending on the pharmacological properties of drugs in various tissue systems, α-adrenoceptors are further sub-classified into α_1-postsynaptic and α_2-presynaptic adrenoceptors (Langer, 1974; Doxy, 1977; Bethelsen and Pettinger, 1977). α_1-adrenoceptor response are blocked by its selective antagonist, prazosin (Cambridge et al., 1977), and α_2-adrenoceptors are preferentially blocked by yohimbine (Starke et al., 1975). The hypothesis of separate α_1- and α_2-adrenoceptors are further supported by ligand binding studies

with labelled catecholamine antagonists (Miach et al., 1980; U'Prichard et al., 1979; Wood et al., 1979). Recently, based on radioligand binding studies Kawahara and Bylund (1985) and Boyajian et al. (1987; 1988) proposed that α_2 receptors may be divided into α_{2A} and α_{2B} subtypes. The occurence of α_{2A} and α_{2B} adrenergic receptor subtypes has been confirmed by Kobilka (1987). In addition an α_{2C} subtype has been identified (Kobilka, 1987). Similarly, α_1-adrenoceptors can be subdivided into α_{1A} and α_{1B} (Bylund, 1988). Based on the functional effects of β-adrenoceptors, Land et al., (1967) subdivided β-adrenoceptors into β_1 and β_2. Examples of β_1 selective antagonists are practolol, atenolol, metoprolol and p-oxprenolol; and β_2-selective agonists are zinteral (NJ 1999), terbutalin and salbutamol (Minneman et al., 1980; Synder et al., 1980). It is possible to measure β-adrenoceptors directly by specific binding of high-affinity antagonists [^{3}H] dihydroalprenolol and [^{125}I] iodohydroxy-benzylpindolol and the agonist [^{3}H] hydroxybenzyl- isoproterenol (William et al., 1978). Some drugs used in the study of adrenoceptors are as shown in Table 1.

Guanine nucleotide-binding regulatory (G) proteins couple hormone and neurotransmitters receptors to second messenger systems. The present classification of adrenoceptors suggests that each of the four major subtypes is coupled to a different class of G-protein. Both β_1 and β_2 are coupled to the stimulation of adenylate cyclase of Gs, whereas α_{2A}, α_{2B} and α_{2C} are coupled to the inhibition of adenylate cyclase of Gi (Bylund, 1988). Recently it is proposed that α_1-adrenoceptors are coupled to an unknown G protein (Gx) leading to the breakdown of phosphotidyl-inositol breakdown (Litosch and Fain, 1986). Thus, the four major adrenoceptor subtypes are coupled to a different G protein and is illustrated as in Figure 5.

Table 1 Some drugs interacting with noradrenergic receptors

α, β - agonist	Noradrenaline
α-agonist	Adrenaline
α_1-agonist	phenylephrine
α_2-agonist	clonidine, UK 14304-18, oxymetazoline BHT-920, BHT-933, xylazine, l-NA tramazoline
α-antagonists	phentolamine, phenoxybenzamine, dibenanime
α_1-antagonists	prazosin, indoramine, WB-4101, labetolol, UK 33274
α_2-antagonists	yohimbine, piperoxan, rauwolscine
β-antagonist	l-propranolol
β_2-antagonist	butoxamine, ICI 118 551
β-agonist	isoprenaline
β_2-agonists	zinterol (MJ 1999), terbutalin, sabultamol
β_1-agonist	tazolol
β_1-antagonists	antenolol, metonolol, practolol, p-oxprenolol

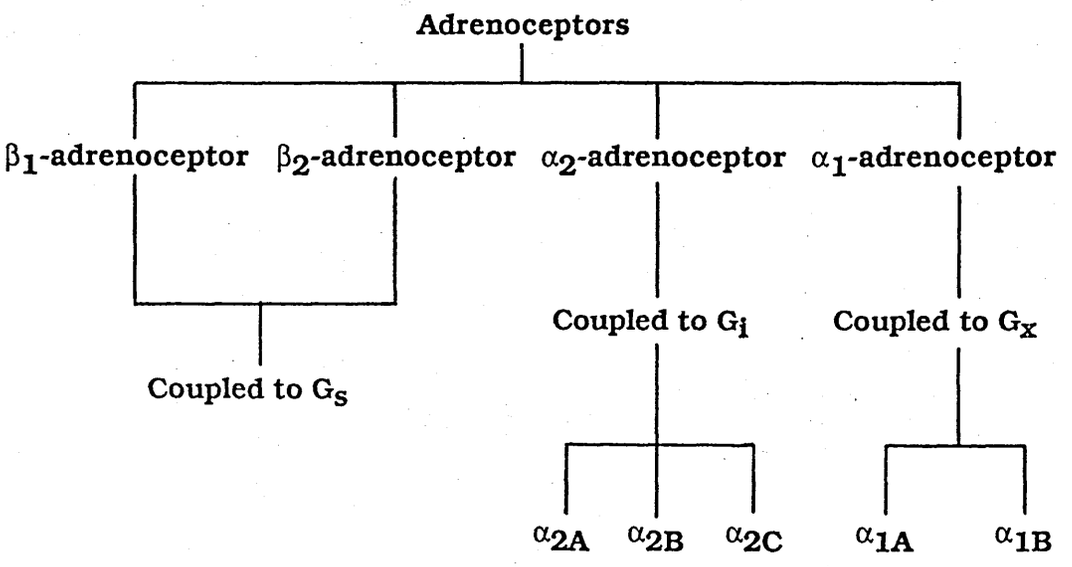


Figure 5 Classification of adrenoceptors

1.2.4 Storage of catecholaminergic neurotransmitter

Most catecholaminergic neurotransmitters are stored in the granular or dense core vesicles found at the nerve terminals. During biosynthesis, dopamine is actively transported into the granular or synaptic vesicles via an ATP+Mg^{2+}-dependent carrier (Bianchi et al., 1984). Noradrenergic vesicles can be identified by the presence of the enzyme dopamine-β-hydroxylase (DBH) in its membrane. This enzyme catalyses the conversion of dopamine to noradrenaline in the granular vesicles. Agents that deplete the neurotransmitter store, e.g. reserpine, also remove the dense core (Van Orden et al., 1967). The vesicular uptake process has a broad substrate specificity for other phenylethylamines, including tryptamine, tyramine and amphetamine; these amines act as indirect sympatomimetic agents and compete with the endogenous catecholamines for the vesicular storage capacity. These phenylethylamines can thus stimulate the release of NA by displacing from its vesicles (Nieoullon et al., 1977; De Beaeroche et al., 1976; Arnold et al., 1977).

There is evidence that DA is not retained as well as NA by large dense core vesicles prepared from bovine splenic nerve (Klein, 1982). From the study using the crude vesicle associated fractions, it is found that [^{3}H]NA retained more stable than [^{3}H]DA because a more stable interaction of the former with ATP was promoted by its β-hydroxy group (Weiner, and Jardetsky, 1964), also [^{3}H]NA was found to be more resistant to the catecholamine depleting effect of reserpine and its analogs (Bianchi et al., 1984).

1.2.5. Release of catecholaminergic neurotransmitters

1.2.5.1 Evidence for the release of noradrenaline via exocytosis

In exocytosis, the expulsion of vesicular contents into the extracellular fluids is achieved by the fusion of the vesicular membrane with that of nerve terminal (Fig. 6) (Bowman and Rand, 1980). Evidence for exocytosis is largely based on chemical analysis and freeze-fracture study using electron microscope. A good example is the proportional release of NA and dopamine-β-hydroxylase (DDH) from sympathetic nerves (Weinshilboum et al., 1971).

The soluble vesicular content of noradrenergic neurons are noradrenaline, ATP, enzyme DBH and large granule chromogranin A. The demonstration that DBH, an enzyme of 300,000 Mr, is released concurrently and proportionately with NA established that the release of NA occurs via exocytotic process (Weinshilboum et al., 1971).

1.2.5.2 Stimulus evoked Ca^{2+}-dependent release of catecholaminergic neurotransmitter

Electrophysiological studies using squid axons, (Katz and Miledi, 1969) have shown that release of neurotransmitter is not only dependent on nerve impulses. Depolarizing agents that affect the ionic-channel permeability may also influence the neurotransmitter release, which has been shown to be Ca^{2+}-dependent (Baker et al., 1973; Kalz and Miledi, 1967; Nachshen, and Blaustein, 1980 and 1982; Leslie et al., 1985 and Daniell and Leslie, 1986). Thus Taube (1977) showed that K^{+}-evoked or electrical stimulation of [^{3}H]NA was abolished when Ca^{2+} was omitted in the buffer medium. In contrast, the spontaneous non depolarization-evoked efflux of neurotransmitter is not calcium dependent.

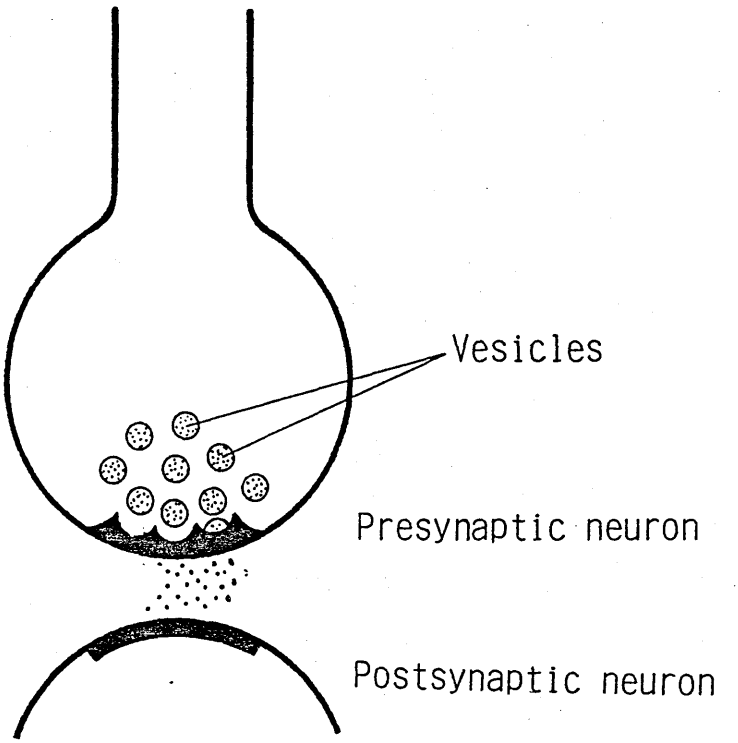


Figure 6 Exocytosis of a vesicle's content at the nerve terminal (From Bowman and Rand, 1980 textbook of pharmacology, 2nd edition, Blackwell Scientific publication, pp 5.23)

Llinas et al. (1972; 1975) studied the influence of Ca^{2+} on synaptic transmission by first injecting aequorin into the pre- and post-synaptic terminals of the squid giant synapse through micropipette. Aequorin is a bioluminiscent protein which reacts with Ca^{2+} to produce light (see Blinks et al., 1976 for review). The pre- and post-synaptic terminals loaded with aequorin were activated by means of external electrodes, and membrane depolarization which was capable of triggering the release of synaptic transmitters also led to a substantial increase in light emission. Thus, their results give direct evidence of the influence of Ca^{2+} in synaptic transmission.

1.3 Regulation of NA release by presynapitc α_2-adrenoceptors

NA released from both the peripheral (Langer, 1974; 1981) as well as central nervous system (see Chesselet, 2984 for review) is able to modulate the stimulated release of NA by either activating the adrenoceptors situated on the presynaptic or postsynaptic membrane, thereby leading to the changes in the physiological response via a negative feedback mechanism. In brain, most studies on presynaptic regulation of the release of NA are performed in vitro using radiolabelled NA with either brain slices or synaptosomes (Middlemiss, 1988). Electrical stimulation or K^{+} depolarization evoked release of radiolabelled NA from the peripheral and central nervous system (CNS) can be inhibited by selective α_2-adrenergic agonists, such as clonidine (Starke et al., 1974; Medgett et al., 1978; Schoffelmeer et al., 1986) and BHT-920 (Van Meel et al., 1981; Leus and Schmann, 1984; Cichini and Singer, 1987). In addition, a more potent α_2-adrenergic agonist such as UK 14304-18 on the regulation of [^{3}H]NA release from the peripheral nervous system has also been pharmacologically

characterized (Cambridge and Davey, 1980; Cambridge, 1981; Van Meel et al., 1981). While selective α_2-adrenergic antagonist such as yohimbine enhances the release of NA from both the peripheral (Langer, 1981) and central nervous system (Starke et al., 1975; Taube et al., 1977). This is because α_2-adrenergic antagonists block the inhibitory effect of NA, acting at presynaptic α_2-adrenoceptors, on its own release. The α_1-adrenergic agonist, phenylephrine and α_1-antagonist, prazosin, did not have any effect on the release of NA from the peripheral as well as central nervous system (Cambridge, et al., 1977; Reichenbacher et al., 1982). In the CNS the β-adrenergic agonist, isoprenaline, does not affect the depolarization evoked release of NA (Taube et al., 1977). Thus release of [^{3}H]NA from the central nervous system is not mediated by β-adrenoceptor. In support of this the β-adrenergic antagonist, propanolol, does not antagonise the inhibitory effect of NA on the stimulated release of [^{3}H]NA from the central nervous system (CNS) (up to 10^{-6}M) (Taube et al., 1977). In contrast, isoprenaline enhances and propranolol is found to antagonise the isoprenaline enhancement of [^{3}H]NA release from the peripheral nervous system (Adler-Graschinisky and Langer, 1975; Celuch et al., 1978; Dahlof et al., 1978). Furthermore at higher concentrations (above 10^{-5}M), propranolol enhanced the inhibitory action of NA on release. A possible explanation for this observation is that by preventing NA from binding to β-adrenoceptors its effective concentration is increased, thus leading to greater inhibition of NA release.(see Langer, 1981 for review).

In conclusion, activation of presynaptic α_2-adrenoceptor leads to the inhibition of stimulated release of [^{3}H]NA from both the peripheral and the central nervous system. However, based on radio

ligand binding studies, it is found that α_2-adrenoceptors found in the CNS are also present at the post-synaptic sites, since the same number of [^{3}H] clonidine is found to bind to the rat cerebral cortex membranes after the pretreatment of the membrane fractions with 6-hydroxydopamine, which function was to destroy presynaptic noradrenoceptors (U'Prichard and Snyder, 1979).

1.3.1 Influence of α_2-adrenoceptors on voltage-sensitive Ca^{2+} and K^+ channels

It has been suggested that α_2-adrenergic agonist inhibit the release of [^{3}H]NA from the peripheral as well as the central noradrenergic neurons by acting on voltage-sensitive Ca^{2+} channels. Thus α_2-adrenergic agonists inhibit depolarization-evoked Ca^{2+}-dependent release whereas the spontaneous efflux is not affected (Gothert et al., 1979; Alberts et al., 1981; Schoffelmeer and Mulder, 1983, 1983b). Further support for this suggestions is provided by electrophysiological studies which demonstrate that α_2-adrenergic agonists, NA and clonidine inhibit the voltage-sensitive Ca^{2+} current in embryonic chick dorsal root ganglion neurons (Dunlap and Fishbach, 1981) and in post-ganglionic neurons of the rat superior cervical ganglion (Hons and Mcaffee, 1980) respectively, thereby reducing the Ca^{2+} inward current.

Electrophysiological studies show that α_2-adrenoceptors situated on the cell bodies of noradrenergic neurons in the locus coeruleus (LC) are the same as the α_2-adrenoceptors found on nerve terminals in the peripheral as well as the central neurons system. For example action potentials of neurons in the LC are inhibited by α_2-adrenergic agonists including adrenaline, noradrenaline (Svensson

et al., 1975; Cedarbaum and Aghajanian, 1976) and clonidine (Cedarbaum and Aghajanian, 1976; Aghajanian and Van der Maalen, 1982) and the inhibitory effect produced by these agonists can be blocked by the α_2-adrenergic antagonist piperoxane but not the β-adrenergic antagonist satolol (Cedarbaum and Aghajanian, 1976). Activation of α_2-adrenoceptors situated on the cell bodies of noradrenergic neurons in the LC by clonidine is found to increase the K^+ conductance leading to hyperpolarization of noradrenergic neurons in the CNS (Aghajanina and Van der Maalen, 1982).

Zimanyi et al. (1988) show that in the presence of selective K^+ channel blockers such as 4-aminopyridine (4-AP) and quinine, the inhibitory effect of α_2-adrenergic agonists including l-NA and xylazine are reduced, while the enhancing effect of α_2-adrenergic antagonist yohimbine on electrically stimulated release of [^{3}H]NA from the peripheral noradrenergic nerve terminals are completely abolished. The effect of 4-AP is to selectively block the voltage-dependent K^+ permeability (Meves and Pichon, 1977), and that of quinine is to block the Ca^{2+}-activated K^+ conductance (Cherubini et al., 1984; Bartschat and Blaustein, 1985). Thus, α_2-adrenergic agonists inhibit the release of NA from the peripheral noradrenergic neurons primarily through hyperpolarization resulting from increased K^+ permeability.

Schoffelmeer and Mulder (1984) suggest that α_2-adrenoceptors located on noradrenergic axonal varicosities, unlike those located on the cell bodies and the peripheral nerve terminal, do not primarily mediate hyperpolarization of the neuronal membrane. Thus NA and clonidine inhibit 13mM K^+-stimulated release of [^{3}H]NA in the presence of 1.2mM Ca^{2+} to the same extent as 56mM K^+-stimulated

release of [^{3}H]NA in the presence of 0.1mM Ca^{2+}. According to them, the diminished inhibitory effect of both NA and clonidine on 56mM K^+-stimulated released of [^{3}H]NA in the presence of 1.2mM Ca^{2+} may be due to the effect of high Ca^{2+} influx during depolarization leading to the oversaturation of release mechanism. Thus, their data strongly argue against the general view that α_2-adrenergic agonists inhibit the K^+-stimulated release of [^{3}H]NA via hyperpolarization of noradrenergic nerve terminals during depolarization in the CNS.

1.3.2 Possible role of cAMP in the regulation of NA release

Depolarization leads to the increase in intracellular cAMP levels in brain slices (Shimizu et al., 1970; Shimizu and Daly, 1972; Zanella and Rall, 1973; Ferrendelli et al., 1976) as well as in synaptosomes (Daly et al., 1980). Indirect evidence for a role of cAMP in synaptic transmission can be studied by using non-hydrolysable membrane penetrating cAMP analogue including db-cAMP and 8-Br-cAMP, and the adenylate cyclase activators, NaF and forskolin (Wermer et al., 1982; Schoffelmeer et al., 1983). Db-cAMP has been reported to enhance the electrical stimulated release of acetylcholine (Goldberg and Singer, 1969) and NA (Wooten et al., 1972) from the peripheral nervous system (PNS). In the CNS, db-cAMP, 8-Br-cAMP, and forskolin enhanced the electrical (Markstein et al., 1984; Schoffelmeer et al., 1985, 1986) K^+ (Wermer et al., 1982) and veratrine (Schoffelmeer et al., 1983) induced release of [^{3}H]NA from rat brain slices (Wermer et al., 1982; Markstein et al., 1984; Schoffelmeer et al., 1982, 1986) and synaptosomes (Schoffelmeer et al., 1985), without affecting the spontaneous efflux of [^{3}H]NA (Schoffelmeer et al., 1983, 1985).

However, Rabe et al. (1982) demonstrated that the forskolin

enhancement of K^+-stimulated release of [^{3}H]NA from rat PC 12 pheochromocytoma cell line is biphasic with facilitation by 0.1-10uM forskolin but inhibition at 100uM. They thus conclude that elevation of intracellular cAMP cannot initiate release, but can enhance depolarization dependent release of NA.

Electrophysiological studies in neurons from suboesophageal ganglia of Helix aspersa demonstrate that intracellular perfusion with cAMP or intracellular application of ATP, at nanomolar concentration cause a slight increase in Ca^{2+} current (ICa) (Yatani et al., 1982). In addition, intracellular injection of cAMP or application of serotonin to the cell body of Aplysia californica caused a prolonged and complete closure of individual K^+ channels (Klein and Kandel, 1978; Siegelbaum et al., 1982) leading to a longer action potential and hence a prolongation of Ca^{2+} influx into the cell. Since Ca^{2+} influx is associated with the release of neurotransmitter, it is then suggested that cAMP and Ca^{2+} may act closely as interrelated second messengers involved in the stimulus-secretion coupling process.

PDE inhibitors, that prevent the breakdown of cAMP, including IBMX and ZK 62771, increase the electrically evoked release of [^{3}H]NA from rat brain slices (Markstein et al., 1984; Schoffelmeer et al., 1985, 1986) and synaptosomes (Schoffelmeer et al., 1985). In contrast, PDE-inhibitors including RO 20-1724, IBMX, 7-benzyl-IBMX and ZK 62771 inhibited the 13mM K^+ (Wemer et al., 1982) and veratrine (Schoffelmeer et al., 1983) induced release of [^{3}H]NA from rat neocortical brain slices. However, ZK 62771 is able to enhance the K^+-stimulated release of [^{3}H]NA in the presence of Na^+ channel blocker, TTX. This indirectly suggests that PDE inhibitors may also possibly act as a Na^+ channel blocker. Till a more selective PDE inhibitor is available, it is

difficult to interpret the cAMP effect with the existing PDE inhibitor. For example, IBMX which is a PDE inhibitor has also been reported to be a potent adenosine receptor antagonist (Stiles, 1986).

α_2-Adrenergic agonists have been reported to inhibit cAMP formation in a number of tissues, including cultures of astrocytes derived from mouse (Van Calker et al., 1978) and rat brain (McCarthy et al., 1979), mouse neuroblastoma X glioma cells, NG 108-15 cells (Sabol and Nirenberg, 1979; Griffi et al., 1985) human (Burns et al., 1982) and hamster adipocytes (Fain and Garcia-Sainz, 1980; Garcia-Sainz et al., 1980). In addition, activation of α_2-adrenoceptors has been shown to mediate inhibition of adenylate cyclase activity stimulated by forskolin in rat cerebral cortical membrane (Kitamura et al., 1985). In contrast, in intact brain slices and synaptosomes, there is no direct evidence to show that α_2-adrenergic agonist inhibits the stimulated release of [^{3}H]NA by decreasing the intracellular cAMP levels. However, inhibitory effect of α_2-adrenergic agonists, including clonidine (Schoffelmeer et al., 1983, 1986), oxymetazolin (Wemer et al., 1982) and the enhancement effect of α_2-adrenergic antagonist phentolamine on electrical (Schoffelmeer et al., 1986), K^+ (Wemer et al., 1982) and veratine (Schoffelmeer et al., 1983) induced release of [^{3}H]NA are strongly reduced in the presence of 8-Br-cAMP and db-cAMP. Furthermore, the inhibitory effect of oxymetazoline and the enhancement effect of phentolamine are also reduced in the presence of NaF, a potent activator of adenylate cyclase (Wemer et al., 1982). Thus, the findings indirectly support the role of cAMP in presynaptic regulation of NA release.

Unlike many peripheral tissue, mammalian brain contains adenylate cyclase (EC 4.6.1.1) activity that is stimulated by Ca^{2+} via the endogenous Ca^{2+} binding protein, calmodulin (CaM) (Brostrom et al., 1977; Cheung, 1980). In turn, cAMP may lead to the phosphorylation of Ca^{2+} channels, thereby enhancing their Ca^{2+} conductance (Reuter, 1983), thus increasing the availability of Ca^{2+} for secretory processes. However, phosphorylation by Ca^{2+} and calmodulin may act in parallel or synergistically with phosphorylation of intracellular cAMP dependent proteins, some of which are located in synaptic vesicles, thereby indirectly leading to the exocytotic process (Nestler and Greengard, 1983). Moreover, in synaptic vesicles and nerve terminal membrane, cAMP and Ca^{2+} have been shown to regulate phospholipase A_2 activity, which may play an essential role in the exocytotic process (Moskowitz et al., 1983, 1984). Thus, it would be interesting to know whether α_2-adrenergic inhibition of stimulated release of [^{3}H]NA is directly affected by the Ca^{2+} influx or by the regulation of intracellular cAMP levels.

Ca^{2+} ionophore, which helps to transport the extracellular Ca^{2+} into the nerve terminals ~~by artificially open up the Ca^{2+} channel~~, induced release of [^{3}H]NA from rat brain slices. Clonidine and phentolamine do not have any effect on, while forskolin and 8-Br-cAMP enhanced the Ca^{2+} ionophore induced release of [^{3}H]NA (Schoffelmeer, 1986). This suggests that forskolin and 8-Br-cAMP can enhance the stimulated release of [^{3}H]NA without effecting voltage sensitive Ca^{2+} channels. Clonidine inhibits and phentolamine further enhanced the forskolin enhancement effect of Ca^{2+} ionophore induced release of [^{3}H]NA, while they remain without any effect on 8-Br-cAMP enhancement effect (Schoffelmeer, 1986). Thus, it is suggested that

forskolin and 8-Br-cAMP operate via separate mechanisms, and that clonidine may inhibit the release of [^{3}H]NA by inhibiting the adenylate cyclase activity stimulated by forskolin, thus decreasing intracellular cAMP formation. Until more direct evidence is obtained, the hypothesis that α_2-adrenergic agonist inhibits the stimulated release of [^{3}H]NA by decreasing cAMP levels in the CNS is inconclusive and remains a speculation.

1.3.3 Possible relationship between adenosine and α_2-adrenoceptors on the regulation of NA release

It is well established that ATP is released together with catecholamines from the adrenergic nerve terminals (Burnstock, 1981). The levels of extracellular adenosine concentrations in the brain tissue were found to be in a low micromolar range (Dunwiddie et al., 1981; Zetterstrom et al., 1982). In electrophysiological experiments, both spontaneous and evoked synaptic transmission were depressed by adenosine (Krentzberg et al., 1983), the effects of which are antagonised by adenosine receptor antagonists, such as the methylxanthines (Jackisch et al., 1985).

In the CNS, the depolarization-evoked release of several neurotransmitters including noradrenaline (Harms et al., 1978; Jonzon and Fredholm, 1984; Jackish et al., 1985; Allagier, 1987), dopamine (Harms et al., 1979; Jarvis and Williams, 1987), acetycholine (Pedata et al., 1983b; Corradetti et al., 1984; Jackisch et al., 1984), serotonin (5-HT) (Harms et al., 1979), glutamate (Dolphin and Archer, 1983) and GABA (Hollins and Stone, 1980) is inhibited by adenosine.

Adenosine decreases $^{45}Ca^{2+}$ uptake into synpatosomes during depolarization (Wu et al., 1982; Silinsky, 1986), which suggests that adenosine inhibits neurotransmitter release by reducing Ca^{2+} influx.

In addition, the adenosine depression of synaptic transmission is blocked by K^+-channel blockers suggesting that adenosine enhances K^+ conductance in nerve terminals (Perkins and Stone, 1980; Scholfield, 1986).

When rat brain slices were treated either with N-ethylmaleimide (NEM) which inactivates many proteins including the guanine nucleotide-binding protein (G-protein) (Allagier, 1987), and pertussis toxin (IAP) which ADP-ribosylate the inhibitory G-proteins (Fredholm and Lindgren, 1987), the inhibitory effects of adenosine on the stimulated release of noradrenergic neurotransmitters from the rabbit hippocampal brain slices was reduced. These findings suggest that the depolarization-evoked release of NA is modulated by adenosine receptors linked to a G-protein.

Radioligand binding studies show that adenosine interacts with specific adenosine receptors in the CNS (Williams and Risley, 1980; Wu et al., 1980; Patel et al., 1982). These receptors are subclassified into A_1 or (Ri) and A_2 (or Ra) receptors. The A_1 or (Ri) receptors have a high (nanomolar) affinity for adenosine while A_2 or (Ra) have a lower (micromolar) affinity for adenosine which either inhibit or activate adenylate activity (Van Calker et al., 1979; Londos et al., 1980) respectively. The 'R' nomenclature was initially suggested since the ribose portion of the adenosine molecule had to be intact for adenylate cyclase activity (Londos and Wolff, 1977).

Adenosine A_1 and A_2 receptors can now be distinguished by various synthetic and enzyme stable adenosine analogues. For example, N^6-substituted derivatives of adenosine including N^6-cyclohexyl adenosine (CHA) and N^6-phenylisopropryl adenosine (R-PIA) are more potent at activating A_1 receptors, which are often linked to a decrease in the formation of cAMP. In contrast, 5'-N-ethylcarboxamido

adenosine (NECA) is more potent than CHA or R-PIA at activating A_2 receptors, leading to increase in intracellular cAMP formation in the cell or tissue studied (Fredholm and Dunwiddie, 1988). In addition adenosine 'P' sites have also been described (Stiles, 1986). The 'P' stie is a putative site on the catalytic unit through which ribose modified adenosine analogs directly inhibit adenylate cyclase activity (Stiles, 1986). However, adenosine 'P' sites are sometimes designated when the purine portion of the adenosine molecule is required for the inhibition of adenylate cyclase activity (Londos and Wolff, 1977, 1980).

Stiles (1986) suggests that in brain slices, activation of A_1-receptor leads to the inhibition of neurotransmitter release, whereas, activation of A_2-receptor leads to the increase of neurotransmitter release. In rabbit hippocampal brain slices, the electrically stimulated release of [^{3}H]NA and [^{3}H]-ACh are inhibited by various adenosine agonists with the following order of potency: CHA > [(-)PIA] > NECA > 2-chloro-adenosine > adenosine > ATP, effects which are reversed by adenosine receptor antagonists, the methylzanthines including theophylline, 8-theophylline and IBMX (Jackish et al., 1984, 1985). It is concluded that adenosine inhibit the electrically-stimulated release of [^{3}H]NA from rabbit hippocampal brain slices via adenosine A_1-receptors. Since NA also inhibits the electrically-stimulated release of [^{3}H]NA via α_2-adrenoceptors, question then arises as to the contributory effect of adenosine on noradrenergic neurotransmitters release. Inhibitory effect of adenosine on electrically-stimulated release [^{3}H]NA from rat brain slices is not antagonised by the α_2-adrenoceptor antagonist, yohimbine (Reichenbacher et al., 1982), and the results suggest that adenosine does not act at a α_2-adrenoceptor. In contrast, Allgaier

et al. (1987) found that the inhibitory effect of the adenosine A_1-receptor agonist, (-)PIA on electrically-stimulated release of [^{3}H]NA from rabbit hippocampal brain slices is further enhanced by yohimbine. The results suggest that adenosine A_1-receptor acts synergistically with α_2-adrenoceptor in the regulation of NA release from rabbit hippocampal brain slices. This controversy may actually be due to the differences between animal species.

1.4 Cyclic AMP in various rat brain regions

Brain was shown to contain a higher level of adenylate cyclase and PDE acitivites than in other tissue systems (Sutherland, et al., 1962). Adenylate cyclase is a membrane bound enzyme which is responsible for the conversion of ATP to cAMP and PDE catalyses the conversion of cAMP to 5'AMP. The concentrations of cAMP in various rat brain regions was then studied by Schmidt, et al. (1971) using microwave irradiation technique, since cAMP was shown to be stable to heat (Sutherland, et al., 1958). Concentrations of cAMP found was: cerebellum = brain stem > hypothalamus > mid-brain > hippocampus = cortex. On the contrary, Weiss and Costa (1968) showed that the PDE-activities in the brain were: cortex-hippocampus > hypothalamus > medulla-pons > cerebellum. This suggests that those parts of the brain which contains higher adenylate cyclase acitivites has lower PDE activities and vice versa.

1.5 Regulation of cAMP levels in intact brain slices

Adenylate cyclase activity in the brain may be activated by hormones and neurotransmitters, which catalyses the conversion of ATP to cAMP and pyrophosphate (PP).

$$ATP \xrightarrow[GTP,\ Mg^{2+}]{\text{Adenylate cyclase}} cAMP + PP$$

Krishna et al. (1968) described a simple method for the assay of adenylate cyclase in tissue homogenates by using either [α-^{32}P]-, [^{14}C]- and [^{3}H]-ATP as substrates, but this is not possible with slices of brain because ATP does not easily penetrate unbroken cells. However, Santos et al. (1968) found that [8-^{14}C] adenine, which is actively transported into slices of brain, is converted to ATP, which in turn is converted to cAMP. Shimizu et al. (1969) found that the technique developed by Santos et al. (1968) is excellent for measuring the neurotransmitter mediated changes in intracellular cAMP content in brain slices. Schmidt et al. (1971, 1972) found that immediately following decapitation there is a rapid and substantial increase in cAMP levels in the brain. In order to obtain a stable and relatively low basal value of cAMP in the brain slices, it is necessary to preincubate the brain slices in the normal buffer medium for about 15 minutes before incubating it with [^{14}C] adenine following by 10 minutes post-incubation period (Schultz and Daly, 1973a). Earlier studies using radiolabelling techniques demonstrate that neurotransmitters such as NA, serotonin and histamine elicited cAMP accumulation in brain slices (Kakiuchi et al., 1968a, 1968b; Shimizu et al., 1970; Rall and Sattin, 1970; Schultz and Daly, 1973a). However, the effects of NA, serotonin and histamine are greatly reduced or absent when tested in incubated homogenates of brain (Klainer et al., 1962; Voigt and Krishna, 1967). The magnitude of biogenic amines response varies between different animal species and the region of the brain from which slices are prepared. For example, NA which elicits marked accumulation of cAMP in cerebral cortical slices from rat (Rall and Sattin, 1970; Shimizu

et al., 1970; Forn and Krishna, 1971) has either no effect or a minimal effect in cortical slices from guinea pig (Kakiuchi et al., 1969; Schultz and Daly, 1973a) and rabbit (Kakiuchi and Rall, 1968b). In addition, NA is more active than histamine in rabbit cerebellum (Kakiuchi and Rall, 1968a) but less active in cerebral cortex (Kakiuchi and Rall, 1968b).

1.5.1 Adenylate cyclase

It is now established that hormone-sensitive adenylate cyclase is regulated by stimulatory receptors (Rs) and inhibitory receptors (Ri) which activate the catalyst adenylate cyclase (C) through the heterotrimeric regulatory GTP binding proteins, Gs (α_s, β and γ) and Gi (α_i, β and γ) (Gilman, 1984; Schramm and Selinger, 1984; Taylor and Merritt, 1986; Thomas and Hoffman, 1987). The hydrophilic α-subunits are associated with the two hydrophobic subunits, β and γ. The actions of Gs and Gi are not symmetric since Gs associates tightly with C, whereas Gi does not (Levitzki, 1987). The structure of hormone-sensitive adenylate cyclase is as illustrated in Figure 7. The regulatory proteins (Gs and Gi), in addition to binding GTP, appear to be associated with GTPase activity which terminate the activation of the G-proteins. G-proteins play a key role in coupling process by which the hormone-bound receptor conveys activation upon the catayltic unit, which in turn catalyses the conversion of ATP to cAMP.

The receptors (Rs and Ri) are hydrophobic with seven membrane-spanning domains (Levitzki, 1987). Activation of Rs by stimulatory hormones (Hs) leads to the dissociation of subunits of Gs, α_s and $\beta\gamma$. The α_s-subunit activates C, resulting in the accumulation of cAMP within the cell (Thomas and Hoffman, 1987).

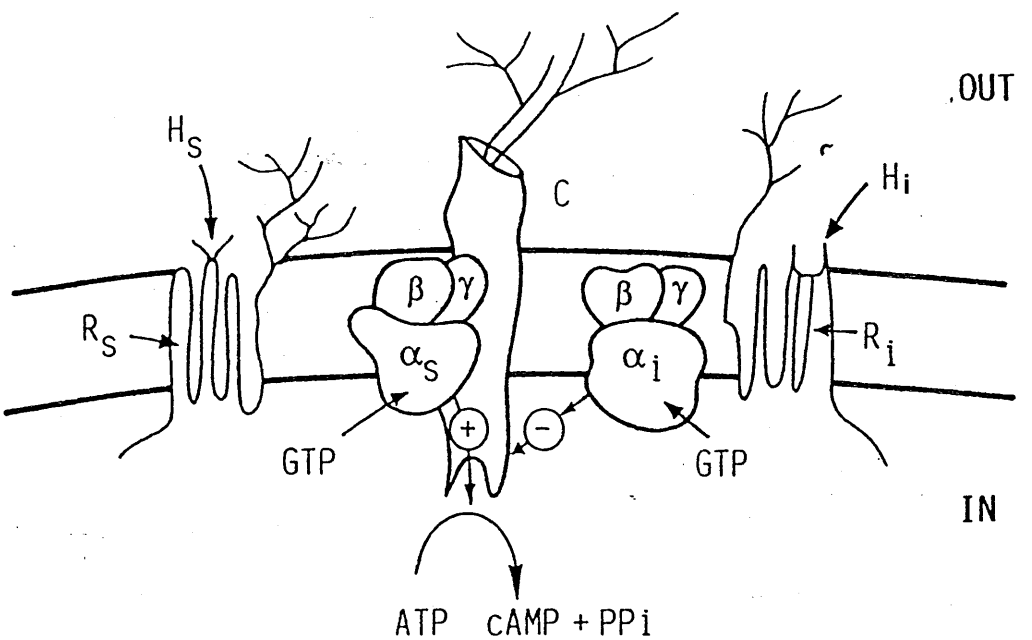


Figure 7 The structure of hormone-sensitive adenylate cyclase

The receptors are highly hydrophobic with seven membrane-spanning domains. The β-subunits of G_s and G_i are embedded in the membrane bilayer. The α-subunits are bound to the β,γ-subunits but are themselves hydrophobic. The α-subunit interacts with R_s and the catalyst C, probably through unique sequences in the cytoplasmic domains.

(From Levitzki, A., Trends in Pharmacological Sciences 8,, 299-303 (1987)).

cAMP may then activate cAMP-dependent protein kinase, resulting in the phosphorylation of specific protein substrates (Nestler and Greengard, 1983). However, activation of inhibitory receptors (Ri) by inhibitory hormones (Hi) leads to the dissociation of subunits of Gi, α_i and $\beta\gamma$. Inhibition of adenylate cyclase probably occurs directly via α_i inhibition of C or indirectly by inactivation of C (Thomas and Hoffmann, 1987). This leads to the fall in intracellular cAMP levels, and decreases in the protein kinase activity. Some examples of stimulatory and inhibitory hormones receptors that coupled to the adenylate cyclase are as depicted in Table 2.

1.5.2 Receptor-mediated cAMP formation

1.5.2.1 α - and β-Adrenergic agents (Noradrenaline)

Stimulation of the locus coeruleus has been shown to increase cAMP levels in the cortex, hippocampus, striatum and hypothalamus leading to the release of NA (Bloom, 1975; Korf and Sebens, 1979). Considerable evidence exists that NA stimulates cAMP formation in many brain regions by activating α- and β-adrenergic receptors, since the NA response on cAMP formation is more potently blocked by α_1-adrenergic antagonist, prazosine and β-adrenergic antagonist, propranolol than by α_2-adrenergic antagonist, yohimbine (Perkin and Moore, 1973; Davis et al., 1978; Daly et al., 1980; Johnson and Minneman, 1986). This is further supported by the findings of Etgen et al. (1987), who demonstrate that β-adrenergic agonist stimulation of cAMP formations in rat hypothalamus brain slices is potentiated by α_1-adrenergic agonist, phenylephrine but not α_2-adrenergic agonist, clonidine. In contrast, Pilc and Enna (1986) found that isoprenaline stimulation of cAMP formation in rat neocortical brain

Table 2 Examples of stimulatory and inhibitory hormone receptors that couple to the adenylate cyclase

Stimulatory receptors (Ra)	Inhibitory receptors (Ri)
β-Adrenergic	α_2-Adrenergic
Adenosine (A_2)	Adenosine (A_1)
Dopamine (D_1)	Dopamine (D_2)
ACTH	Muscarinic
Glucagon	Opiates (Enkephalin/morphine (μ)
Prostaglandin (E_1)	Somatostatin
Vasointestinal peptide (VIP)	Angiotensin (II)
Vasopressin (V_2)	

slices is potentiated by clonidine but not phenylephrine. Furthermore, they reported that isoprenaline and α-adrenergic agonist, 6-fluoronorepinephrine stimulation of cAMP formation is more potently blocked by yohimbine than by prazosin They thus suggest that NA stimulation of cAMP formation via the synergistic effect of α_2- and β-adrenoceptors.

Destruction of presynaptic noradrenergic nerve terminals with 6-hydroxydopamine results in enhanced responsiveness of cAMP-generating systems by both α- and β-adrenoceptors (Huang et al., 1973; Kalisker et al., 1973). The findings suggest that these receptors may also be located post-synaptically.

Activation of α_2-adrenoceptors have been shown to inhibit cAMP formation in a number of tissues, including human (Burns et al., 1982) and hamster adipocytes (Garcia-Sainz et al., 1980), mouse Neuroblastoma X glioma cells, NG 108-15 cells (Sabol and Nirenberg, 1979) and cultures of astrocytes derived from mouse (Van Calker et al., 1978) and rat brain (McCarthy et al., 1979). So far, there is no direct evidence which shows that activation of α_2-adrenoceptors linked to the inhibition of cAMP formation in brain slices since the α_2-adrenergic agonist, clonidine inhibition of NA stimulation of cAMP formation in rat neocortical brain slices is not reversed by α_2-adrenergic antagonist (Pilc and Enna, 1986).

NA stimulation of cAMP formation in rat cerebral cortex and hypothalamus may involved PGE_1 since the effect of NA is decreased by the protoglandin synthetase inhibitors indomethacin, aspirin and acetominophan and this could be reversed by PGE_1 (Partington et al., 1980).

Daly et al. (1980) demonstrate that in slices of rat cerebral cortex, the α-adrenergic agonist, 6-fluoronorepinephrine and the β-adrenergic agonist, 2-fluoronorepinephrine stimulation of cAMP formation can be blocked by 8-phenyl theophylline and propranolol, respectively. The finding suggests that α-adrenergic response is dependent on adenosine. Furthermore, adenosine or histamine potentiated NA and 6-fluoronorepinephrine but not β-adrenergic agonist, 2-fluoronorepinephrine stimulation of cAMP formation. The results show that α-adrenoceptor acts as a receptor modulator leading to the potentiation of adenosine, histamine and β-adrenoceptors stimulation of cAMP formation. In contrast, α-adrenoceptor does not elicit any response in cerebellum (Daly et al., 1980).

NA at high concentrations may also activate dopamine-sensitive cyclases. This is the reason why NA is able to stimulate cAMP levels in slices of caudate nucleus (Forn et al., 1974).

1.5.2.2 Adenosine

Adenosine stimulates cAMP formation 20-30 fold in guinea pig cortex slices, and the effect can be blocked by methylxanthines including theophylline and caffeine (Satin and Rall, 1970). However, in guinea pig particulate preparation, adenosine is only able to stimulate cAMP formation 4 fold (Daly et al., 1980).

It was initially presumed that adenosine increased cAMP formation by increasing the availability of substrate ATP for enzymes adenylate cyclase. However, using deoxy ATP, which does not give rise to adenosine in the assay medium, Cooper et al. (1980) show that adenylate cyclase in rat cerebral cortex is inhibited by low concentration of adenosine. In addition, adenosine analogue such as

phenylisopropyladenosine (PIA) and N^6-cyclohexyladenosine (CHA) are able to exert their effect on adenylate cyclase activity in a biphasic manner i.e. in the presence of GTP, low concentration 10nM of the analogue inhibits and high concentration above 1uM stimulates adenyl cyclase activity. The inhibitory effect of the adenosine analogue is antagonised by IBMX (Ki, 0.45uM). Van Calker et al. (1979) also demonstrate a high affinity inhibitory site (nanomolar) and a low affinity stimulatory site (micromolar) from the studies on adenosine stimulation of cAMP formation in glial cell cultures, which they termed A_1 and A_2 respectively. In rat brain slices, adenosine uptake inhibitors such as dipyridamole, hexobendine and p-nitrophenylthioguanosine were found to significantly potentiate the response to low concentrations of adenosine stimulation of cAMP formation. The finding suggests that adenosine stimulates cAMP formation by activating the adenosine receptors located on the extracellular sites.

Adenosine have also been shown to potentiate biogenic amines including NA, serotonin and histamine (Sattin and Rall, 1970; Schultz and Daly, 1973; Daly et al., 1980; Daum et al., 1982), glutamate and aspartate (Shimizu et al., 1974) and K^+ (Huang et al., 1971) stimulation of cAMP formation in brain slices, and their effects can be blocked by adenosine antagonists, methylxanthines.

1.5.3 Forskolin stimulation of adenylate cyclase

The diterpene forskolin, isolated from the roots of the Indian medicinal plant Coleus forskohlii (Bhat et al., 1977) has been shown to directly stimulate the catalytic unit of the adenylate cyclase by passing the guanine nucleotide regulatory proteins, Gs and Gi (see Daly, 1984 for review).

Low concentrations of forskolin, which alone have no effect on adenylate cyclase activity, are able to potentiate agonist stimulations, thereby increasing the potency and/or efficacy of the agonists. This suggests that forskolin may interact synergistically with Gs (Daly et al., 1982; Seamon and Daly, 1983). A two-site model of forskolin action in C6-2B rat astrocytoma cells was proposed by Borovsky et al. (1984), who suggested that a low affinity site which mediates the direct action of forskolin to increase intracellular cAMP accumulation and a high-affinity site which mediates the potentiation of forskolin exist.

The actual mechanism(s) and site(s) of forskolin action are still unknown. It has been suggested that forskolin may stimulate adenylate cyclase activity via an unknown regulatory protein that is not linked to hormone receptors and Gs (Brooker et al., 1983).

1.5.4 Effect of depolarization on cAMP formation

Electrical stimulation (KaKiuchi et al., 1969; Zanella and Rall, 1973) as well as membrane depolarization by various depolarizing agents including high concentrations of K^+, batrochotoxin, veratrine, ouabain (Shimizu et al., 1970, 1973; Shimizu and Daly, 1972) cause an increase in intracellular cAMP formation in guinea pig cerebral cortex slices. The effects of these depolarizing agents are found to be depended on Ca^{2+} concentrations since the absence of Ca^{2+} concentrations inhibit depolarization induced cAMP formation. In addition, Schwabe et al. (1978) demonstrate that the addition of Ca^{2+} chelator, EGTA to guinea pig cortical slices acutely reduce extracellular calcium increased basal levels of cAMP formation. Similarly, the addition of theophylline, an adenosine antagonist (Schwabe et al., 1978) has also been found to inhibit electrical

(KaKiuchi et al., 1969) as well as ouabain, batrachotoxin, veratrine and high concentrations of K^+ (KaKiuchi et al., 1969; Shimizu et al., 1970; Shimizu and Daly, 1972) stimulation of cAMP formation in guinea pig cerebral cortex slices. The findings thus suggest that depolarization which increases Ca^{2+} influx may lead to the release of endogenous adenosine which in turn stimulates cAMP formation via external adenosine receptors.

1.5.5 Ca^{2+}/Calmodulin regulation of neuronal cAMP levels

Neuronal cAMP levels have been shown to be regulated by calmodulin (CaM), a Ca^{2+}-binding protein since the latter is able to stimulate both adenylate cyclase (Brostrom et al., 1977; Wolff and Brostrom, 1979) and phosphodiesterase (Cox et al., 1981) activities. Native calmodulin (CaM) is inactive and contains four high affinity Ca^{2+}-binding domains (Watterson et al., 1980) and six auxiliary ion-binding sites (Cox, 1988). Divalent ion such as Mg^{2+} can modulate the affinity of CaM by occupying the auxiliary sites which are more specific for Mg^{2+}, and less so for Ca^{2+} (Cox, 1988).

Brostrom et al. (1977) demonstrate that Ca^{2+} exhibits a biphasic response on adenylate cyclase activities in rat cerebral cortical membrane when CaM is present in the assay medium; that is, low Ca^{2+} concentrations ($>$ 0.1mM) activates and high Ca^{2+} concentrations ($<$ 0.5mM) inhibit the activity. In addition, the inhibitory effect of high Ca^{2+} concentrations on the adenylate cyclase activity in the presence of CaM can be reversed by the addition of EGTA to the assay medium.

The exact mechanism(s) of CaM are still unknown since there are evidences which suggest that CaM may either stimulate cAMP formation by

directly activating the catalytic subunit (C) independent of the GTP-binding protein (Seamon and Daly, 1982), or interacts with guanyl nucleotides in an additive (Seamon and Daly, 1982) or synerigstic (Heideman et al., 1982) manner.

1.6 Modulation of voltage-sensitive ion channels by guanine nucleotide regulatory proteins (G-proteins)

A family of pertussis toxin (IAP) sensitive G-proteins including Gi_1, Gi_2, Gi_3 and Go have been identified in tissues (Falloon et al., 1986; Mitchell et al., 1986; Graziano and Gilman, 1987; Milligan, G., 1988) which when coupled to receptors may translate external messengers into intracellular responses by directly activating the voltage-sensitive ion channels instead of the adenylate cyclase. The brain contains two Gi-type G-proteins, namely Gi_1 and Gi_2 (Neer et al., 1984) and it is highly enriched in G_o (Neer et al., 1984; Sternweis and Robishaw, 1984). Similarly, neuroblastoma-glioma hybrid cells are also found to contain Gi-type G-proteins and high concentrations of Go (Milligan et al., 1986).

Electrophysiological techniques including whole cell patch-clamp recording from dorsal root ganglion (DRG) neurons demonstrate that NA and γ-aminobutyric acid (GABA) inhibitory effect on voltage-dependent calcium channels are blocked by preincubation of cells with pertussis toxin (Ui, 1984) or intracellular administration of guanosine 5'-O-(2'-thiodiphosphate) (GDP-γ-S), a non-hydrolysable analogue of GDP that competively inhibits the binding of GTP to G-proteins (Eckstein et al., 1979; Holz et al., 1986). Angiotensin (II) stimulation of Ca^{2+} current in the adrenocortical cell line Y1 (Kojima et al., 1986) is abolished in Y1 cells pretreated with pertussis toxin, while

intracellular application of cAMP does not stimulate the Ca^{2+} current in the cells. Thus, the finding suggests that G-proteins act as signal transducers by directly linking the hormones or neurotransmitters to voltage-sensitive Ca^{2+} channel independently of cAMP (Fig. 8A).

Sasaki et al. (1987) demonstrated that when hormone receptors activation of the ganglion cells of the sea slug Aplysia by either acetylcholine (Ach.), histamine or DA leads to an increase in K^+ conductance, which can be irreversible and selectively blocked by intracellular injection of pertussis toxin. Furthermore, intracellular application of guanosine-5'-O-(3-thiotriphosphate) (GTP-γ-S), a poorly hydrolysed analogue of GTP alone, caused extremely slow and irreversible opening of K^+ channels. This suggests that G-proteins may be involved in the receptor regulation of K^+ channels (Fig. 8B).

K^+ induced depolarization leads to an increase in Ca^{2+} influx, which in turn activates K^+ channels causing hyperpolarization and promoting repolarization (Blatz and Magleby, 1986) thereby switching off Ca^{2+} dependent cellular responses.

1.6.1 Modulation of ion channels by Ca^{2+} mobilizing receptors possibly mediated via unknown G-proteins

Occupancy of Ca^{2+} mobilizing receptors by either adrenaline (α_1), acetycholine (M), substance P, NA (α_1), thyroliberin (TRH), 5-hydroxytryptamine (5-HT), caenilein or vasopressin (V_1) involve the breakdown of membrane-bound phosphoinositides to a number of inositol phosphate and diacylglycerol (Taylor and Merritt, 1986). These reactions are catalysed by phospholipase C. Consequences of the breakdown of phosphoinositides include mobilization of intracellular Ca^{2+} (Berridge and Irvine, 1984; Taylor, 1987), which in turn may

activate protein kinase C (Nestler and Greengard, 1983; Hemmings et al., 1986). In mast cells, activation of Ca^{2+}-mobilizing receptors lead to the stimulation of phospholipase C, the effect of which is inhibited by pertussis toxin (Nakamara and Ui, 1984). Similarly, in adipocytes, α_1-adrenergic stimulation of phospholipase C is inhibited by pertussis toxin, while cholera toxin does not affect the coupling of Ca^{2+}-mobilizing receptors to phospholipase C (Taylor and Merrit, 1986). The finding suggests that Gs is not involved in this coupling process. Furthermore, a crude plasma membrane preparation of blow fly salivary glands produced $In(1,4,5)P_3$ in response to 5-HT only in the presence of GTP. In addition, stable analogues of GTP, GTP-γ-S and 5'-guanylylimidodiphosphate [Gpp(NH)p], which stimulate the formation of $Ins(1,4,5)P_3$ are able to potentiate the effects of 5-HT (Litosch et al., 1985). The results suggest a close link between an unknown pertussis toxin sensitive G-protein and phospholipase C. Also, the increase in intracellular Ca^{2+} via receptor/G-protein mediated hormone sensitive phospholipase C may also lead to the activation of K^+ conductance thereby reducing Ca^{2+} influx (Fig. 8C).

1.6.2 Modulation of ion channels by intracellular messengers

Protein kinases including cAMP-dependent protein kinase (cAMP-PK), cGMP-dependent protein kinase (cGMP-PK) and the Ca^{2+}/phospholipid-dependent protein kinase (protein kinase C) (PKC) have been shown to modulate voltage sensitive Ca^{2+} channels (Rosenthal and Schultx, 1987; Fig. 8D).

Ca^{2+} channels of hippocampal neurons are stimulated by cAMP-PK (Gray et al., 1987). Electrophysiological studies using the whole cell clamp technique demonstrate that intracellularly applied cAMP or the

catalytic subunit of cAMP-PK enhances the Ca^{2+} activated K^{+} currents in snail neurons (Ewald et al., 1985). Similarly, intracellular infusion of cGMP or of cGMP-PK via patch pipette in snail neurons also enhances Ca^{2+} currents and increases the 5-hydroxytryptamine (5-HT) induced Ca^{2+} current (Paupardin-Tristch et al., 1986). Injection of PKC into mollusc neurons has also been found to enhance Ca^{2+} currents (De Reimer et al., 1985). Nichols et al. (1987) demonstrate that activation of protein kinase C by tumour-promoting phorbol esters enhanced the K^{+}-stimulated release of [^{3}H]NA and [^{3}H]Acetycholine from rat cerebral cortex synaptosomes preparations in a Ca^{2+}-dependent manner. The results suggest that phosphorylation of ion channels by protein-kinases may also regulate the release of neurotransmitters.

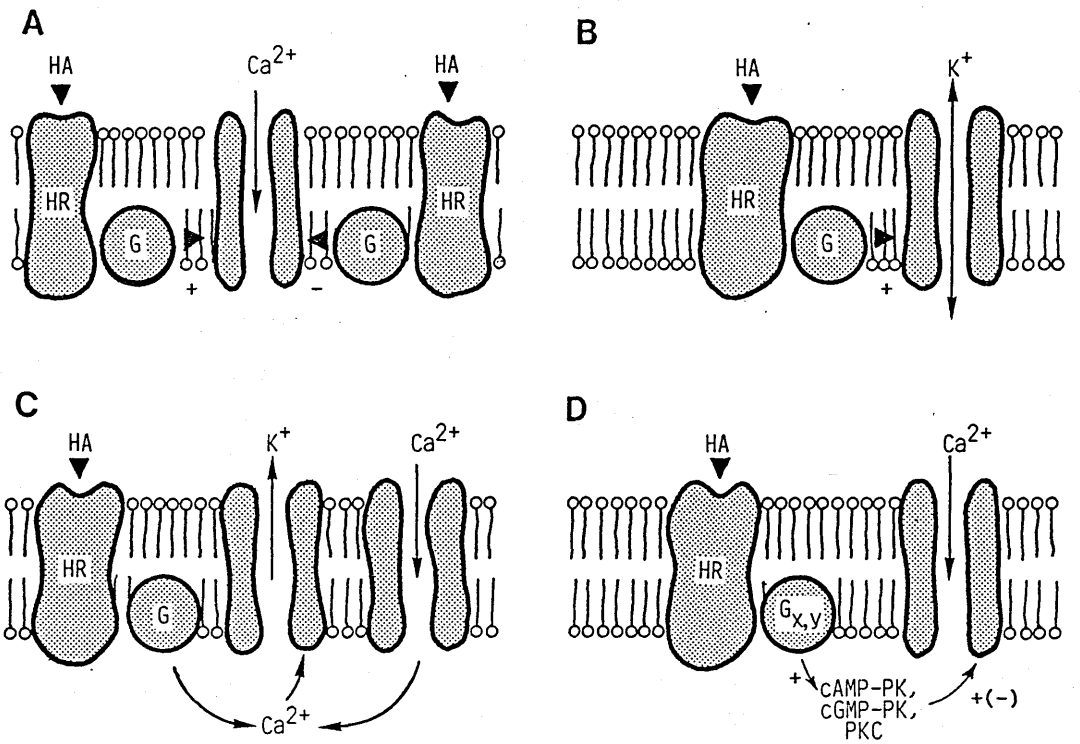


Figure 8 Modulation of ion channel activity via cell surface receptors

(A) G proteins mediate either hormonal stimulation or hormonal inhibition of Ca^{2+} channel activity

(B) Receptor control of K^+ channel activity via G-protein

(C) Receptor control of K^+ channel activity by an intracellular messenger

(D) Hormonal modulation of Ca^{2+} channel activity achieved by protein kinases including cAMP-PK, cGMP-PK or protein kinase C

Abbreviations: HA, hormonal agonist (hormone or neurotransmitter); HR, hormone receptor; G, G protein

(From Rosenthal, W. and Schultz, G. (1987) Trends in Pharmacological Sciences 8, 351-354.)

1.7 Aim of project

The aim of this project was to examine the hypothesis that presynaptic α_2-adrenergic inhibition of noradrenaline release is mediated via changes in cAMP formation. This hypothesis is based on the observations that α_2-adrenergic agonist in a number of tissues have been shown to be linked to the inhibition of cAMP formation. Answers to the following two questions, relating to this hypothesis were sought (i) Does increasing the concentration of cAMP in occipital cortex tissue chops reverse the α_2-adrenergic inhibition of NA release and (ii) Is there a relationship between the effect of α_2-adrenergic agonists on NA release and cAMP formation in rat brain occipital cortex tissue chops?

2. MATERIALS & METHODS

2.1 Materials

Materials used were obtained from the following sources:

Amersham International, Amersham Buckinghamshire

1-[7,8]-[^{3}H]-Noradrenaline (30-50Ci/mmol)

8-[^{3}H]-Adenine (20-25Ci/mmol)

U-[^{14}C]-cAMP (261mCi/mmol)

BOC Limited, Brentford

95% O_2 - 5% CO_2

Sigma, Poole, U.K.

Noradrenaline

Clonidine

Propranolol

Prazosin

Yohimbine HCl

Isoprenaline

Adenosine

Forskolin

3-Isobutyl-1-methylxanthine

Desipramine

Pargyline

Neutral alumina

Imidazole HCl

N^{6}-cyclohexyl adenosine

Ethyleneglycol-bis-(β-amino-ethyl)N,N'-tetra acetic acid (EGTA)

The α_2-adrenergic agonists BHT-920 and UK-14304-18 were kindly donated by Syntex Research Centre, Riccarton, Edinburgh and Pfizer Central Research, Sandwich, Kent, respectively.

Kindly donated by Dr. D. Pollock, Department of Pharmacology, University of Glasgow.

Phenylephrine

Kindly donated by Roche Products Ltd., Welwyn Garden City, Hertfordshire

RO-20-1724

BDH Chemicals Ltd., Poole, England

Ascorbic acid

Folin-Ciocaltean reagent

Sodium dodecyl sulphate

N-2-Hydroxyethylpiperazine-N-2-ethane sulphonic acid (HEPES)

BioRad

Dowex AG 50W-X-4; 200-400 mesh (hydrogen form)

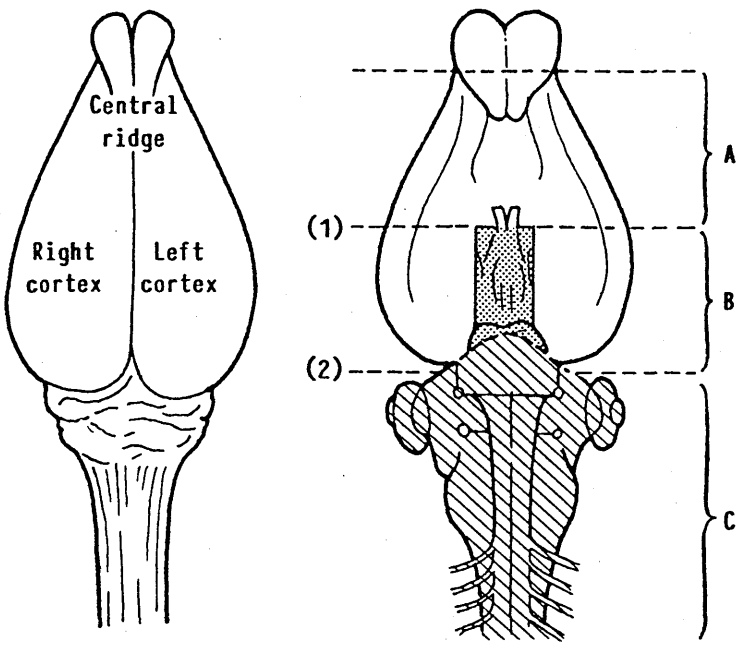
Research Biochemical Inc., MA., U.S.A.

8-cyclopentyl-1,3-dipropylxanthine

2.2 Dissection of rat brain

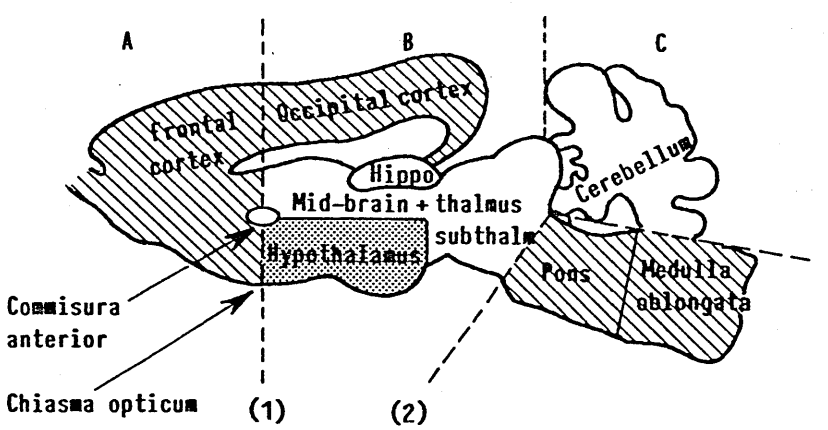
Male Wistar rats (230-250g) were sacrificed by CO_2 anaesthesia followed by decapitation. Brains were removed and placed in a chilled glass petri dish.

For the dissection of occipital cortex, a transverse section (Fig. 9C, dashed line, 1) was made to remove the front brain (A). The occipital cortex could be easily removed by gently peeling back using a spatula beginning at the central ridge (Fig. 9A). The white and grey matter attached to the cortex was removed using forceps. The



(A) Frontal view of rat brain

(B) Ventral view of rat brain



(C) Saggital section of rat brain, showing the diagrammatic representation of dissection procedure. Dotted line indicates position of initial sections (Glowinski and Iversen, 1966)

Figure 9 Dissection of occipital cortex and hypothalamus

hypothalamus was dissected out by the method of Glowinski and Iversen, 1966 (Fig. 9C). The anterior commissure was taken as a horizontal reference and the line between the posterior hypothalamus and the mammillary bodies as the caudal limit.

2.3 Preparation of tissue chops

Tissue from occipital cortex or hypothalamus was chopped using a McIlwain tissue chopper to produce 0.26 x 0.26mm slices cut at 90^{o} to each other. The tissue chops were then washed once with HEPES buffer in order to remove tissue debris, and then used for uptake and release of [^{3}H]NA or for cAMP assay.

2.4 HEPES buffered salines

The HEPES buffer used for both the centrifugation assay and the cAMP assay consisted of (in millimolar concentrations) : NaCl, 140; KCl, 5; $CaCl_2$, 0.5; HEPES, 15; D-Glucose, 10; K_2HPO_4, 1.5; $MgSO_4$.7HO, 1.5; pargyline, 0.1; ascorbic acid, 0.1. The buffer was equilibrated with 95% O_2-5% CO_2, and the pH adjusted to 7.4 with 1M NaOH. It was then kept at 4^{o}C. For cAMP assay, pargyline and ascorbic acid were omitted from the buffer. High potassium concentrations were obtained by replacing NaCl with the equivalent amount of KCl to maintain isomolarity.

2.5 HPLC-ED Assay of endogenous catecholamines from rat brain regions

Endogenous NA and DA were assayed by HPLC-ED as shown in Figure 10. Brain samples were weighed and then homogenised in 0.5ml 0.1M PCA in Eppendorf tubes using a mini drill (Expo Drills Ltd.,

London, U.K.) fitted with a teflon-tipped pestle. The tubes were then centrifuged at 10,000g for 5 minutes in an Eppendorf 5412 bench centrifuge. After that 80ul aliquots of supernatant were injected onto a Partisil 10 ODS column (Whatman Ltd., 25cm x 4.6mm i.d.) via a six port rotary injection valve (Model 7120, Rheodyne Instruments Ltd., U.S.A.) fitted with a 20ul sample loop. A mobile phase of 0.027M citric acid, 0.05M sodium acetate, 0.06M sodium hydroxide, pH5.2 containing 0.057g sodium octyl sulphonate per litre and 2.5% (v/v) methanol was used for the separation of catecholamines (Keller et al., 1976). The mobile phase was filtered through Sartorius filters (0.45m pore size) before use, degassed with helium and delivered at a constant flow rate of 1.1ml/min using an LC3-XP pump (Pye Unicam Ltd., Cambridge, U.K.). Catecholamines were detected with a TL-5 electrochemical detector cell (silicon-grease based carbon paste, CP-S, Bio-Analytical Systems Incl.) which consisted of a glassy-carbon working electrode, Ag/AgCl reference electrode, a platinum auxillary electrode and a LC-4 controller Unit. The operating potential was 0.65v and the current produced by oxidation of catecholamines was transduced by the LC-4 Controller Unit into a peak on a Philips PM8251 single pen chart recorder (Philips Ltd.). The retention times of NA and DA were 4 and 12 minutes respectively. The unknown concentrations of NA and DA were calculated from the peak heights produced by the samples compared with the peak height produced by standard solutions of NA and DA (4-20ng/100ul in 0.1M-$HClO_4$) (Fig. 11).

2.6 Uptake of [^{3}H]NA into occipital cortex tissue chops

The effect of desipramine (DMI), an uptake inhibitor, on the uptake of NA by chopped brain slices was examined using the method of

HPLC - ED system

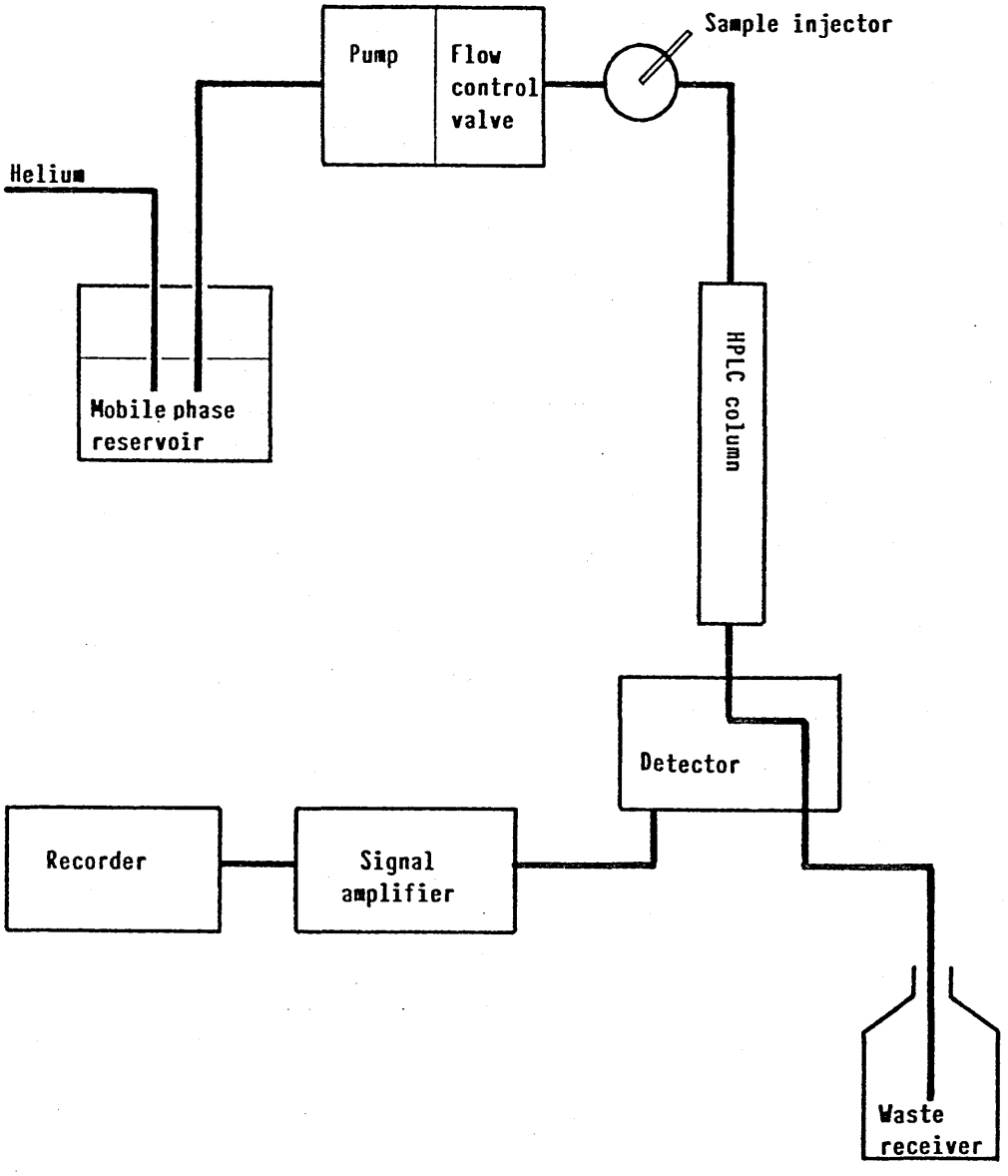


Figure 10 Flow diagram of HPLC-ED system

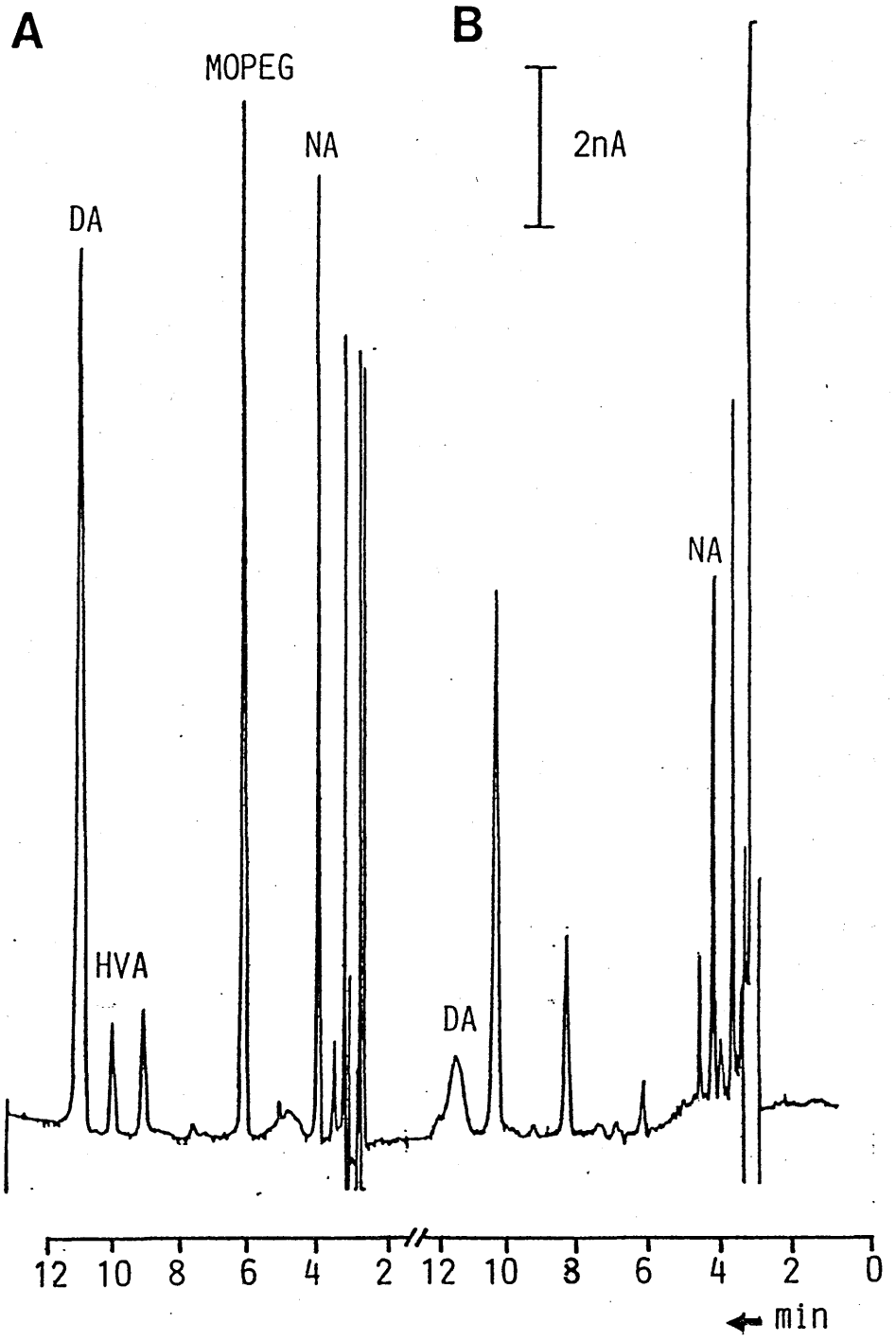


Figure 11.
Chromatogram of separation of NA and DA by HPLC
A) Standard and B) Sample (occipital cortex), Flow rate,1.1ml/min; Dopamine (DA) concentration,10ng/20µl; Noradrenaline (NA) concentration. 10ng/20µl.

2.7 Determination of [^{3}H]NA release

Tissue chops were prelabelled by incubating with 0.3 x 10^{-7}M [^{3}H]NA (specific activity, 30-50Ci/mmol) in HEPES buffer containing 0.5mM Ca^{2+} for half an hour at 37^{o}C. The tissue chops were then washed by gently turning the test tube upside down three times, allowing the tissue to settle and carefully removing the supernatant. The tissue chops were resuspended in fresh HEPES buffer. This process was repeated eight times. For the study on Ca^{2+} dependent release, tissue chops were washed with HEPES buffer containing 0.1mM EGTA, without Ca^{2+}. Aliquots (12ul) of the resuspended tissue chops (5-10mg) were pipetted into Eppendorf tubes using a Gilson pipette with the tip cut off. 50ul DMI 10^{-6}M (final concentrations) was added to each tube to prevent re-uptake of the released [^{3}H]NA. Total volume in the Eppendorf tube was 0.5ml.

Release of [^{3}H]NA was evoked by the addition of 20mM or 30mM K^+ and the tubes were incubated for 6 minutes at 37^{o}C either in the presence or absence of drugs as indicated in the legends of the relevant figures. For K^+-evoked Ca^{2+}-dependent study, different concentrations of Ca^{2+} were added to the tubes as mentioned in legends of the relevant figures. The tubes were shaken at regular intervals. In some cases, the tissue chops were preincubated for periods up to 30 minutes at 37^{o}C, shaken at regular intervals with appropriate drugs as indicated in the relevant figures and tables before evoking release with 20mM K^+ as described above. The reaction was terminated by centrifuging the Eppendorf tubes at 2000 x g for 5 minutes at 4^{o}C. The supernatant was transferred to vials containing 4ml "Ecoscint" (liquid scintillant) for scintillation counting. [^{3}H]NA left in the pellet was extracted by

Carmichael and Israel (1973). The assay was modified to separate the labelled tissue from the medium by centrifugation. 12ul aliquots of the tissue chops (5-10mg) suspended in minimal volume of HEPES buffer was pipetted into Eppendorf tubes using a Gilson pipette with the tip cut off. Desipramine (DMI) over the range 10^{-8}M-10^{-4}M was added to the tubes except the control, and these were incubated for 10 min at 37^{o}C with regular manual shaking at 2-3 minute intervals. The tubes were then centrifuged for 5 min at 250 x g, at 4^{o}C, and the supernatant was discarded. After that, fresh medium containing 0.3 x 10^{-7}M [^{3}H]NA (specific activity, 30-50Ci/mmol) in the presence of an appropriate dilution of DMI was added to the tubes, which were further incubated for half an hour at 37^{o}C and shaken at regular intervals. The total volume in the tubes was 0.5ml. The tubes were centrifuged at 2000 x g at 4^{o}C for 5 minutes and the supernatant in each tube was discarded. The pellets were washed by adding fresh medium to the tubes, shaking the tubes gently, and allowing the tissues to settle and the supernatant was discarded. The process was repeated three times. The [^{3}H]NA in the pellet was extracted by homogenising the tissue with 500ul 0.1M perchloric acid (PCA). The tubes were centrifuged at 2000 x g at 4^{o}C for 5 minutes. The PCA extracts were transferred into vials containing 4ml "Ecoscint" (scintillation fluid), and the [^{3}H]NA was determined in a scintillation counter. NA uptake was initially expressed as counts per minute (CPM)/mg protein, and then converted into % of control value. The protein content of the chopped brain slices in the tube was determined by method of Lowry et al. (1951).

homogenisation with 0.5ml 0.1M PCA, and then centrifuged at 2000 x g for 5 min. The [^{3}H]NA content in the PCA supernatant was determined by liquid scintillation counting.

2.7.1 Calculation of [^{3}H]NA release

The [^{3}H]NA released under each condition was expressed as:

% of total tissue tritium

$$= \frac{\text{counts per minutes (cpm) supernatant}}{\text{cpm(pellet)} + \text{cpm(supernatant)}} \times 100$$

or $$\frac{S}{P + S} \times 100$$

The results were expressed as K^+-induced release of [^{3}H]NA in the presence or absence of drugs minus the basal (unstimulated) release of [^{3}H]NA in the presence or absence of drugs.

The [^{3}H]NA release expressed as a % of the control value was calculated as follows:

$$[^3\text{H}]\text{NA release as \% of control} = \frac{\text{\% of total tissue tritium in the presence of drugs}}{\text{\% of total tissue tritium}} \times 100$$

2.8 cAMP assay

cAMP formation in tissue chops was followed by measuring the production of [^{3}H]cAMP from [^{3}H] adenine using the method of Shimizi et al. (1969). Tissue chops were incubated for 15 minutes at 37^{o}C in 9ml HEPES buffer. The tissue chops were allowed to settle, the supernatant removed and the tissue chops were incubated for 40 min at 37^{o}C in fresh medium (9ml) containing 10^{-7}M [^{3}H] Adenine (specific activity 20-25Ci/mmol). The tissue chops were rinsed five times by gently shaking the tubes, allowing the tissues to settle, and then removing the supernatant. A further incubation of tissue chops with

fresh HEPES buffered saline was carried out for 10 min at 37°C. The incubation medium was discarded, and the tissues were suspended in minimal volume of HEPES buffer. 12ul aliquots of the tissue chops (5-10mg) were pipetted into Eppendorf tubes using a Gilson pipette with the tip cut off. cAMP formation was stimulated for 10 min at 37°C by the addition of isoprenaline, noradrenaline, adenosine, potassium or forskolin in the concentrations stated in the relevant figures or tables. For the studies with α_2-adrenergic agonist, the tissue chops were preincubated with the relevant agonist for 5 minutes before the addition of the compound used to stimulate cAMP formation. The reaction was terminated by centrifuging the tubes for 3 minutes, 2000 x g, at 4°C. The supernatant was decanted. [^{3}H]cAMP in the tissue pellet was extracted by homogenisation with 1ml 10% TCA containing [^{14}C]-AMP(5Ci/100ml 10%.TCA) as internal standard. The Eppendorf tubes containing 1ml 10% TCA extracts were centrifuged for 10 minutes 2000 x g at 4°C. 50ul of the TCA supernatant was used to determine the total radioactivity in the tissue. [^{3}H]cAMP in the rest of the TCA supernatant was then purified using Dowex and alumina columns by method of Solomon et al. (1974). Results are expressed as percentage conversion; i.e. the percentage of total [^{3}H] adenine taken up by the tissue converted to [^{3}H] cAMP.

2.9 Preparation of Dowex 50 and aluminium columns for adenylate cyclase assay (Solomon, 1979)

2.9.1 Supplies

(1) Columns used were 5ml syringes, diameter 1 cm, containing a sintered filter disk.

(2) Racks were constructed to hold 24 columns each. They were made in such a way that the two columns fitted onto each other in pairs, with the columns in the upper and lower racks vertically aligned.

(3) The lower columns were properly spaced so that the effluent containing [^{3}H]cAMP and [^{14}C]-AMP could drain into 24 scintillation vials arranged directly below the columns.

2.9.2 Packing Dowex 50 columns

Dowex AG 50W-X4 (200-400 mesh) was placed in a conical flask, washed twice with 1M HCl, and equilibrated with 100ml 1M HCl for 30 minutes. The acid-treated resin was washed with distilled water until the effluent was colour free. Columns were then filled with the resin to about 4cm. In order to prevent bacterial and fungal growth, columns were stored with 2ml 1M HCl at room temperature. Columns were washed with 10ml distilled water before use.

2.9.3 Packing Alumina Columns

0.6g dry neutral alumina was placed in each column and washed with approximately 10ml 1.0M imidazole HCl buffer, pH 7.5, and stored at room temperature. Before use columns were washed with 6ml 0.1M imidazole buffer.

2.9.4 Column recycling

Dowex 50 columns: The columns were regenerated by washing with 4ml 1M HCl, followed by 10ml distilled water.

Alumina columns: Columns were regenerated with 3ml 1M imidazole/HCl buffer, pH 7.5, followed by 6ml 0.1M imidazole/HCl buffer.

2.10 cAMP purification

1ml TCA extracts of the samples were applied to Dowex columns. ATP and ADP were eluted with 2.0ml 0.1M HCl, followed by 2.0ml distilled water. cAMP was then eluted onto the alumina columns with 6ml distilled water. The alumina columns were washed with 1ml 0.1M imidazole HCl and [^{3}H]cAMP eluted with 3.0ml 0.1M imidazole/HCl buffer directly into vials containing 12ml Ecoscint.

2.11 Preparation of a dual-label quench curve by external standard method

The Beckman LS1800 scintillation spectrophotometer uses a high energy gamma source, such as ^{137}Cs as an external reference standard and is equipped with the H# method for quench correction.

The quench curve was calibrated using 10 sealed samples of [^{3}H] or [^{14}C] of known dpm, supplied by Amersham, containing different amounts of quenching agent in scintillation fluid. The counting efficiency of [^{3}H] or [^{14}C] was then obtained using the formula below:

$$\text{counting efficiency (CE)} = \frac{\text{cpm}}{\text{dpm}} \times 100\%$$

As a result of this preliminary quench curve, the counter was then programmed to obtain dpm from cpm. H# values were set at a minimum of 39 and a maximum of 219. Counting efficiencies for [^{3}H] and [^{14}C] were found to be approximately 40% and 70% respectively.

2.12 Scintillation counting

The [^{3}H]NA released was determined by adding in 0.5ml of supernatant or 0.5ml of 0.1M PCA tissue extracts to scintillation vial containing 4ml Ecoscint, and counted in Beckman LS1800 liquid

scintillation spectrophotometer for 5 minutes. In the case of cAMP assay, radioactivity of [^{3}H]cAMP and [^{14}C]cAMP was counted in larger volume of imidazole buffer (3.0ml), therefore it required the use of 12ml of Ecoscint to ensure homogeneity.

2.13 Protein estimation

Protein in the tissue pellet was estimated using a modification of the method of Lowry et al. (1951) as follows:

(i) Tissue in the pellet was dissolved in 500µl of 2% (w/v) SDS in 0.1M NaOH overnight, and 100µl aliquots placed in test tubes.

(ii) To each tube was added 3ml of a mixture of 50ml solution A + 1ml solution B.

Solution A consisted of 2% (w/v) anhydrous Na_2CO_3 and 0.2% (w/v) SDA in 0.1M NaOH, and solution B consisted of 0.5% $CuSO_4$ $5H_2O$ in 1.0% (w/v) trisodium citrate.

(iii) After 10 minutes, 0.5ml Folin-Ciocalteau reagent diluted 1:2 (v/v) with water was added to the tubes, and then mixed with a vortex mixer.

(iv) The test tubes were left for at least 30 minutes in order to allow colour development to stabilize before reading the absorbance at 650 of the samples using a Beckman Dual Beam spectrophotometer (Beckman Ltd.).

(v) Protein was estimated by reference to a protein calibration curve (0-100ug protein) using bovine serum albumin as a standard (Fig. 12).

2.14 Statistical Evaluation

Standard deviation (S.D.) is a measure of the difference of each

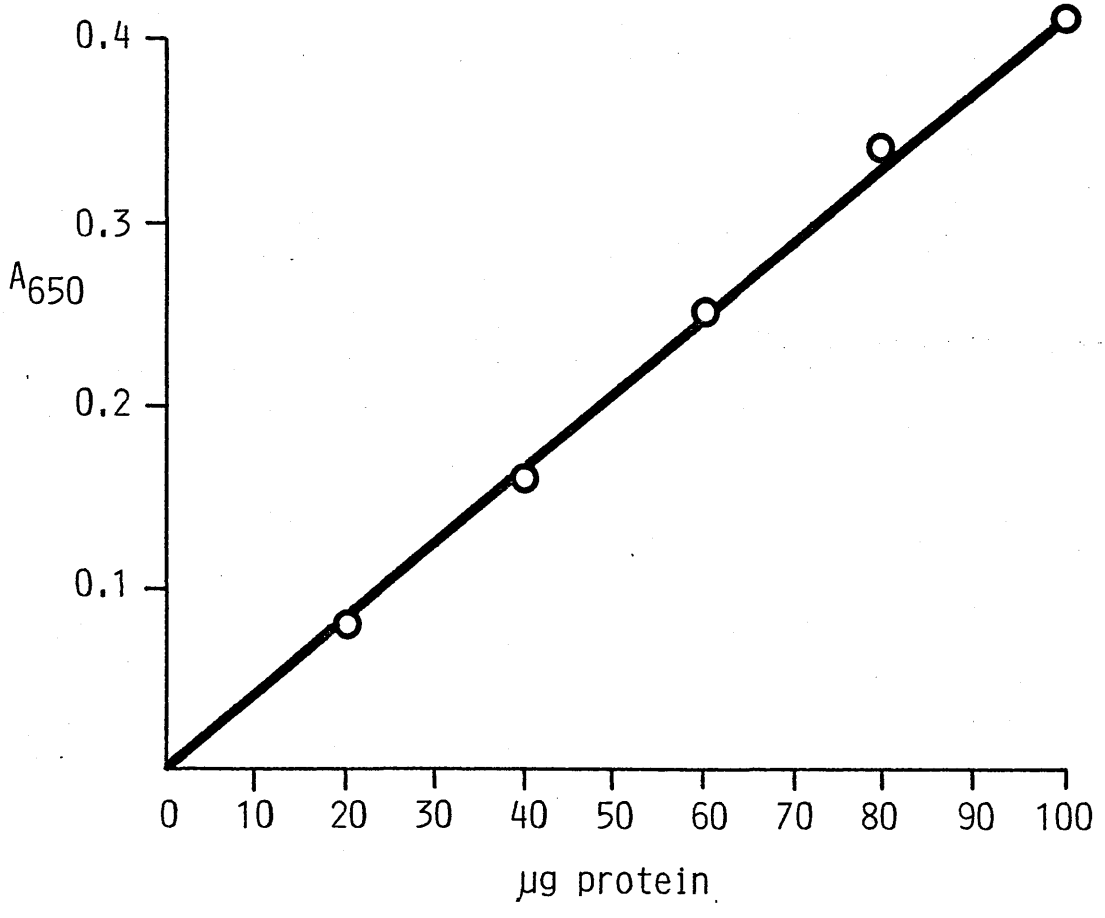


Figure 12 Calibration curve for protein estimation

observation from the mean. It was calculated using the formula:

$$SD = \sqrt{\frac{(\bar{x}-x)^2}{n-1}}$$

where,

x = individual observation

$\bar{x}$ = mean of number of observations

$(\bar{x}-x)^2$ = sum of the squares of differences (or deviation from the mean)

n = number of observations

$\frac{(\bar{x}-x)^2}{n-1}$ = variance

Standard errors mean (S.E.M.) was used to describe the significance of the difference between the means. It was obtained by dividing the S.D. of each sample by the square root of the number of observations in the sample (n). It was calculated by using the formula below:

$$S.E.M. = \frac{S.D.}{\sqrt{n}}$$

Statistical significance of the effects of two alternative treatments or experiments comparing in pairs was determined by the t-test or sometimes referred to as Student's t-test. The factor t, the significance of a deviation of a statistic from zero was calculated by using the formula:

$$t = d \div \frac{S.D.}{\sqrt{n}}$$

where,

d = mean of the differences between the two alternative treatments

$\frac{S.D.}{\sqrt{n}}$ = S.E.M. of the differences

Entering the t value into the table of the t-distribution (Pollard, 1977) at the appropriate degree of freedom gave the probability value of a chance effect.

3 RESULTS

3.1 Preliminary studies

3.1.1 NA and DA content in the occipital cortex and hypothalamus of rat brain

Occipital cortex (O.C.) and hypothalamus were dissected as described in the Methods Section. The wet weights of O.C. and hypothalamus were 37.9 ± 14.8mg (n = 7, mean ± S.D.) and 90.1 ± 17.3mg (n = 7, ± mean S.D.) respectively.

The NA and DA contents of O.C. and hypothalamus were measured using HPLC with electrochemical detection. The NA contents of O.C. and hypothalamus were 502 ± 347ng/g wet weight (n = 7, mean ± S.D.) and 1404 ± 251ng/g wet weight (n = 7, mean ± S.D.) respectively, while the DA contents were 59 ± 18ng/g wet weight (n = 7, mean ± S.D.) and 220 ± 16ng/g wet weight (n = 7, mean ± S.D.) respectively. Thus both NA and DA contents in the hypothalamus are higher than those found in the O.C.

3.1.2 Effect of desipramine (DMI) on the uptake of [^{3}H]NA in O.C. tissue chops

The centrifugation assay used in this study has the advantage of enabling release to be followed in a small volume, thus economising on tissue and material. However, the released NA could be rapidly taken up into the nerve endings, thus reducing the net release of NA. Therefore, DMI, an inhibitor of NA uptake was included in the assay to prevent the re-uptake of released NA. DMI (10^{-6} M) inhibited about 90% of [^{3}H]NA uptake (Fig. 13). Thus, this concentration was included in all [^{3}H]NA release experiments.

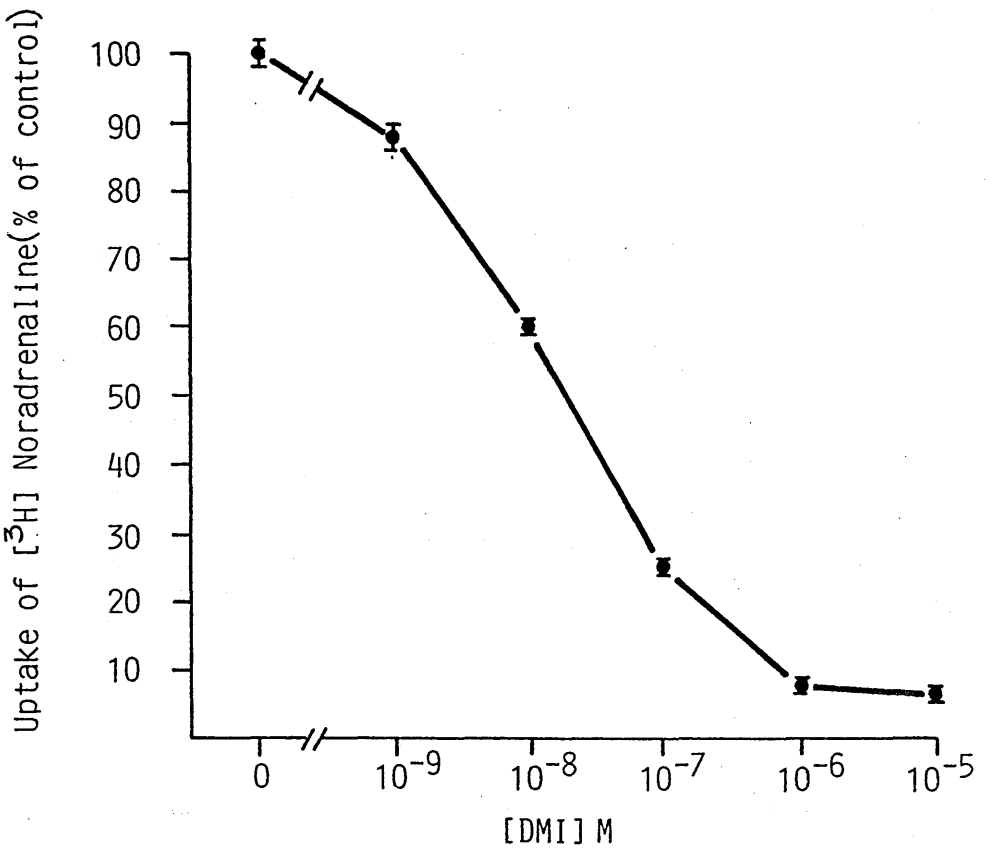


Figure 13 Effect of DMI on [^{3}H]NA uptake into O.C. tissue chops

Aliquots of tissue chops were preincubated for 10 min at 37°C with oxygenated HEPES buffer containing DMI. The tissue chops were loaded with [^{3}H]NA in HEPES buffer containing DMI, range (10^{-8}M-10^{-4}M) for 1/2 h. The total uptake of tissue tritium averages 10^{-4} cmp/mg protein. Protein assay was performed by method of Lowry et al. Each point represents the mean ± S.E.M. of 4 separate experiments, each of which was performed in triplicate.

3.1.3 K^+-stimulated Ca^{2+}-dependent release of [^{3}H]NA from O.C. and hypothalamic tissue chops

K^+-evoked release of [^{3}H]NA was examined in O.C. (Fig. 14) and hypothalamic (Fig. 15) tissue chops. Release of [^{3}H]NA increased with increasing K^+ concentration and reached a maximum over the range 80mM - 120mM K^+ for both tissues. High K^+ was obtained by replacing NaCl with the equivalent amount of KCl.

Ca^{2+}-dependent K^+-stimulated release of [^{3}H]NA was studied in O.C. tissue chops. Figure 16 shows that high K^+ (40mM or 20mM) stimulation of [^{3}H]NA is more dependent on Ca^{2+} concentrations than low K^+ (5mM). The effect of K^+ stimulation on Ca^{2+} - dependent release from O.C. tissue chops was examined either in HEPES buffer equilibrated with 95% O_2 - 5% CO_2 and the pH subsequently adjusted to 7.4 (Fig. 16B) or in non-oxygenated HEPES buffer (Fig. 16A). K^+ stimulation of [^{3}H]NA release is not dependent on the oxygenation of buffer since very little difference was observed between maximal release in oxygenated (Fig. 14) compared with non-oxygenated (Fig. 14) buffer. However, lower basal release was observed in oxygenated HEPES buffer (Fig. 16A) compared with non-oxygenated HEPES buffer (Fig. 16B).

3.1.4 Time course of the effect of clonidine on the release of [^{3}H]NA from O.C. tissue chops

The effect of clonidine, an α_2-adrenergic agonist, on the release of [^{3}H]NA was studied under depolarizing (30mM K^+) and non-depolarizing (5mM K^+) conditions by incubating tissue chops with clonidine (10^{-6}M) for the different times shown in Figure 17. Clonidine (10^{-6}M) did not inhibit the basal release of [^{3}H]NA over

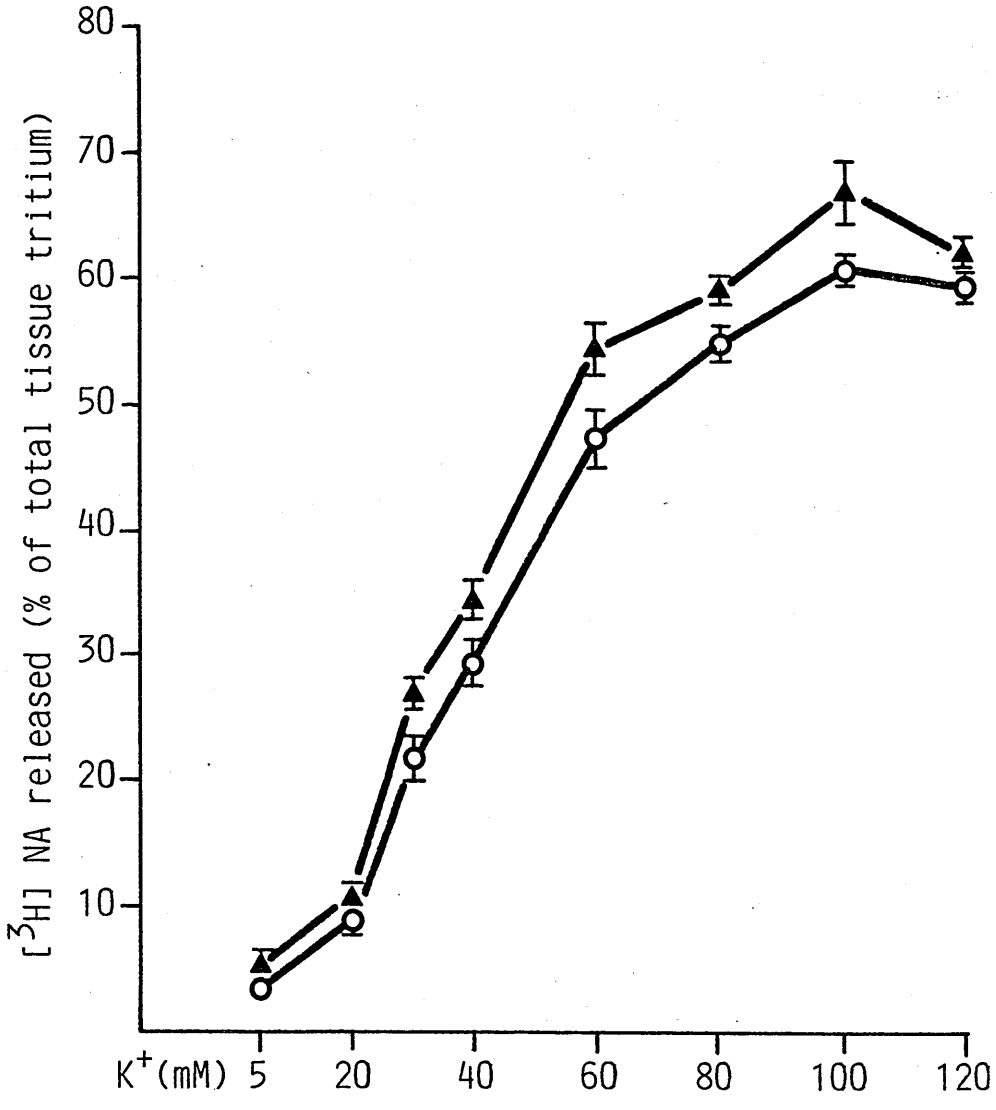


Figure 14 Effect of K^+ on the release of [^{3}H]NA from O.C. tissue chops

Tissue chops were preloaded with [^{3}H]NA. Release of [^{3}H]NA was evoked by incubating the tissue chops with different K^+ concentrations as shown above either in non-oxygenated (▲) or oxygenated (95% O_2 - 5% CO_2) (O) HEPES buffer medium containing 10^{-6}M DMI for 6 min at 37°C. Each point is the mean of 5 experiments ± S.E.M., each of which was performed in triplicate.

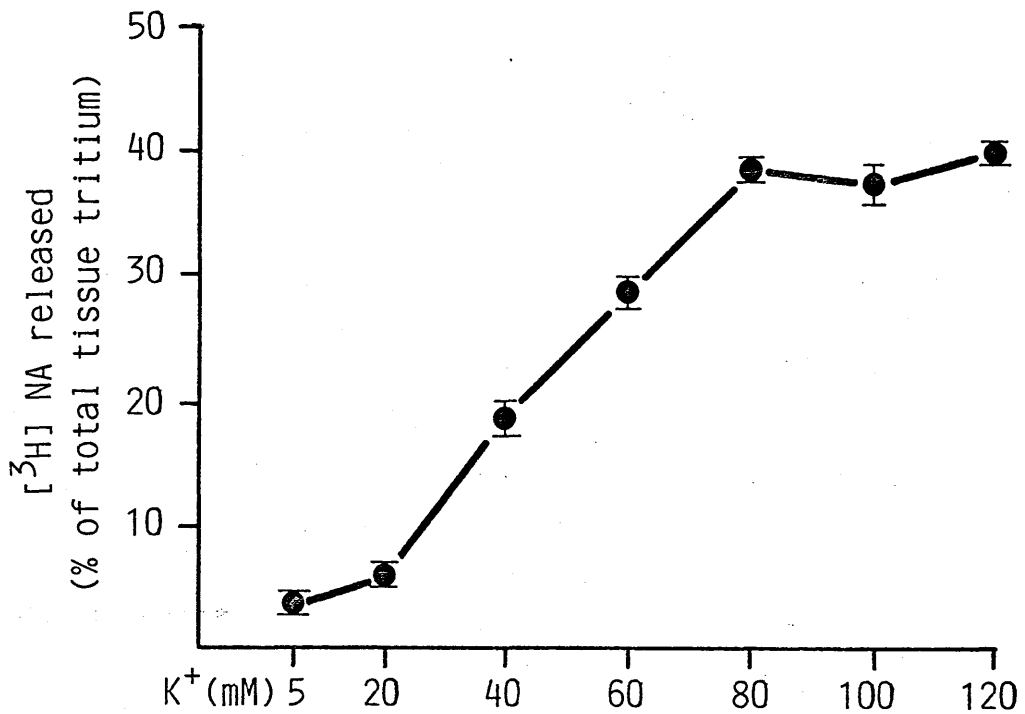


Figure 15 Effect of K^+ on the release of [^{3}H]NA from rat hypothalamic tissue chops

Tissue chops were preloaded with [^{3}H]NA. Release of [^{3}H]NA was evoked by incubation of tissue chops under the conditions described in Fig. 14. Results are means ± S.E.M. of 4 experiments, each of which was performed in triplicate.

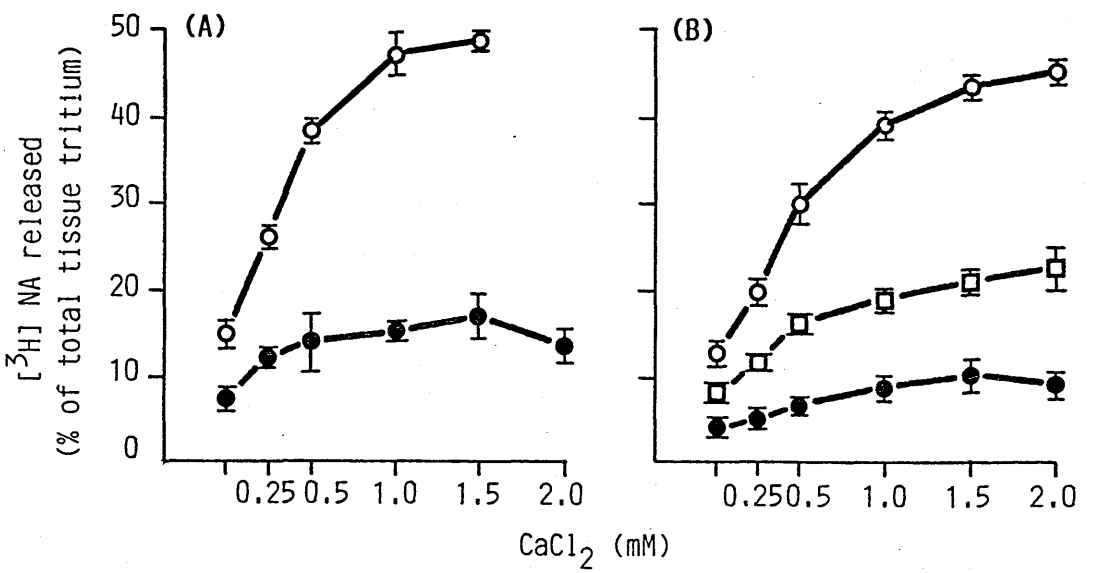


Figure 16 Effect of Ca^{2+}-dependent K^{+}-evoked release of [^{3}H]NA from O.C. tissue chops

Tissue chops were preloaded with [^{3}H]NA under two conditions (A) non-oxygenated or (B) oxygenated HEPES buffer. Tissue chops were then washed with HEPES buffer without Ca^{2+} containing 0.1mM EGTA according to the conditions in (A) and (B) respectively. 5mM (●), 20mM (□) and 40mM (○) K^{+}-evoked release of [^{3}H]NA in the presence of different Ca^{2+} concentrations described above were performed under conditions described in Fig.14. Results are means ± S.E.M. of 4 experiments.

a 15 min incubation period,while it inhibited 30mM K^+-evoked release of [^{3}H]NA at all time points (Fig. 17). The above observation suggests that clonidine inhibition of K^+-evoked release of [^{3}H]NA was an instantaneous effect, and remained effective over the time range.

3.1.5 Effect of pretreatment of tissue chops with clonidine on K^+-evoked release of [^{3}H]NA from O.C. tissue chops

Clonidine (10^{-6} M) added to the incubation tube at the same time as 30mM K^+ inhibited release of [^{3}H]NA from O.C. tissue chops by 40.% (Fig. 18A). Pretreatment of tissue chops with clonidine for 10 min (Fig. 18B), 20 min (Fig. 18C) or 30 min (Fig. 18D) at 37^{o}C before evoking [^{3}H]NA release with 30mM K^+ for 6 min at 37^{o}C did not alter the extent to which clonidine inhibited [^{3}H]NA release. These results show that it is not necessary to pretreat the tissue chops with clonidine to obtain maximum inhibition of [^{3}H]NA release.

3.1.6 Effect of clonidine on 40mM, 30mM and 20mM K^+-evoked release of [^{3}H]NA from O.C. tissue chops

The effect of clonidine on K^+-evoked release of [^{3}H]NA was investigated either in non-oxygenated (Fig. 19A) or oxygenated (Fig. 19B) HEPES buffer medium. K^+-evoked release of [^{3}H]NA was expressed as the difference between percentage release in the presence of 40mM, 30mM or 20mM KCl and 5mM KCl. When the experiment was performed in non-oxygenated HEPES buffer medium, clonidine (10^{-6} M) did not inhibit 40mM K^+-stimulated release of [^{3}H]NA, while only 20.6% and 33.1% inhibition were observed at 30mM and 20mM K^+ respectively (Fig. 19A). However, when the experiment was performed in oxygenated HEPES buffer medium, clonidine (10^{-6} M) inhibited 40mM, 30mM and 20mM K^+-evoked release of [^{3}H]NA by 17.1%, 37.3% and 52.5% respectively (Fig. 19B).

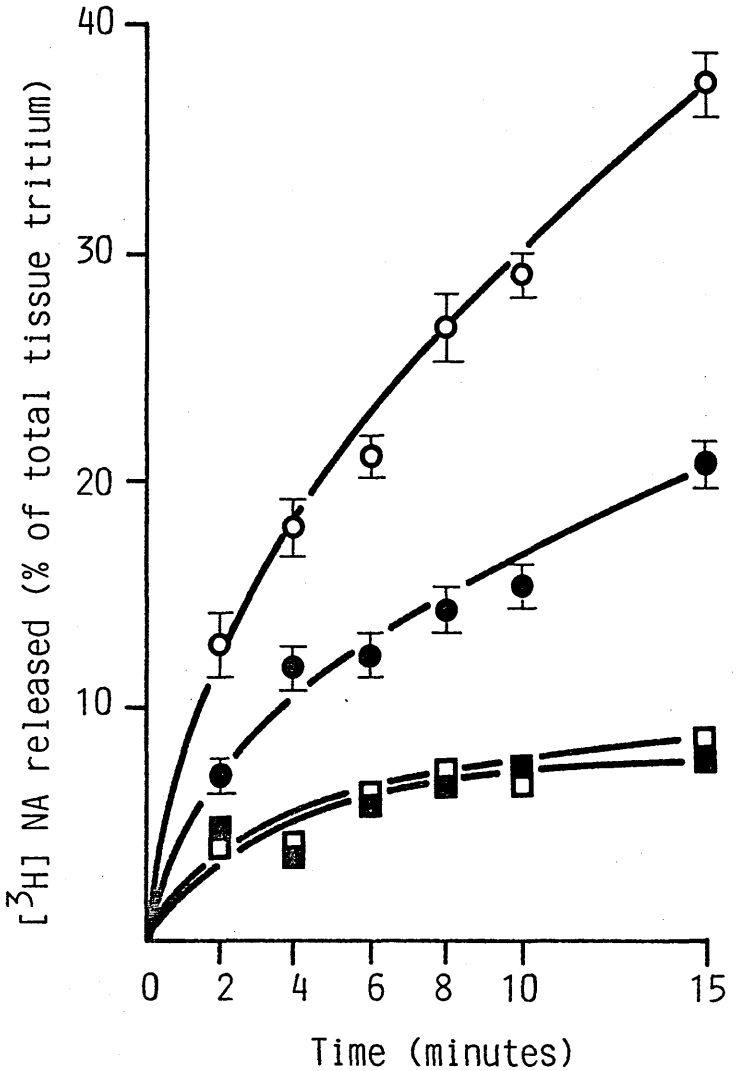


Figure 17 Effect of clonidine on [^{3}H]NA released from O.C.tissue chops : Time course study

Aliquots of tissue chops preloaded with [^{3}H]NA were incubated in oxygenated (95% O_2 - 5% CO_2) HEPES buffer for up to 15 min either in the presence (■, ●) or absence (□, ○) of 1 1µM clonidine with either 5mM K^+ (■, □) or 30mM K^+ (●, ○). Results are means ± S.E.M. of 3 experiments.

The above observations show that oxygenation of HEPES buffer medium is prerequisite to a better clonidine inhibition, and that lower K^+-depolarization (20mM K^+) gave a greater extent of clonidine inhibition. Therefore, oxygenated HEPES buffer was used in all the experiments on α_2-adrenergic regulation of [^{3}H]NA release.

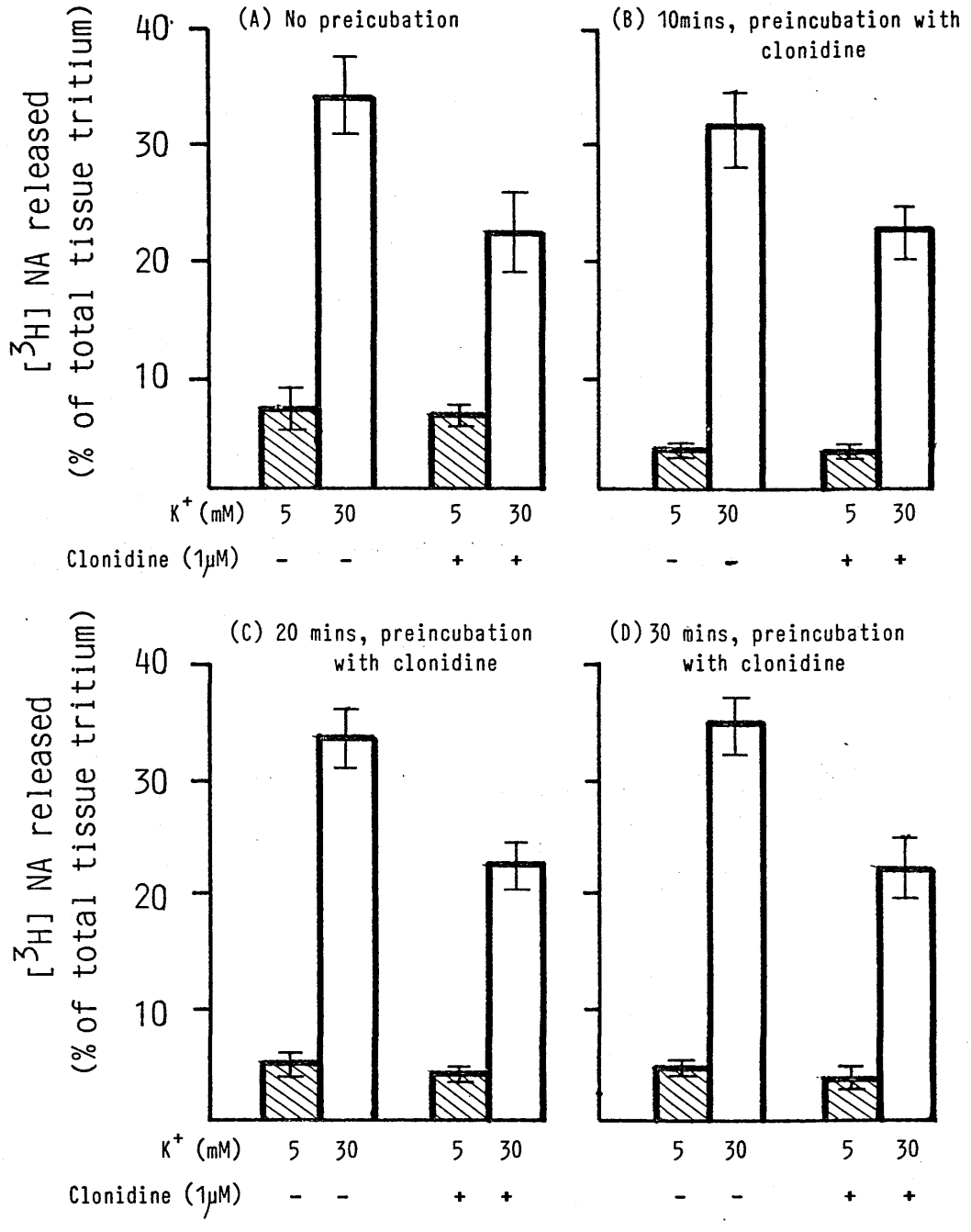


Figure 18 Effect of clonidine on K^+ stimulated [^{3}H]NA release from O.C. tissue chops under different preincubation conditions

Tissue chops were preloaded with [^{3}H]NA. The experiments were carried out under four conditions: (A) No preincubation, (B) 10 min, (C) 20 min and (D) 30 min preincubation of tissue chops with 1μM clonidine. 5mM (▨) or 30mM (□) K^+-evoked release of [^{3}H]NA either in the presence or absence of 1μM clonidine for 6 min . Results are means ± S.E.M. of 3 experiments.

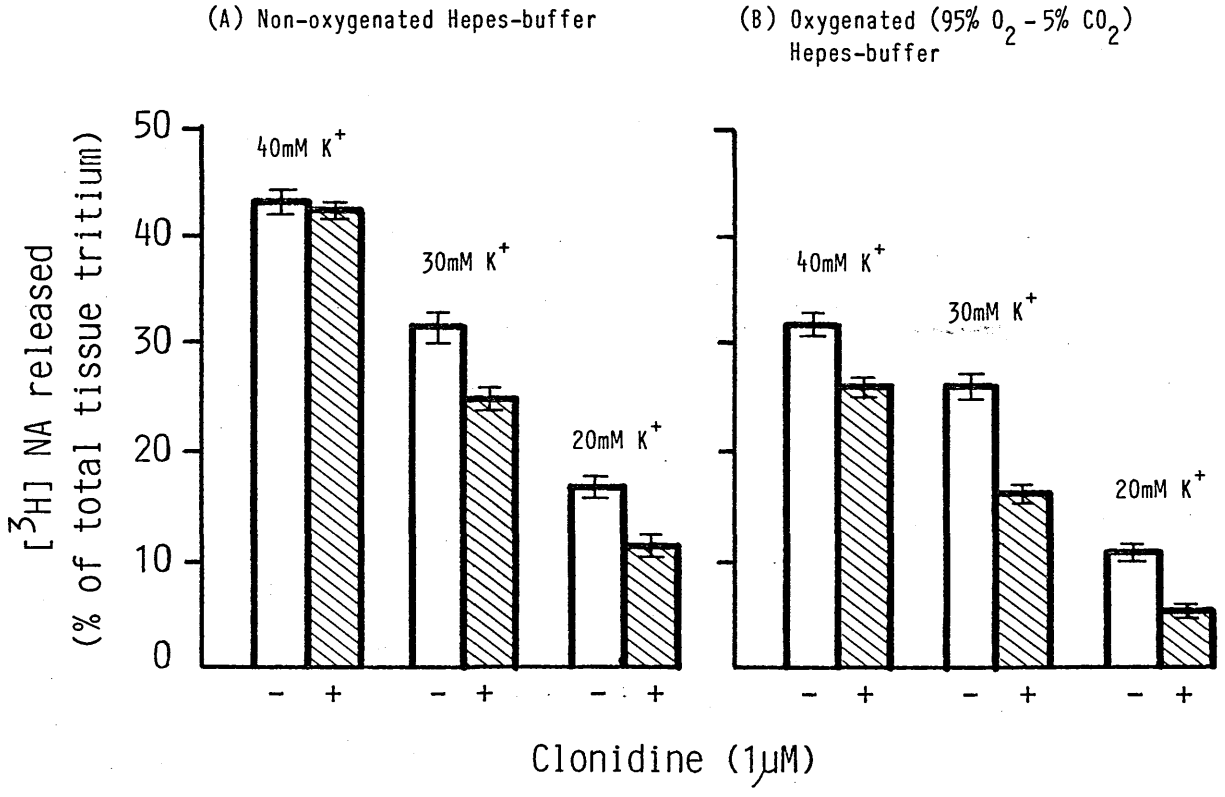


Figure 19 Inhibitory effect of clonidine on 40mM, 30mM and 20mM K^+-evoked release of [^{3}H]NA from 0.C tissue chops

Aliquots of tissue chops preloaded with [^{3}H]NA in either (A) non-oxygenated, or (B) oxygenated (95%)$_2$-5%CO_2) HEPES buffer. K^+-evoked release of [^{3}H]NA either in the presence (▨) or absence (□) of 1μM clonidine described in (A) and (B) was examined by incubating the tissue chops for 6 min. Results are means ± S.E.M. of 5 experiments.

3.2 α_2 adrenergic regulation of [^{3}H]NA release

The results from the preliminary studies show that the α_2-adrenergic agonist, clonidine inhibits [^{3}H]NA release stimulated by K^+ from rat O.C. tissue chops. α_2-Adrenergic agonists have been reported to inhibit cAMP formation in a number of tissues, including human (Burns et al., 1982) and hamster adipocytes (Garcia-Sainz et al., 1980) mouse Neuroblastoma X glioma cells, NG 108 - 15 cells (Sabol and Nirenberg, 1979), and cultures of astrocytes derived from mouse (Van Calker et al., 1978) and rat brain (McCarthy et al., 1979), thus the major aim of this study was to test the hypothesis that the α_2-adrenergic agonist inhibition of NA release is mediated by cAMP. Two approaches were adopted in the study:

(1) If the α_2-adrenergic agonist inhibition of NA release is mediated by cAMP then it would be predicted that increasing intracellular cAMP levels would (a) stimulate NA release and (b) reverse the inhibitory effect of the α_2-adrenergic agonist. Therefore intracellular levels of cAMP were increased using the cAMP analogue db-cAMP, Forskolin, (which is known to alter intracellular cAMP levels) and with PDE inhibitors, that prevent the breakdown of cAMP.

(2) The effect of α_2-adrenergic agonists on cAMP formation in O.C. tissue chops was examined to see if there is a correlation between conditions under which α_2-adrenergic agonists inhibit both cAMP formation, and the release of NA.

In this thesis the effects of α_2-adrenergic agonists, clonidine, BHT-920 and UK143-4-18 were compared with the effects of NA, on both release of [^{3}H]NA, and cAMP formation in rat O.C. tissue chops.

3.2.1 α_2-adrenergic modulation of K^+-evoked release of [^{3}H]NA from O.C. and hypothalamic tissue chops

BHT-920, clonidine, UK14304-18 and NA inhibited the release of 20mM K^+-evoked release of [^{3}H]NA in a dose-dependent manner (Fig. 20). 20mM K^+ was used to stimulate [^{3}H]NA release in O.C. tissue chops since it was found (Fig. 19) that clonidine was most effective at this concentration. The IC_{50} values (concentration of drug which exhibits half the maximum inhibitory effect) on the release of [^{3}H]NA) were 1 x 10^{-7}M, 3.5 x 10^{-7}M, 5.5 x 10^{-7}M and 7.5 x 10^{-7}M for UK14304-18, clonidine, BHT-920 and NA respectively (Fig. 20). This suggests a potency order of UK14304-18 > clonidine > BHT-920 > NA. Maximum inhibition of the release of [^{3}H]NA was achieved by a 10^{-5}M concentration of each α_2-agonist. NA was the most effective as it inhibited 20mM K^+-evoked release of [^{3}H]NA by about 84%, compared with 70%, 53% and 37% inhibition achieved by UK14304-18, clonidine and BHT-920 respectively (Fig. 20). On the contrary, yohimbine (10^{-5}M), an α_2-adrenergic antagonist, enhanced 20mM K^+-stimulated release of [^{3}H]NA by 25% (Fig. 20).

Clonidine and NA also inhibited the 30mM K^+ stimulated release of [^{3}H]NA from hypothalamic tissue chops (Fig. 21). Unlike O.C. tissue chops, maximum inhibition of [^{3}H]NA release was not reached by 10^{-4}M concentration for both NA and clonidine. Clonidine and NA had the same inhibitory effect on 30mM K^+-evoked release of [^{3}H]NA at submicromolar (10^{-6}M - 10^{-7}M) concentrations. However, NA (10^{-4}M) was more effective than clonidine (10^{-4}M) as it inhibited K^+-stimulation of [^{3}H]NA release by 60% compared with an inhibition of 40% observed with clonidine (Fig. 21). On the contrary, yohimbine (10^{-5}M) only slightly enhanced [^{3}H]NA release (Fig. 21).

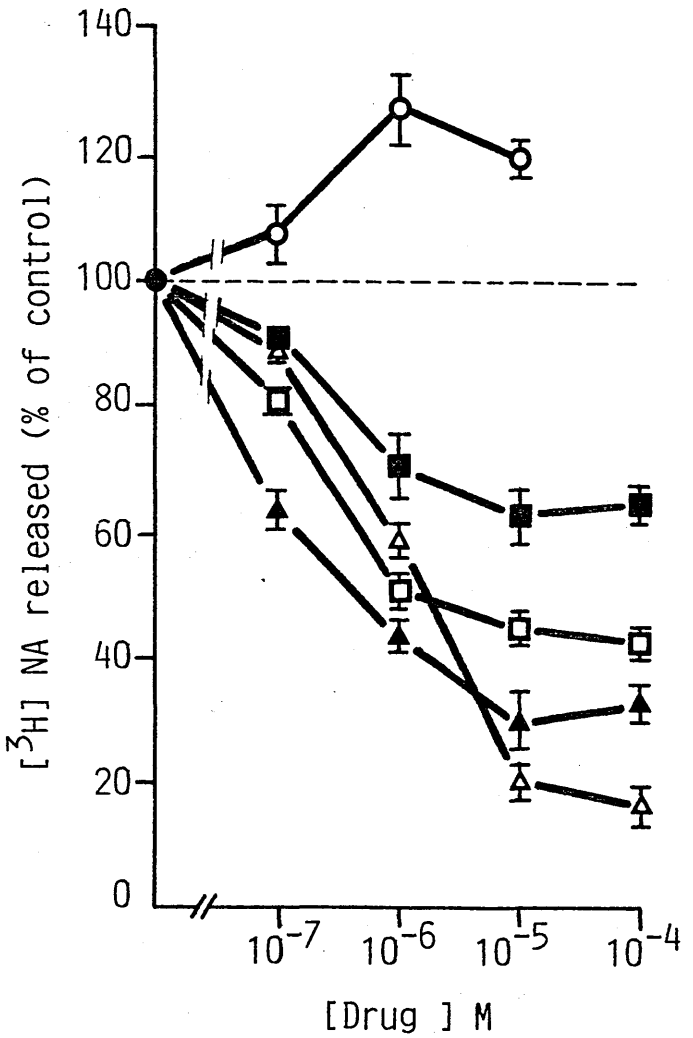


Figure 20 Dose response curves for α_2-adrenergic agonists and antagonist (yohimbine) on the release of [^{3}H]NA from O.C. tissue chops

Aliquots of tissue chops preloaded with [^{3}H]NA were stimulated with 20mM K^+ in the presence of α_2-agonist, BHT-920 (■), clonidine (□), UK14304-18 (▲) and NA (△) or α_2-antagonist, yohimbine (○) for 6 min. Control release was 9.6 ± 0.5% of total tissue tritium. Results are means ± S.E.M. of 4 experiments. Each was performed in triplicate.

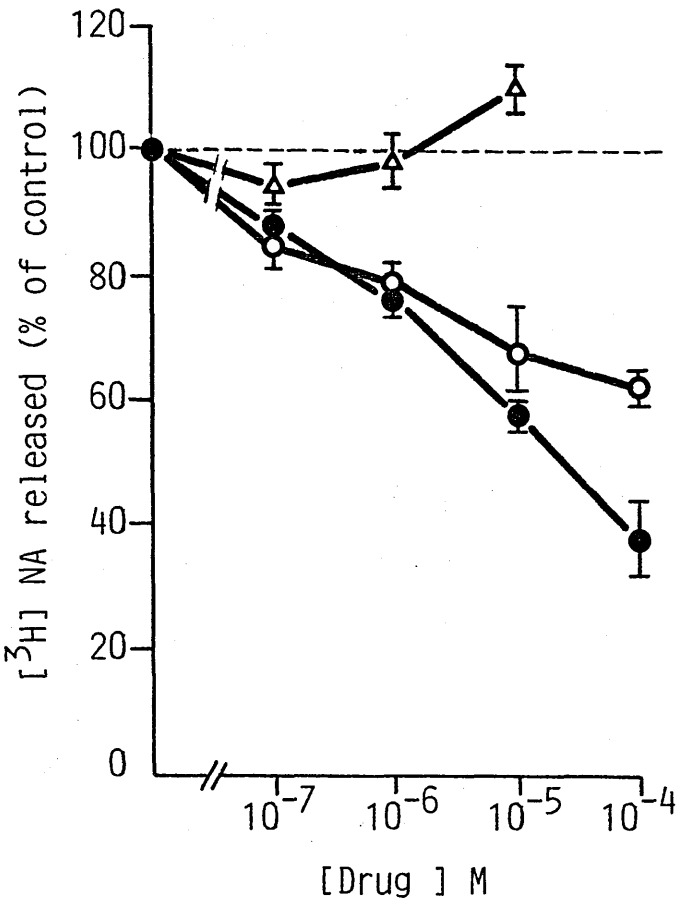


Figure 21 Dose response curves for α_2-adrenergic agonists and antagonist on the release of [^{3}H]NA from hypothalamic tissue chops

Aliquots of tissue chops preloaded with [^{3}H]NA were stimulated with 30mM K^+ in the presence of α_2-adrenergic agonist, clonidine (O) or NA (●) or α_2-adrenergic antagonist, yohimbine (Δ) for 6 min. Control release was 16.8 ± 0.45% of total tissue tritium. Results are means ± S.E.M. for 4 experiments.

The above observations suggest that O.C. tissue chops is a better system to use in the study of α_2-adrenoceptors regulation of [^{3}H]NA release since more potent inhibitions by α_2-adrenergic agonists are observed.

3.2.2 The effect of yohimbine on the inhibition of [^{3}H]NA release from O.C. and hypothalamic tissue chops by α_2-adrenergic agonists

The inhibiton of 20mM K^+-evoked release of [^{3}H]NA from O.C. tissue chops by α_2-adrenergic agonists (10^{-6}M), BHT-920, NA, clonidine and UK14304-18 were reversed by the α_2-adrenergic antagonist, yohimbine, in a dose-dependent manner (Fig. 22). Complete reversal of inhibition was achieved by yohimbine (10^{-5}M). In contrast, neither the β-adrenergic antagonist propranolol (Fig. 23) nor α_1-adrenergic antagonist prazosin (Fig. 24) reversed the inhibitory effect of NA on K^+ stimulation of [^{3}H]NA release from O.C. tissue chops. This suggests that the inhibitory effect of NA on K^+-stimulation of [^{3}H]NA release is mediated by α_2-adrenergic receptors. Interestingly, in the presence of propranolol at 10^{-6}M or 10^{-5}M concentration, the inhibitory effect of NA (10^{-6}M) on K^+-stimulation of [^{3}H]NA released was increased (Fig. 23). The inhibition of K^+-stimulation of [^{3}H]NA released by clonidine (10^{-5}M) in hypothalamic tissue chops was also reversed by yohimbine (Fig. 25).

3.2.3 Effect of preincubation with forskolin on K^+-evoked release of [^{3}H]NA from O.C. tissue chops

Preincubation of tissue chops with 10^{-5}M forskolin for 30 min enhances K^+-evoked release of [^{3}H]NA by 26.7% (Table 3). On the

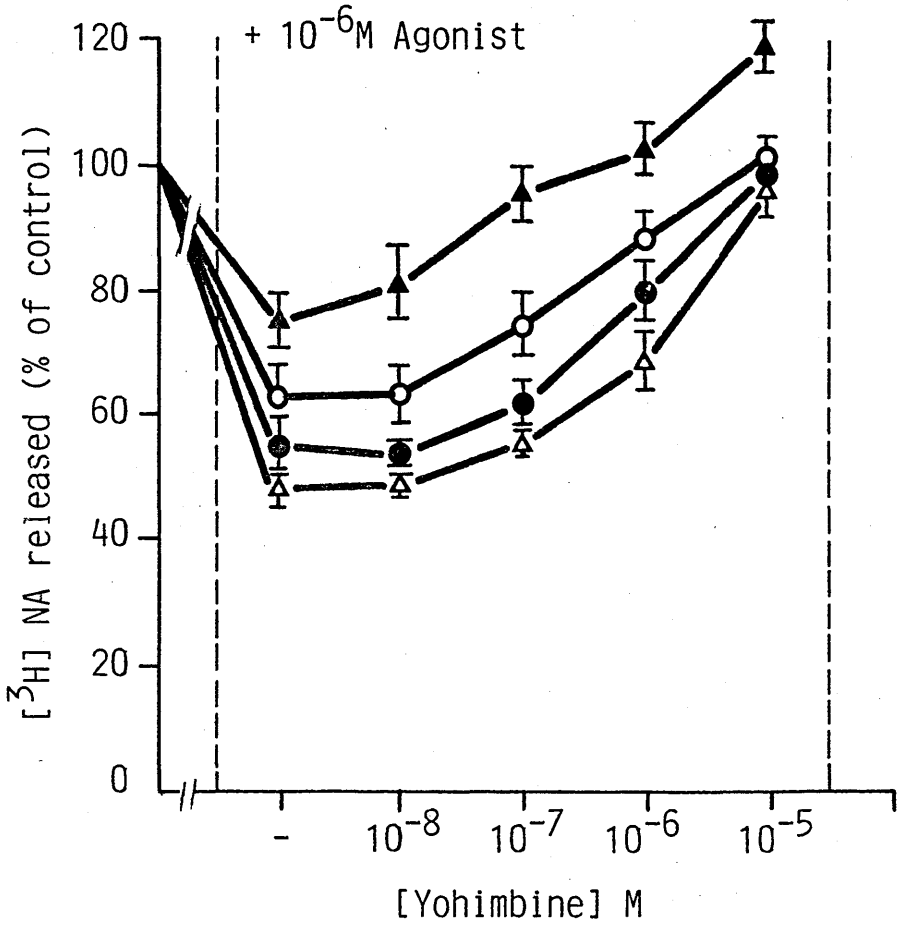


Figure 22 Reversal effect of α_2-adrenergic antagonist (yohimbine) on α_2-adrenergic agonist inhibitory response on K^+-evoked release of [^{3}H]NA from 0.C tissue chops

Aliquots of tissue chops preloaded with [^{3}H]NA were stimulated with 20mM K^+ in the presence of 10^{-6} α_2-adrenergic agonist, NA (O), clonidine (●), UK14304-18 (△) or BHT-920 (▲) and increasing concentration of yohimbine for 6 min. Control release was 9.8 ± 0.3% of total tissue tritium. Results are means ± S.E.M. of 4 experiments, each of which was performed in triplicate.

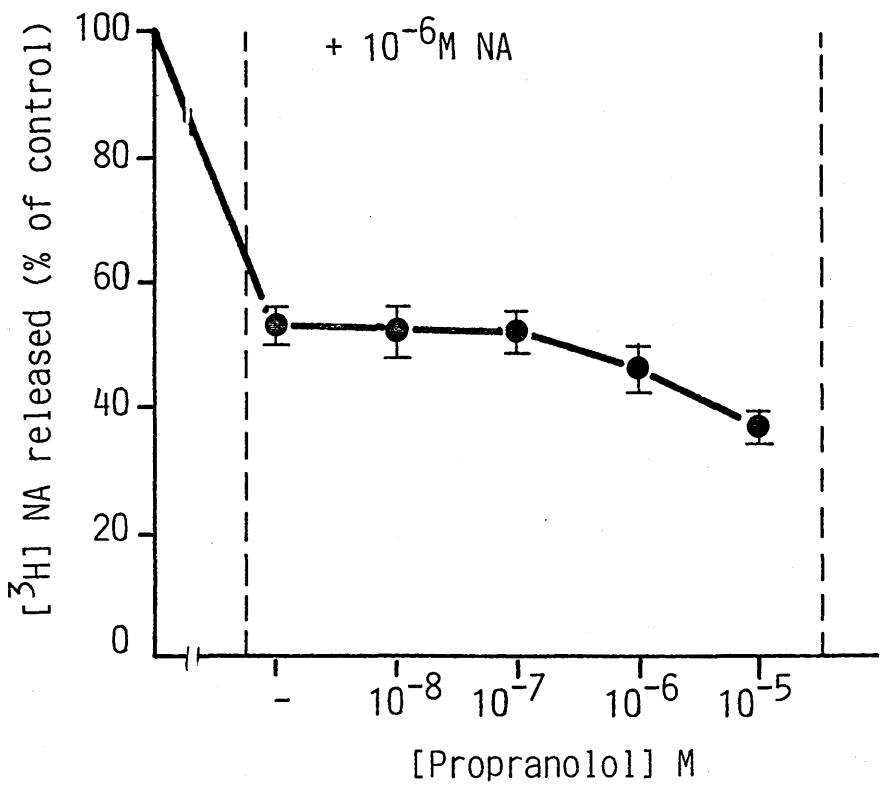


Figure 23 Effect of β-adrenergic antagonist (propranolol) on the inhibitory response of NA on K^+-evoked release from O.C. tissue chops

Aliquots of tissue chops preloaded with [^{3}H]NA were stimulated with 20mM K^+ in the presence of 10^{-6}M NA with increasing concentrations of propranolol for 6 min. Control release was 10.2 ± 0.5% of total tissue tritium. Results are means ± S.E.M. of 4 experiments.

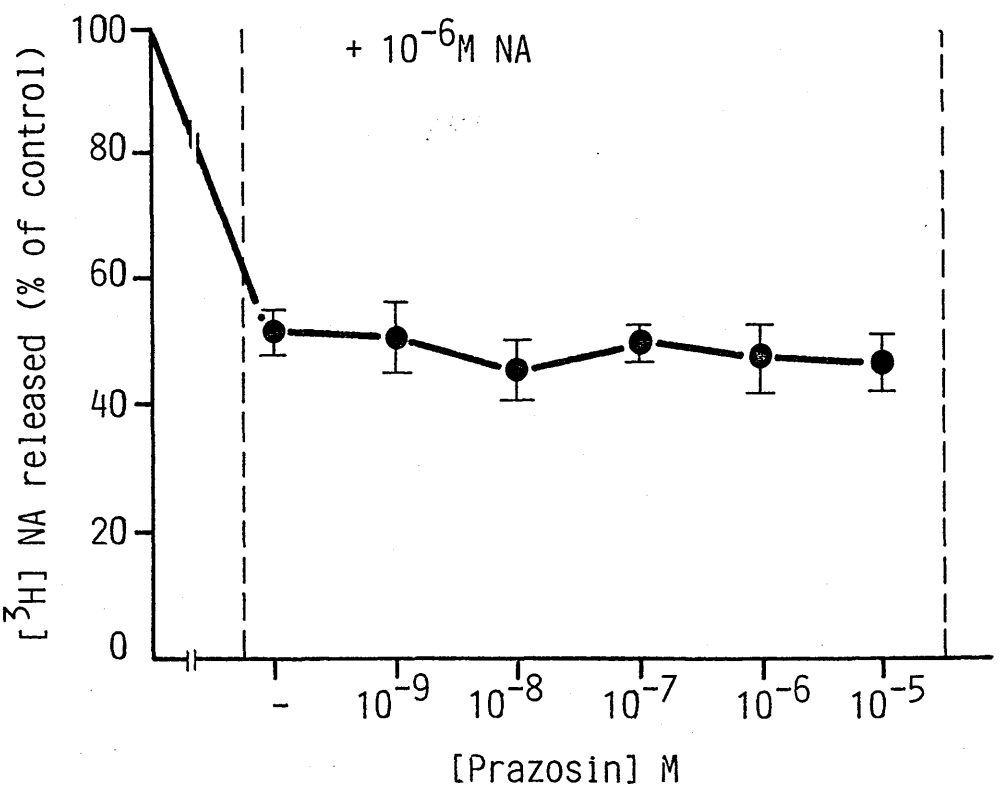


Figure 24 Effect of α_1-adrenergic antagonist (prazosin) on the inhibitory response of NA on K^+-evoked release of [^{3}H]NA from O.C. tissue chops

Aliquots of tissue chops preloaded with [^{3}H]NA were stimulated with 20mM K^+ in the presence of 10^{-6} M NA and increasing concentration of prazosin for 6 min. Control release was 9.3 ± 60% of total tissue tritium. Results are means ± S.E.M. of 3 experiments.

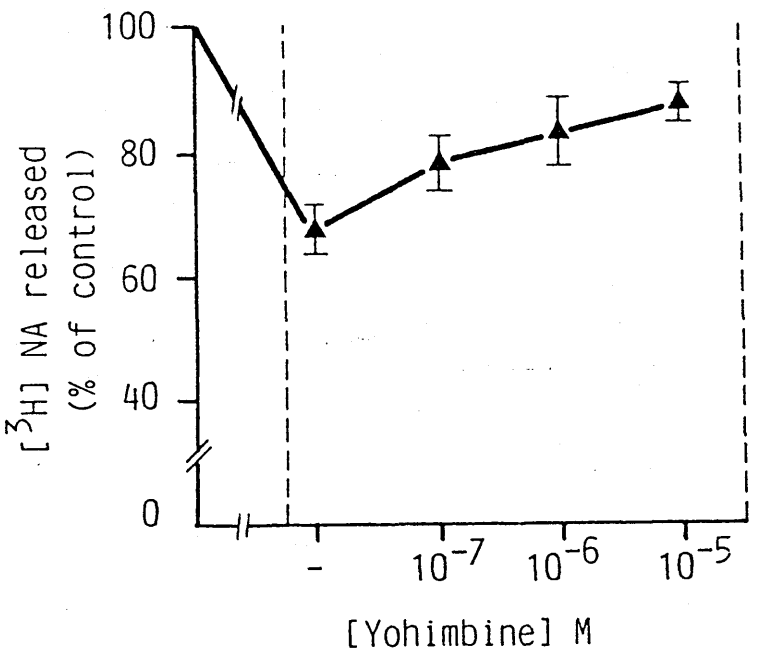


Figure 25 Effect of yohimbine on clonidine inhibition of K^+-evoked release of [^{3}H]NA from hypothalamic tissue chops

Tissue chops were preloaded with [^{3}H]NA. Release of [^{3}H]NA from hypothalamic tissue chops was stimulated by 30mM K^+ under condition described in Fig. 22. Control release was 15.2 $\pm$ 0.38% of total tissue tritium. Results are means $\pm$ S.E.M. of 3 experiments.

contrary, only 18.0% enhancement of stimulated release was observed if the tissue chops were incubated with forskolin for 15 min, while 5.6% was observed if forskolin was added without preincubation (Table 3). This suggests that increases in intracellular cAMP stimulate release since forskolin has been shown to stimulate adenylate cyclase by acting on the catalytic unit (Seamon and Daly, 1980; Seamon et al., 1981; and Daly et al., 1982).

3.2.4 Effect of db-cAMP, forskolin and PDE-inhibitors (IBMX and RO20-1724) on α_2-adrenergic agonist modulation of K^+-evoked release of [^{3}H]NA from O.C. tissue chops

Stimulation of [^{3}H]NA release by 20mM K^+ was also increased by 28.2% (Table 4) even when db-cAMP (10^{-3}M) and IBMX (10^{-4}M) were added without preincubation. These concentrations of db-cAMP and IBMX did not affect clonidine inhibition of K^+ stimulation of [^{3}H]NA release under these conditions. Preincubation of tissue chops with IBMX (10^{-4}M), db-cAMP (10^{-3}M) and forskolin (10^{-5}M) for 30 minutes enhanced K^+-evoked release of [^{3}H]NA by 19.0%, 29.0% and 20.8% respectively (Table 5). In addition db-cAMP was capable of partially reversing clonidine (10^{-6}M) inhibition of K^+-evoked release of [^{3}H]NA (Table 5).

In the presence of IBMX (10^{-4}M) K^+ stimulation of [^{3}H]NA release by forskolin and db-cAMP was enhanced. Under these conditions only db-cAMP was able to partially reverse the inhibiton of [^{3}H]NA release by the α_2-adrenergic agonists clonidine, BHT-920, UK14304-18 and NA (Table 6). No reversal of [^{3}H]NA release could be observed when tissue chops were preincubated for 30 min with IBMX (10^{-4}M) and forskolin (10^{-5}M) (Table 6). On the contrary, db-cAMP and forskolin in the

Table 3 Effect of forskolin on [^{3}H]NA release at various preincubation time from the O.C. tissue chops

Condition	%[^{3}H]NA released ± S.E.M.	% Enhancement of [^{3}H]NA released
(a) No preincubation		
Control	7.2 ± 0.61	
Forskolin	7.6 ± 0.81	5.6
(b) 15 min preincubation		
Control	8.8 ± 0.09, b'	
Forskolin	10.4 ± 0.79, b	18.2
(c) 30 min precinubation		
Control	8.6 ± 0.22, c'	
Forskolin	10.9 ± 0.25, c	26.7

Tissue chops preloaded with [^{3}H]NA were treated with 10^{-5} M forskolin under the following conditions: (a) without preincubation; (b) 15 min precubation and (c) 30 min preincubation, before stimulating release with 20mM K^+ for 6 min. Enhancement of release is expressed to their relative control. results are means ± S.E.M. of 6 experiments.

b Significantly different from control b' ($p < 0.05$)
c Significantly different from control c' ($p < 0.01$)

Table 4 Influence of IBMX, db-cAMP and clonidine (without pretreatment of tissue chops with IBMX and db-cAMP) on K^+ stimulation of [^{3}H]NA released from O.C. tissue chops

Preincubation condition	% of [^{3}H]NA released ± S.E.M.	% Inhibition of [^{3}H]NA released	% Enhancement of [^{3}H]NA released
None (control	7.8 ± 0.49		
Clonidine	4.9 ± 1.67	37.2	
db-cAMP + IBMX	10.0 ± 0.20, a		28.2
db-cAMP + IBMX + clonidine	5.7 ± 2.25	43.0	

10^{-4}M IBMX, 10^{-3}M db-cAMP and 10^{-6}M clonidine were added to aliquots of tissue chops preloaded with [^{3}H]NA and stimulated with 20mM K^+ for 6 min. Data is expressed as the difference between % release in the presence of 20mM K^+ and 5mM K^+. Results are means ± S.E.M. of 6 experiments.

[a] Significantly different from control ($p < 0.05$)

Table 5 Effect of forskolin, db-cAMP, IBMX and clonidine on the release of [^{3}H]NA from O.C. tissue chops

Preincubation condition	% of [^{3}H]NA released ± S.E.M. (n)	% Inhibition of [^{3}H]NA released	% Enhancement of [^{3}H]NA released
condition	10.1 ± 0.50 (10)		
clonidine	4.6 ± 0.30 (10)	54.5, b	
IBMX	12.0 ± 0.28 (8)		19.0
IBMX+clonidine	5.7 ± 0.49 (8)	52.5	
forskolin	12.2 ± 0.20 (5)		20.8
forskolin + clonidine	5.1 ± 0.15 (5)	58.2	
db-cAMP	13.0 ± 0.50 (5)		29.0
db-cAMP + clonidine	8.0 ± 0.20 (5)	38.5, a	

Tissue chops preloaded with [^{3}H]NA were preincubated with 10^{-4}M IBMX, 10^{-3}M db-cAMP or 10^{-5}M forskolin for 30 min. Release was stimulated by 20mM K^+ either in the presence or absence of clonidine for 6 min, and is expressed as the mean ± S.E.M. of (n) experiments, of the difference between percentage release in the presence of 20mM K^+ and 5mM K^+. Enhancement of release is expressed relative to control. Inhibition of release is expressed relative to the respective condition in the absence of clonidine.

a Significantly different from b ($p < 0.01$)

Table 6 Effect of forskolin or db-cAMP in the presence of IBMX on α_2-adrenergic agonist regulation of [^{3}H]NA release from O.C. tissue chops

Preincubation condition	% of [^{3}H]NA released ± S.E.M. (n)	% Inhibition of [^{3}H]NA released	% Enhancement of [^{3}H]NA released
control	10.1 ± 0.50 (10)		
clonidine	4.5 ± 0.24 (10)	55.4, a*	
BHT-920	7.1 ± 0.46 (6)	29.7, b*	
UK 14304-18	4.1 ± 0.34 (6)	59.4, c*	
NA	5.7 ± 0.49 (6)	43.6, d*	
IBMX + forskolin	13.2 ± 0.39 (4)		30.7
IBMX + forskolin + clonidine	6.2 ± 0.50 (4)	53.0	
IBMX + forskolin + BHT-920	10.0 ± 0.33 (4)	24.2	
IBMX + forskolin + UK 14304	6.0 ± 0.36	54.5	
IBMX + forskolin + NA	8.1 ± 0.32 (4)	39.0	
IBMX + db-cAMP	14.6 ± 0.6 (6)		40.0
IBMX + db-cAMP + clonidine	9.4 ± 0.5 (6)	33.3, a	
IBMX + db-cAMP + BHT-920	11.7 ± 0.21 (4)	17.0, b	
IBMX + db-cAMP + UK 14304-18	8.8 + 0.30 (4)	37.6, c	
IBMX + db-cAMP + NA	9.7 ± 0.20 (4)	31.2, d	

Tissue chops preloaded with [^{3}H]NA were preincubated alone or with 10^{-3}M db-cAMP or 10^{-5}M forskolin in the presence of 10^{-4}M IBMX for 30 min. Release was stimulated by 20mM K^+ either in the presence or absence 10^{-6}M α_2-adrenergic agonist for 6 min. Results are means ± S.E.M. of (n) experiments. Enhancement of release is expressed relative to control. Inhibition of release is expressed relative to the respective condition in the absence of α_2-adrenergic agonist.

a, b, c and d significantly different from a*, b*, c* and d* respectively ($p < 0.01$)

presence of RO 20-1724 neither enhanced K^+-evoked release of [^{3}H]NA nor partially reverse the inhibition caused by UK 14304-18 (Table 7).

3.2.5 Influence of db-cAMP and forskolin in the presence of IBMX on clonidine inhibitory response of K^+-evoked release of [^{3}H]NA from hypothalamic tissue chops

Preincubation of hypothalamic tissue chops with either db-cAMP (10^{-3}M) and IBMX (10^{-4}M) or forskolin (10^{-5}M) and IBMX (10^{-4}M) gave rise to a slight enhancement of about 7.0% each on 30mM K^+-evoked release of [^{3}H]NA (Table 8A). Similarly, a 25% enhancement of [^{3}H]NA release was observed when the release of [^{3}H]NA was stimulated with 25mM K^+ following the preincubation of hypothalamic tissue chops with db-cAMP or forskolin in the presence of IBMX (10^{-4}M). Clonidine (10^{-5}M) inhibited 25mM K^+-evoked release of [^{3}H]NA by 37.3% (Table 8B) and 30mM K^+-evoked release of [^{3}H]NA by 30.0% (Table 8A). Preincubation of hypothalamic tissue chops for 30 min with either db-cAMP or forskolin in the presence of IBMX did not reverse clonidine inhibiton of K^+-evoked release of [^{3}H]NA release (Table 8A, B). The failure of db-cAMP in the presence of IBMX to partially reverse clonidine inhibition of NA release represents a difference between hypothalmus and occipital cortex tissue chops.

3.2.6 Effects of adenosine and cyclohexyl-adenosine (CHA) on the release of [^{3}H]NA from O.C. tissue chops

Adenosine inhibited 20mM K^+-evoked release of [^{3}H]NA in a dose-dependent manner (Fig. 26), with an IC_{50} value of 6×10^{-9}M. Maximum inhibition of 20-30% of K^+-stimulated release of [^{3}H]NA was

Table 7 Effect of forskolin or db-cAMP in the presence of RO 20-1724 on UK 14304-18 regulation of [^{3}H]NA release from O.C. tissue chops

Preincubation condition	% of [^{3}H]NA released ± S.E.M. (n)	% Inhibition of [^{3}H]NA released
control	10.1 ± 0.5 (10)	
UK 14304-18	4.1 ± 0.34 (6)	59.4
RO 20-1724	8.2 ± 0.60 (5)	18.8
RO 20-1724 + UK 14304-18	3.6 ± 0.46 (5)	56.1
RO 20-1724 + db-cAMP	9.6 ± 0.90 (5)	
RO 20-1724 + db-cAMP + UK 14304-18	4.5 ± 0.42 (5)	53.1
RO 20-1724 + forskolin	10.8 ± 0.33 (5)	
RO 20-1724 + forskolin + UK 14304-18	4.8 ± 0.48 (5)	55.6

Tissue chops with [^{3}H]NA were preincubated alone or with 10^{-3} M db-cAMP or 10^{-5} M forskolin in the presence of 10^{-4} M RO 20-1724 for 30 min. Release was stimulated by 20mM K^+ either in the presence or absence 10^{-6} M UK 14304-18 for 6 min. Results are means ± S.E.M. of (n) experiments. Inhibition of release is expressed relative to the respective conditions in the absence of UK 14304-18.

Table 8A, B Effect of db-cAMP, forskolin and clonidine on K^+-evoked release of [^{3}H]NA from hypothalamic tissue chops

Table A 30mM K^+ stimulation of [^{3}H]NA released

Preincubation condition	% of [^{3}H]NA released ± S.E.M.	% Inhibition of [^{3}H]NA released	% Enhancement of [^{3}H]NA released
None (control)	17.7 ± 0.21		
clonidine	12.4 ± 0.68	30.0	
IBMX + db-cAMP	19.0 ± 0.36, a		7.3
IBMX + forskolin	18.9 ± 0.89, a		6.8
IBMX + db-cAMP + clonidine	13.4 ± 1.04	29.5	
IBMX + forskolin + clonidine	12.5 ± 0.56	33.9	

[a] Significantly different from control ($p < 0.05$)

Table B 25mM K^+ stimulation of [^{3}H]NA released

Preincubation condition	% of [^{3}H]NA released ± S.E.M.	% Inhibition of [^{3}H]NA released	% Enhancement of [^{3}H]NA released
None (control	11.0 ± 0.47		
clonidine	6.9 ± 0.57	37.3	
IBMX + db-cAMP	13.8 ± 0.57, b		25.5
IBMX + forskolin	13.6 ± 0.61, b		23.6
IBMX + db-cAMP + clonidine	8.7 ± 0.18	37.0	
IBMX + forskolin + clonidine	7.7 ± 0.19	43.4	

[b] Signifcantly different from control ($p < 0.05$)

Aliqots of tissue chops previously preloaded with [^{3}H]NA were preincubated either alone or with 10^{-3} M db-cAMP or 10^{-5} M forskolin in the presence of 10^{-4} IBMX for 30 min at 37°C. Release was affected with either 30mM K^+ (Table A) or 25mM K^+ (Table B) in the absence or presence of 10^{-5} M clonidine for 6 min. Results are means ± of 6 experiments.

achieved by 10^{-5}M adenosine. CHA which is more potent as an A_1 than A_2 agonist, also inhibited the K^+-stimulation of [^{3}H]NA release in a dose-dependent manner (Fig. 26), with an IC_{50} value of 8 x 10^{-10}M. Maximum inhibition of 40% of K^+-stimulated release of [^{3}H]NA was achieved by 10^{-6}M CHA. This suggests that CHA is more potent and effective than adenosine in inhibiting [^{3}H]NA release. Slight reversal of inhibition was observed with higher concentrations (10^{-5}-10^{-4}M) of CHA.

3.2.7 Effects of PDE-inhibitors, IBMX and RO20-1724 on the adenosine inhibitory response of [^{3}H]NA release from O.C. tissue chops

IBMX (10^{-6}M - 10^{-4}M) did not affect the 20mM K^+-stimulated release of [^{3}H]NA (Fig. 27B). However, this concentration range of IBMX reversed the inhibition of 20mM K^+-stimulated release of [^{3}H]NA caused by 10^{-6}M adenosine (Fig. 27A) IBMX (10^{-4}M) in the presence of adenosine (10^{-6}M) enhanced the K^+-stimulated release of [^{3}H]NA by about 15% (Fig. 27A). This observation is in agreement with reports that IBMX acts as an adenosine antagonist as well as a PDE inhibitor. This suggestion is supported by the observation that RO 20-1724, a PDE-inhibitor which does not act as an adenosine receptor antagonist did not reverse the inhibitory effect of 10^{-6}M adenosine on K^+-stimulation of [^{3}H]NA release (Fig. 28A).

3.2.8 Effect of adenosine A_1 antagonist, CPDPX on adenosine inhibitory response of K^+-evoked release of [^{3}H]NA from O.C. tissue chops

8-Cyclopentyl-1,3-dipropylxanthine, CPDPX (10^{-7}M - 10^{-5}M), which is approximately 150 times more selective as an A_1 than A_2

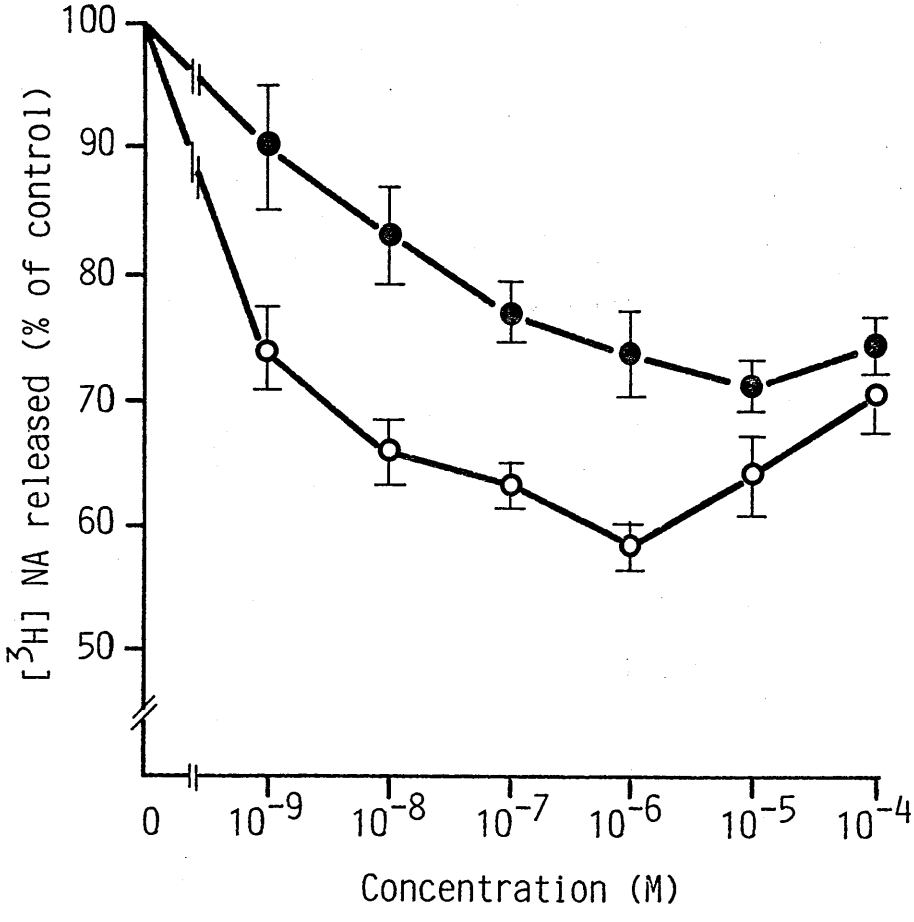


Figure 26 Dose response curves for adenosine and cyclohexyl-adenosine (CHA) on the release of [^{3}H]NA from O.C. tissue chops

Aliquots of tissue chops preloaded with [^{3}H]NA were stimulated with 20mM K^+ either in the presence of adenosine (●) or CHA (O) for 6 min. Control release was 9.2 ± 0.5% of total tissue tritium. Results are means ± S.E.M. of 4 experiments.

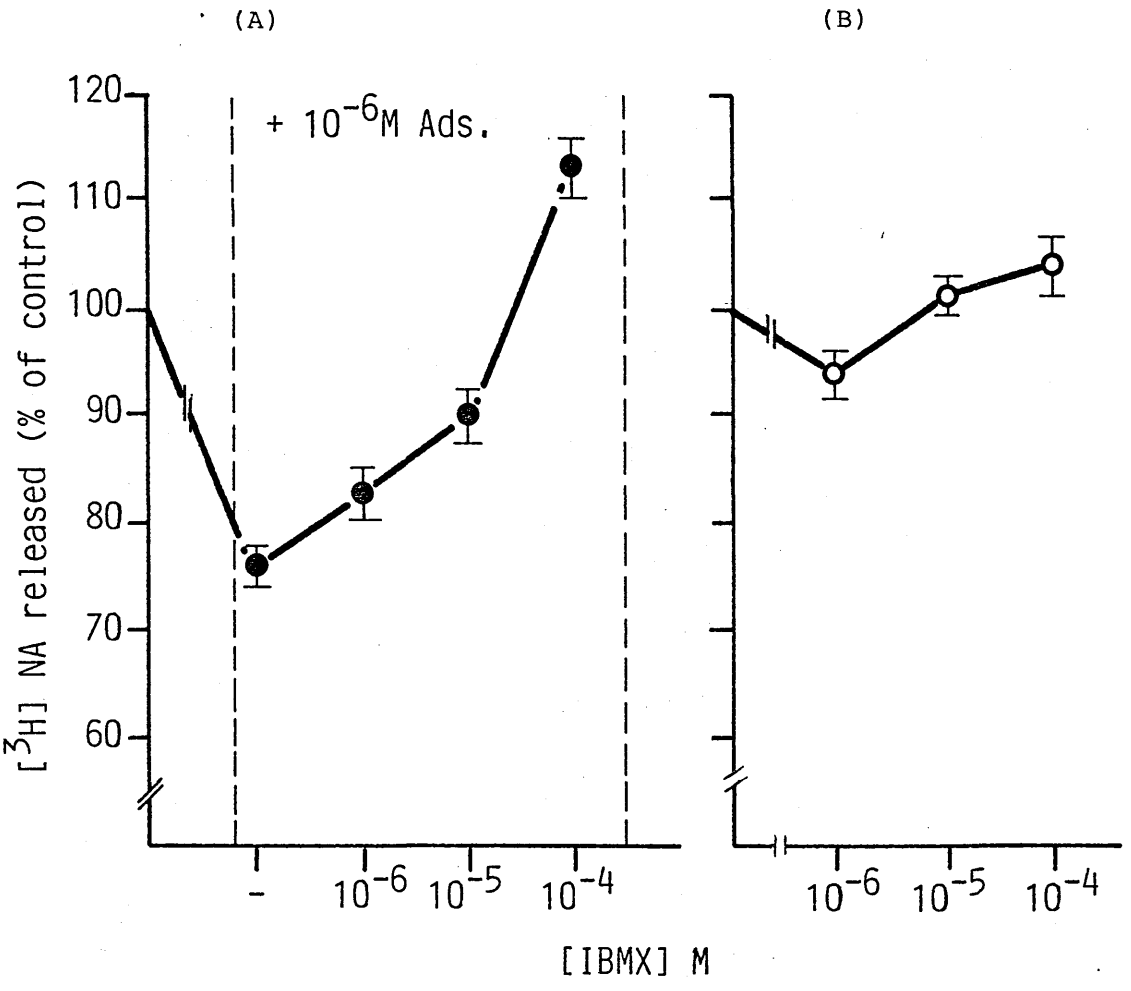


Figure 27 Effect of IBMX on the inhibitory response of adenosine on K^+-evoked release of [^{3}H]NA from O.C. tissue chops

Aliquots of tissue chops preloaded with [^{3}H]NA were stimulated with 20mM K^+ in the presence of 10^{-6}M adenosine with increasing concentration of IBMX (●) or IBMX alone (○) for 6 min Control release was 9.8 ± 0.28 % of total tissue tritium. Results are means ± S.E.M. of 3 experiments.

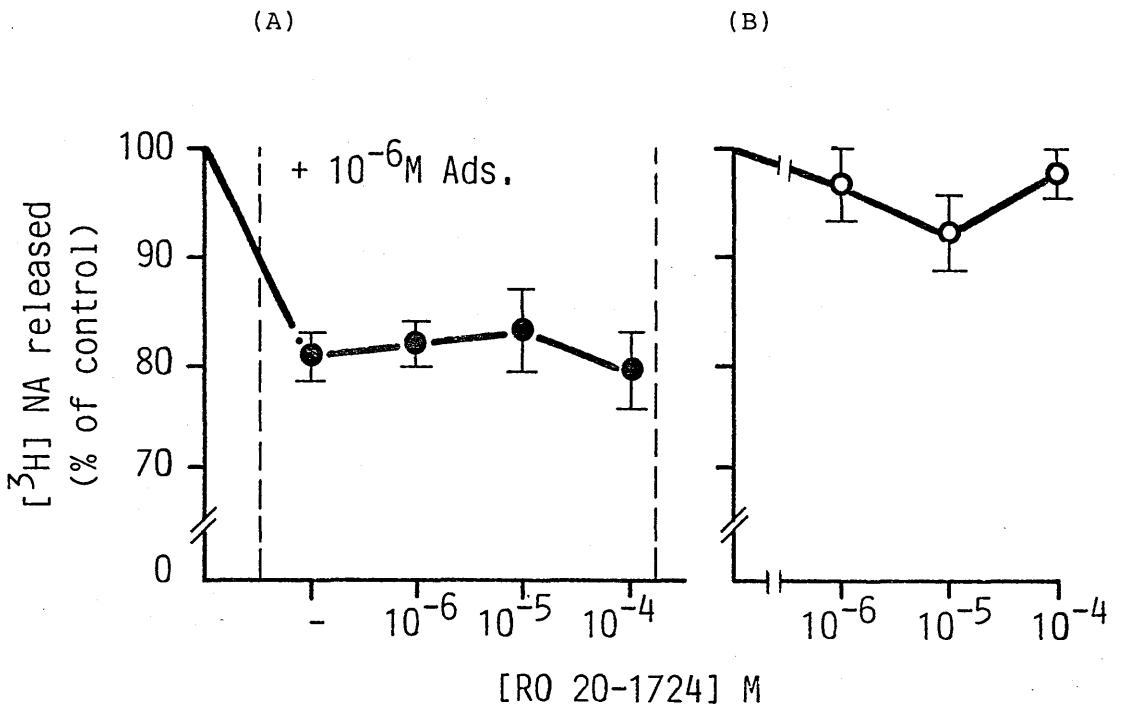


Figure 28 Effect of RO 20-1724 on the inhibitory response of adenosine on K^+-evoked release of [^{3}H]NA from O.C. tissue chops

Aliquots of tissue chops preloaded with [^{3}H]NA were stimulated with 20mM K^+ in the presence of 10^{-6} M adenosine with increasing concentration of RO 20-1724 (●) or RO 20-1724 alone (○) for 6 min. Control release was 10.5 ± 0.25% of total tissue tritium. Results are means ± S.E.M. of 3 experiments.

antagonist (Martinson et al., 1987), reversed the inhibition of 20mM K^+-stimulated release of [^{3}H]NA caused by 10^{-6}M adenosine (Fig. 29A). Furthermore, CPDPX (10^{-5}M) in the presence of adenosine (10^{-6}M) enhanced the K^+-evoked release of [^{3}H]NA by about 10% (Fig. 29A), as was also observed with IBMX (10^{-4}M) (Fig. 27A).

3.2.9 NA and adenosine modulation of K^+-evoked release of [^{3}H]NA from O.C. tissue chops

NA (10^{-6}M) and adenosine (10^{-6}M) inhibited 20mM K^+-evoked release of [^{3}H]NA by 40% and 20% respectively (Fig. 30). The α_2-adrenergic antagonist, yohimbine, at a concentration of 10^{-5}M, slightly reduced the adenosine inhibition of K^+-stimulation of [^{3}H]NA release, while it was able to completely reverse the NA inhibition of K^+-stimulation of [^{3}H]NA release (Fig. 30). On the contrary, the adenosine antagonist, 10^{-4}M IBMX, did not affect NA inhibition of K^+-evoked release of [^{3}H]NA, while it was able to completely reverse the adenosine inhibition of K^+-stimulation of [^{3}H]NA release. The partial reversal of adenosine inhibition by yohimbine suggests that α_2-adrenoceptors may also play a regulatory role in adenosine effect of [^{3}H]NA release.

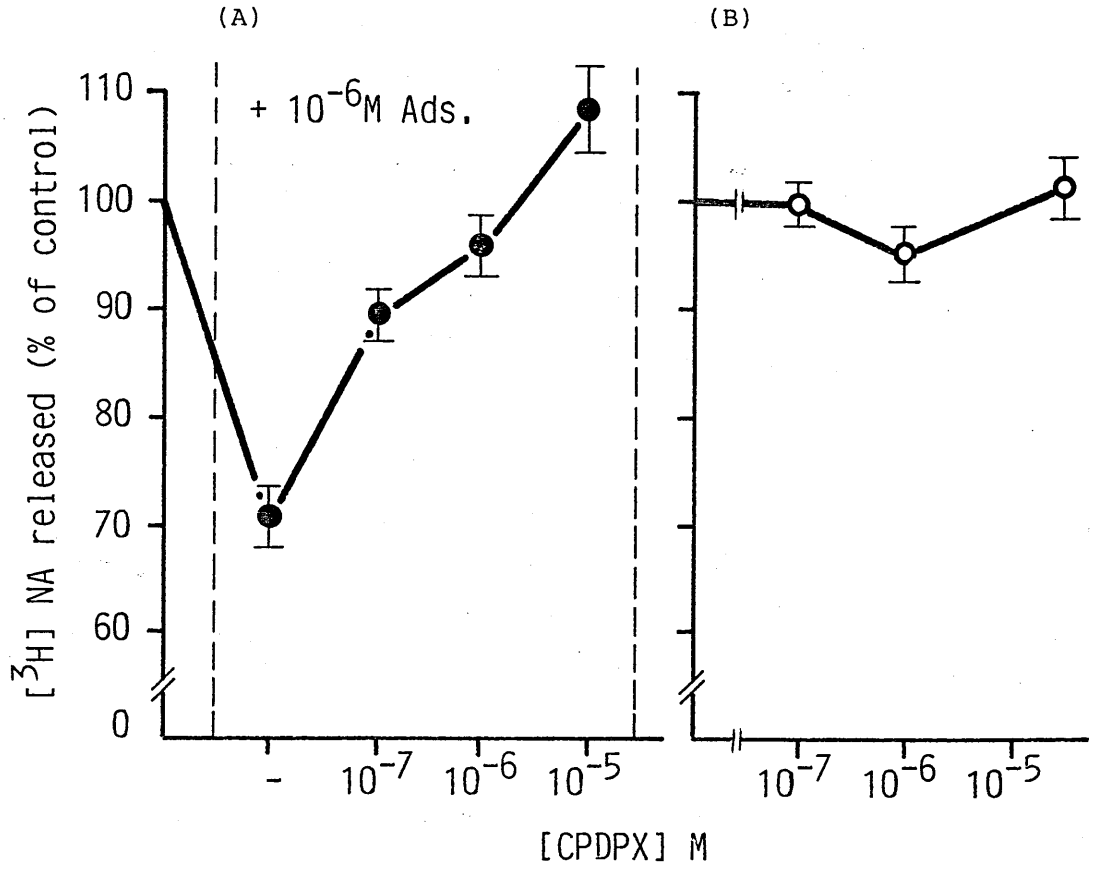


Figure 29 Effect of CPDPX on the inhibitory response of adenosine on K^+-evoked release of [^{3}H]NA from O.C. tissue chops

Aliquots of tissue chops preloaded with [^{3}H]NA were stimulated with 20mM K^+ in the presence of adenosine with increasing concentration of CPDPX (●) or CPDPX alone (○) for 6 min. Control release was 10.5 ± 0.30% of total tissue tritium. Results are means ± S.E.M. of 3 experiments.

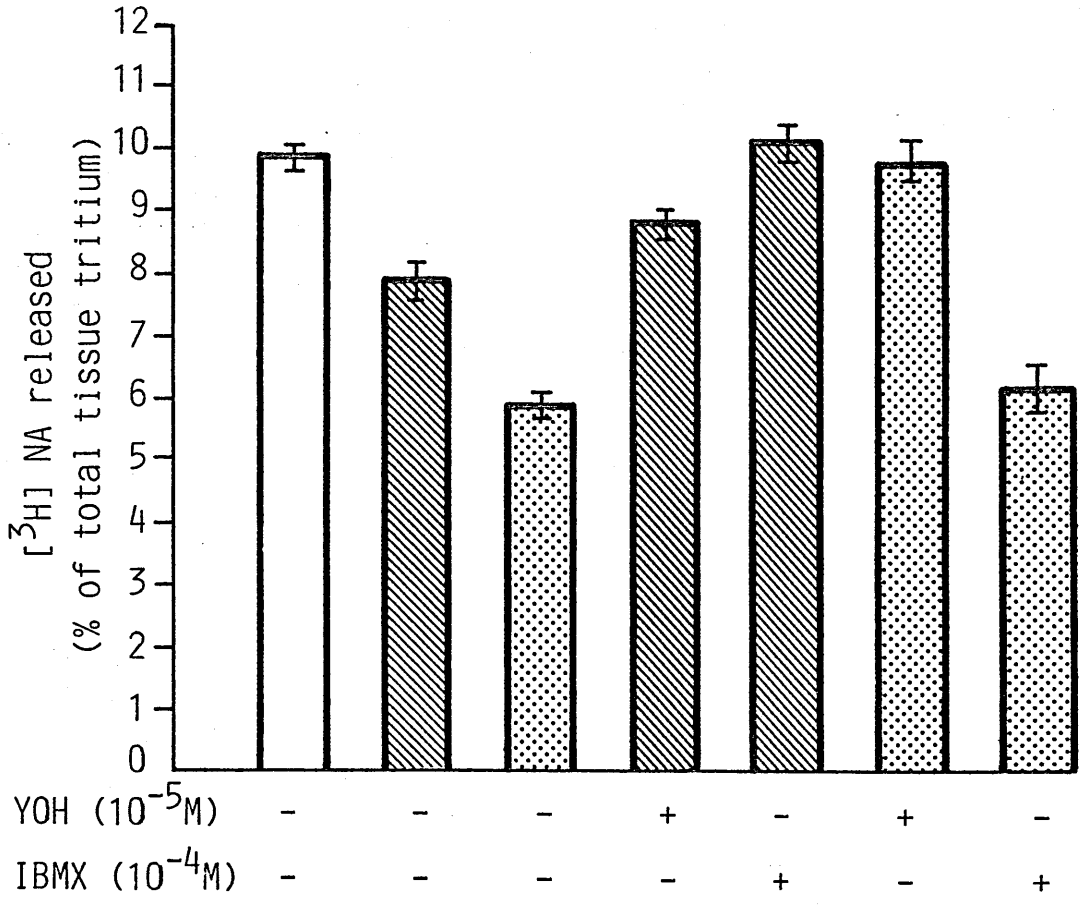


Figure 30 NA and adenosine modulation of K^+-evoked release of [^{3}H]NA in O.C. tissue chops

Tissue chops preloaded with [^{3}H]NA were stimulated with either 20mM K^+ alone (□) or in the presence of adenosine (▨) or NA (▩) with or without yohimbine (YOH) or IBMX as indicated on the figure for 6 min. Results are means ± S.E.M. of 4 separate experiments.

3.3 Regulation of cAMP formation in O.C. tissue chops

3.3.1 Stimulation of cAMP formation by forskolin, NA and isoprenaline in the absence or presence of IBMX and RO 20-1724

Forskolin, NA and isoprenaline stimulated cAMP formation in O.C. tissue chops (Fig. 31) with EC_{50} values (i.e. concentration of drug which stimulates half maximal cAMP formation) of 5×10^{-6}M, 4×10^{-6}M and 55×10^{-9}M respectively. Maximum responses were achieved by 10^{-4}M forskolin, 10^{-5}M NA and 10^{-4}M isoprenaline, with 5-9 fold, 2-3 fold and 1.5 fold stimulations being observed respectively.

IBMX (10^{-4}M) had no effect on either the potency or efficacy of forskolin to stimulate cAMP formation. Thus the inclusion of a PDE-inhibitor did not affect forskolin stimulation. On the contrary, NA and isoprenaline no longer stimulated cAMP formation in the presence of IBMX (Fig. 32). These observations were unexpected since the inclusion of a PDE-inhibitor would be expected to enhance, rather than inhibit cAMP formation. Interestingly, 10^{-4}M RO 20-1724 although elevating the basal levels, did not affect the EC_{50} values of forskolin, NA and isoprenaline stimulation of cAMP formation (Fig. 33).

3.3.1.1 Effects of α and β-adrenergic antagonists on NA or isoprenaline stimulation of cAMP formation

The α_1, α_2 and β-adrenergic antagonists prazosin, yohimbine and propranolol blocked the NA (10^{-5}M) stimulation of cAMP formation in O.C. tissue chops in a concentration dependent manner (Fig. 34) with IC_{50} values of 6×10^{-9}M, 10^{-8}M and 3×10^{-8}M respectively. This suggests a potency order of prazosin > yohimbine > propranolol. At 10^{-5}M concentrations, prazosin, yohimbine and

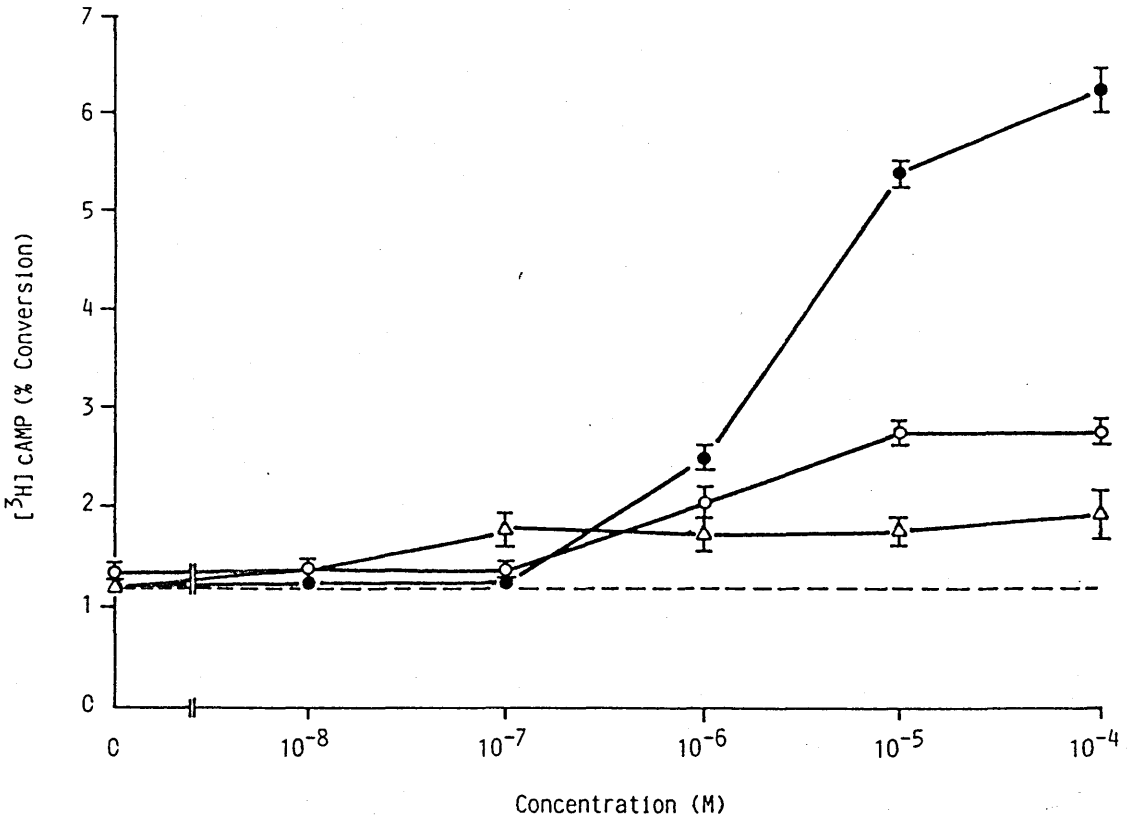


Figure 31 Dose response curves of forskolin, NA and isoprenaline stimulation of [^{3}H]cAMP formation in O.C. tissue chops

Tissue chops were labelled with [^{3}H] adenine then incubated with forskolin (●), NA (O) and isoprenaline (Δ) for 10 min. Results are means ± S.E.M. of 6 experiments

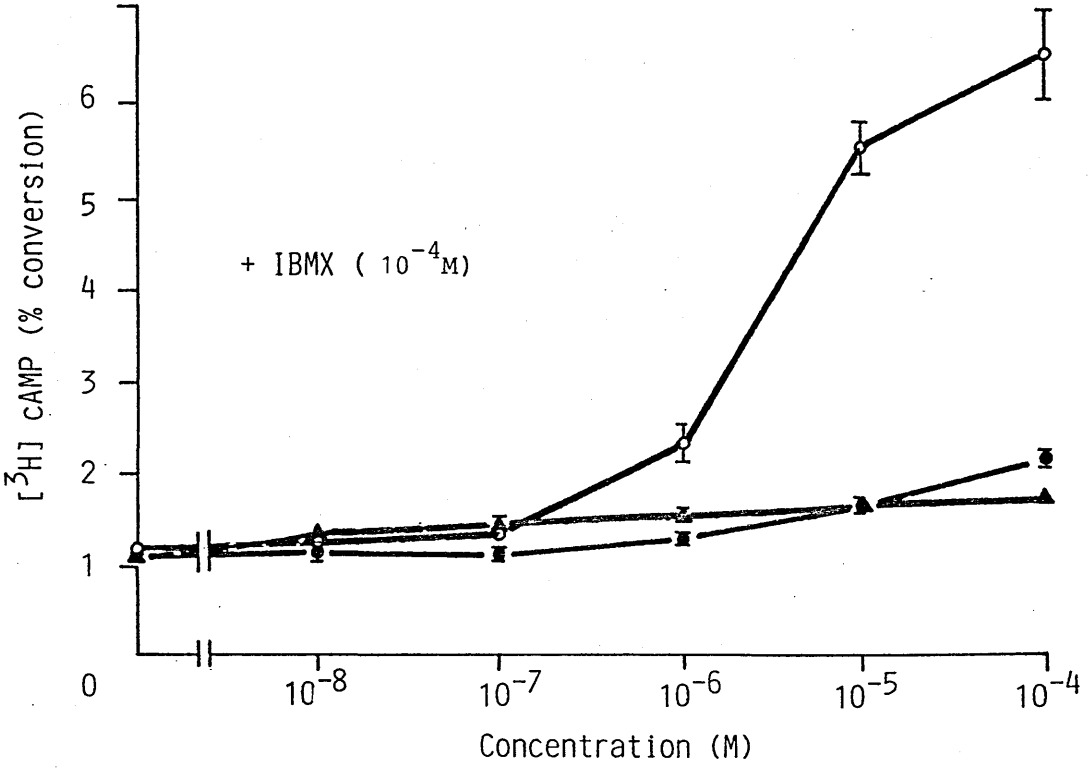


Figure 32 Influence of IBMX on dose response curves of forskolin, NA and isoprenaline stimulation of [^{3}H]cAMP formation in O.C. tissue chops

Tissue chops were labelled with [^{3}H] adenine then incubated with forskolin (O), NA (●) and isoprenaline (▲) in the presence of 10^{-4}M IBMX for 10 min. Results are means ± S.E.M. of 3 experiments.

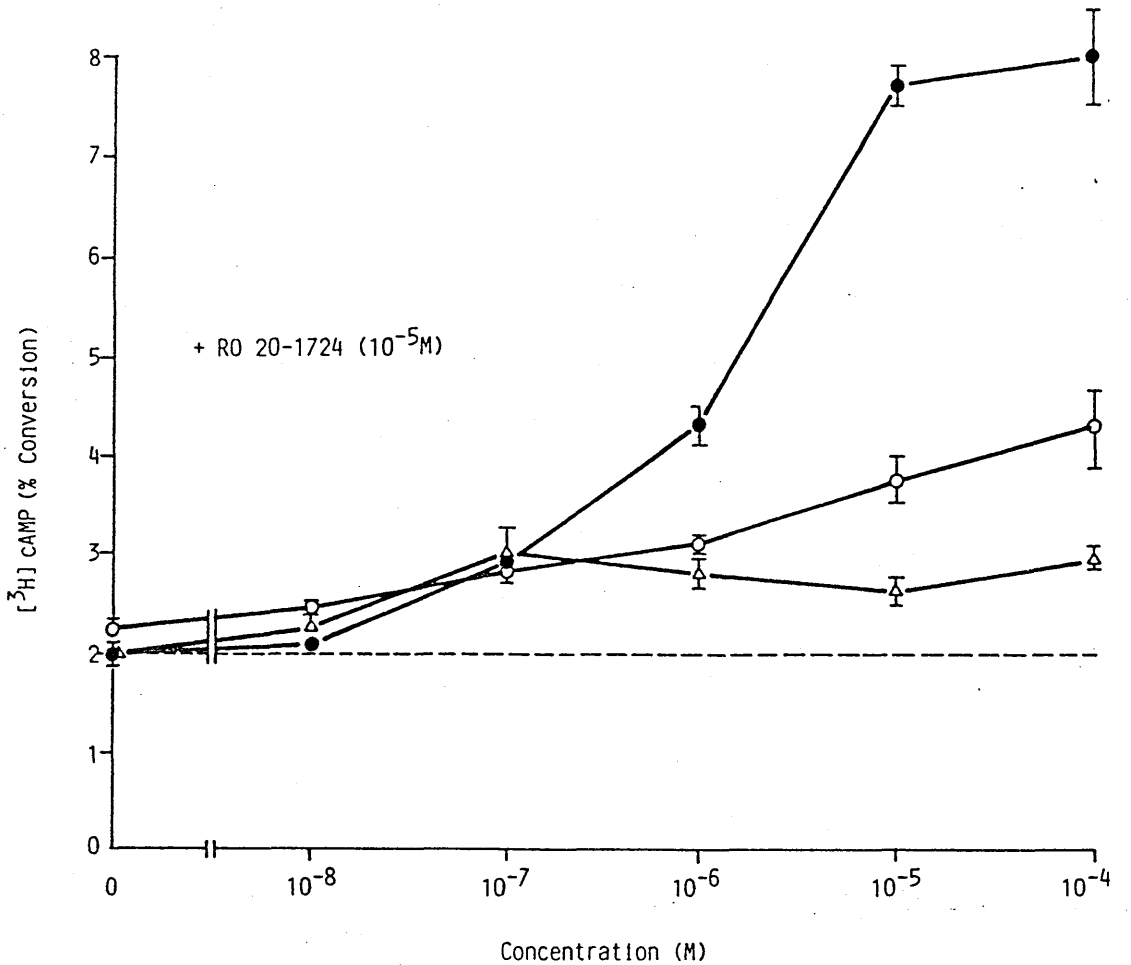


Figure 33 Influence of RO 20-1724 on dose response curves of forskolin, NA and isoprenaline stimulation of [^{3}H]cAMP formation in O.C. tissue chops

Tissue chops were labelled with [^{3}H] adenine then incubated with forskolin (●), NA (O) and isoprenaline (Δ) for 10 min in the presence of 10^{-5} M RO 20-1724. Results are means ± S.E.M. of 3 experiments.

propranolol inhibited NA (10^{-5}M) stimulation of cAMP formation by 30%, 35% and 60% respectively. This suggests that although prazosin was the most potent adrenergic antagonist propanolol was more effective than yohimbine or prazosin at inhibiting the NA (10^{-5}M) stimulation of cAMP formation (Fig. 34). The results suggest that NA stimulation of cAMP formation was due to the synergistic effect of α_1, α_2 and β -adrenoceptors.

Propranolol at submicromolar concentrations (10^{-11}M - 10^{-7}M) blocked isoprenaline (10^{-6}M) stimulation of cAMP formation, and complete inhibition was observed at 10^{-6}M and 10^{-5}M propranolol (Fig. 35). This shows that isoprenaline is a β-adrenergic agonist since its stimulation of cAMP formation is blocked completely by the β-adrenergic antagonist, propanolol.

3.3.1.2 Stimulation of cAMP formation by adenosine in the absence or presence of IBMX and RO 20-1724

Adenosine stimulated cAMP formation in chopped brain slices in a concentration-dependent manner (Fig. 36) with an EC_{50} value of 6×10^{-5}M. A maximum stimulation of 2.7 - 3.5 fold was achieved by adenosine (10^{-3}M). In the presence of IBMX (10^{-4}M), the dose response of adenosine was shifted to the right, suggesting that IBMX inhibits cAMP formation by acting at adenosine A_2 receptors (Fig. 36). In contrast, RO 20-1724 (10^{-5}M) elevated the basal level of cAMP formation, but it did not affect the EC_{50} value (6×10^{-5}M) of adenosine stimulation of cAMP formation (Fig. 36).

3.3.1.3 Effects of α - and β-adrenergic antagonists on adenosine combined with NA stimulation of cAMP formation

Low concentrations of adenosine (10^{-7}M) did not affect the basal as well as NA (10^{-5}M) stimulation of cAMP formation (Fig. 37).

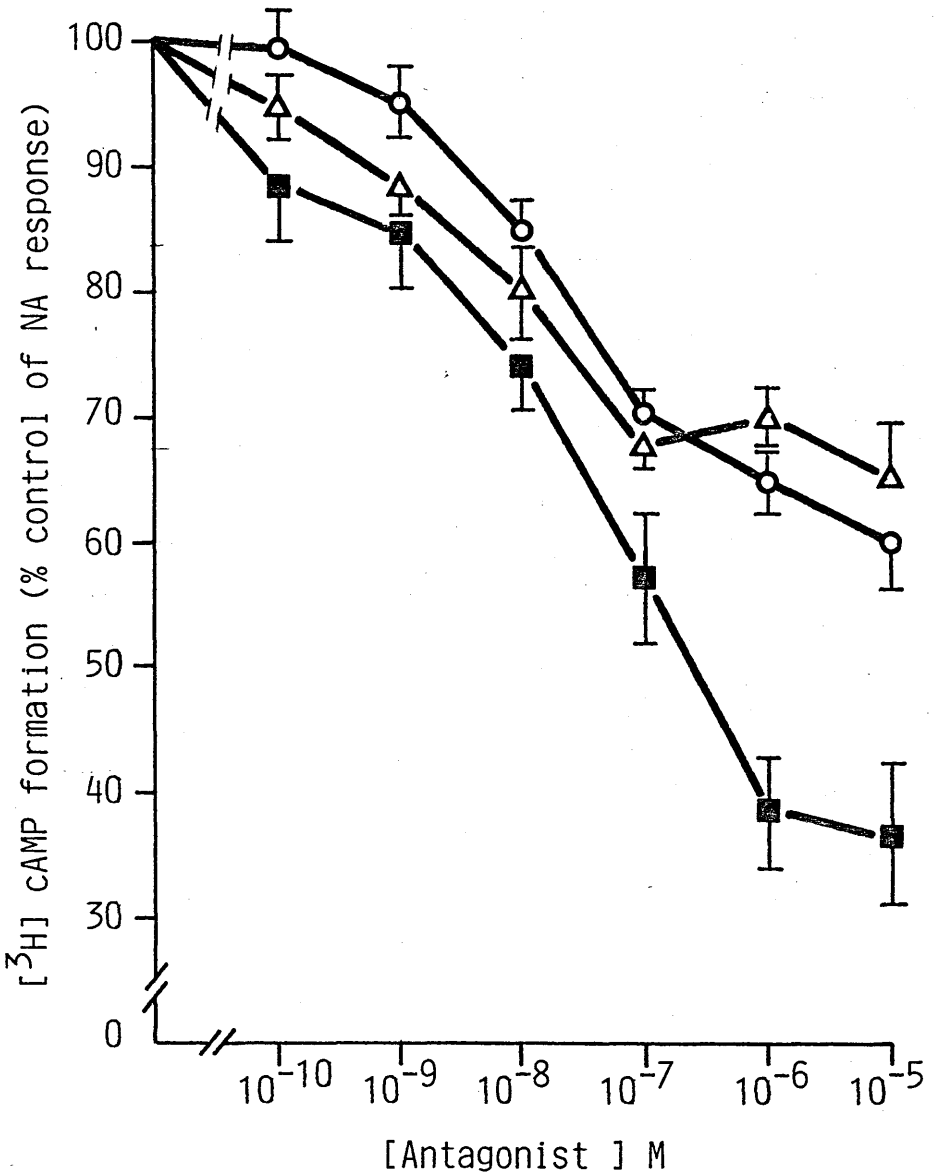


Figure 34 Effect of prazosin, yohimbine and propranolol on NA stimulation of cAMP formation in O.C. tissue chops

Tissue chops were labelled with [^{3}H] adenine then incubated with different concentrations of prazosin (Δ), yohimbine (O) propranolol (■) in the presence of 10^{-5}M NA for 10 min. The basal level of cAMP formation was 0.64 ± 0.04% conversion, and 10^{-5}M NA stimulated cAMP formation by 1.5 ± 0.08% conversion. Therefore 10^{-5}M NA stimulation increased cAMP level by 0.87% conversion, and this was expressed as 100% control on the figure. Results are means ± S.E.M. of 4-6 experiments.

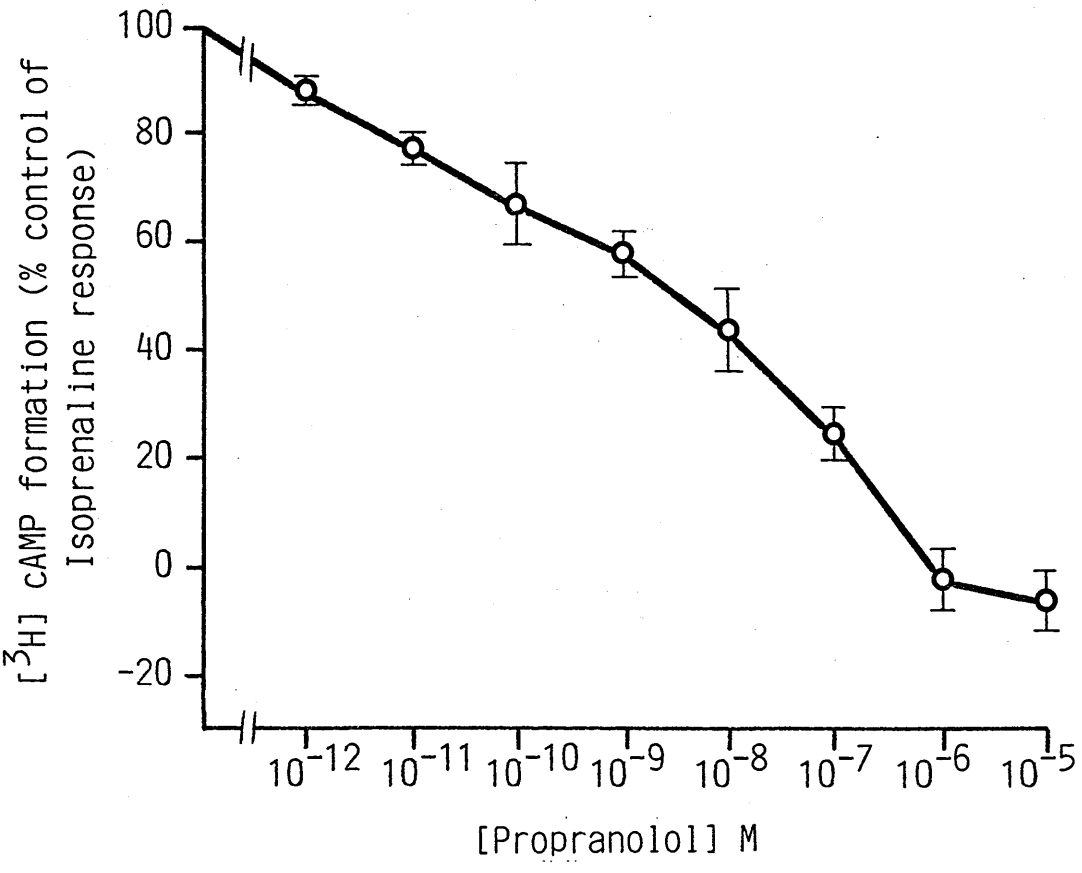


Figure 35 Effect of propranolol on isoprenaline stimulation of cAMP formation in O.C. tissue chops

Tissue chops were labelled with [^{3}H] adenine then incubated with different concentrations of propranolol in the presence of 10^{-6}M isoprenaline for 10 min. Basal level of cAMP formation was 0.56 $\pm$ 0.02% conversion, and isoprenaline stimulation of cAMP formation was 0.98 $\pm$ 0.03% conversion. Therefore, isoprenaline stimulation increased cAMP formation by 0.42% conversion, and this is expressed as 100% of control on the figure. Result is the representative of 2 experiments.

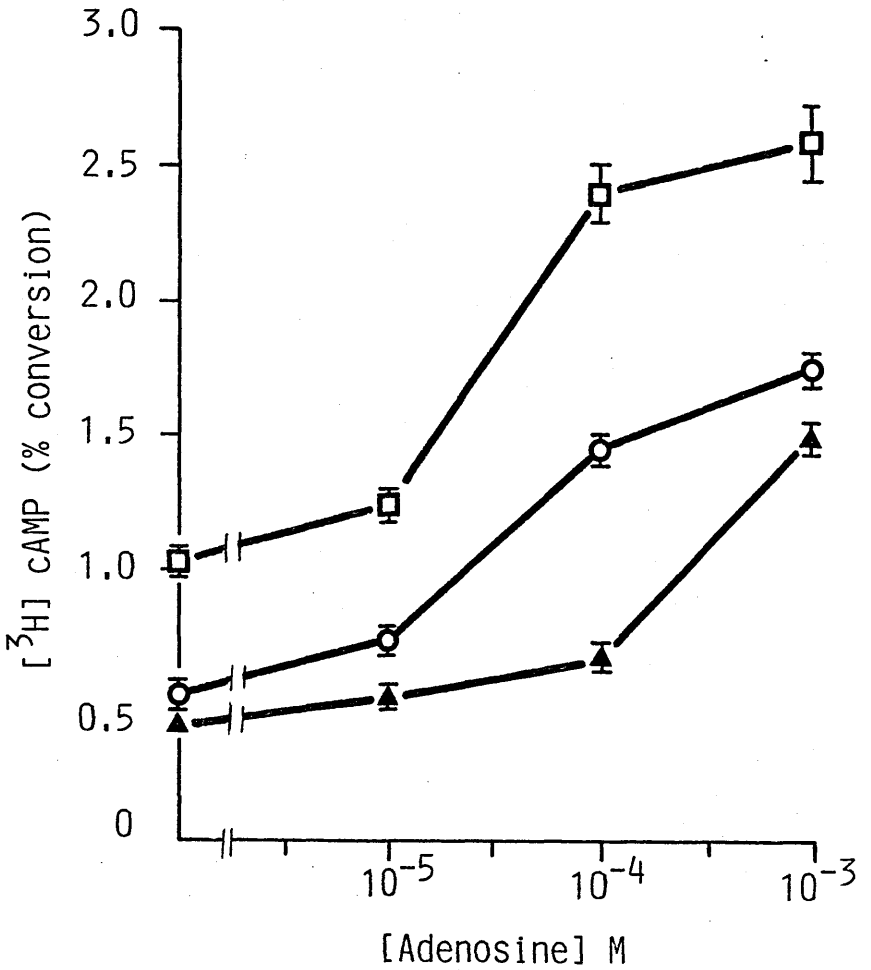


Figure 36 Influence of IBMX and RO 20-1724 on adenosine stimulation of cAMP formation in O.C. tissue chops

Tissue chops were labelled with [^{3}H] adenine then incubated with different concentrations of adenosine (O) either in the presence of 10^{-4}M IBMX (▲) or 10^{-5}M RO 20-1724 (□) for 10 min. Results are means ± S.E.M. of 3 experiments.

However, adenosine at 10^{-4}M potentiated NA (10^{-5}M) stimulation of cAMP formation by 0.7 fold, taking into account the individual NA and adenosine stimulation of cAMP formation (Fig. 37). α_2-Adrenergic antagonist, yohimbine, α_1-adrenergic antagonist, prazosin and β-adrenergic antagonist, propranolol blocked the adenosine (10^{-4}M)combined with NA (10^{-5}M) stimulation of cAMP formation in a concentration dependent manner (Fig. 38) with IC_{50} values of 8 x 10^{-9}M, 2.5 x 10^{-9}M and 6 x 10^{-7}M for yohimbine, prazosin and propranolol respectively. This suggests a potency order of yohimbine > prazosin > propranolol (Fig. 38). At 10^{-5}M concentration yohimbine, prazosin and propranolol inhibited adenosine (10^{-4}M) combined with NA (10^{-5}M) stimulation of cAMP formation by 40%, 30% and 22% respectively. This suggests that yohimbine is a more potent and effective adrenergic antagonist at inhibiting adenosine (10^{-4}M) combined with NA (10^{-5}M) stimulation of cAMP formation than prazosin and propranolol. In contrast, propranolol was shown to be the most effective adrenergic antagonist at inhibiting NA stimulation of cAMP formation (Fig. 34). In other words the adenosine component is more sensitive to α-adrenergic than β-adrenergic antagonists.

3.3.1.4 Influence of forskolin on NA, isoprenaline and adenosine stimulation of cAMP formation

Forskolin (10^{-7}M) which did not affect the basal level of cAMP formation potentiated NA (10^{-5}M), isoprenaline (10^{-6}M) and adenosine (10^{-4}M) stimulation of cAMP formation (Fig. 39) by 0.7, 0.8 and 1.5-fold respectively. However, forskolin (10^{-6}M and 5 x 10^{-6}M) which itself stimulated cAMP formation was only able to increase NA, isoprenaline and adenosine stimulation of cAMP formation in an additive manner (Fig. 39).

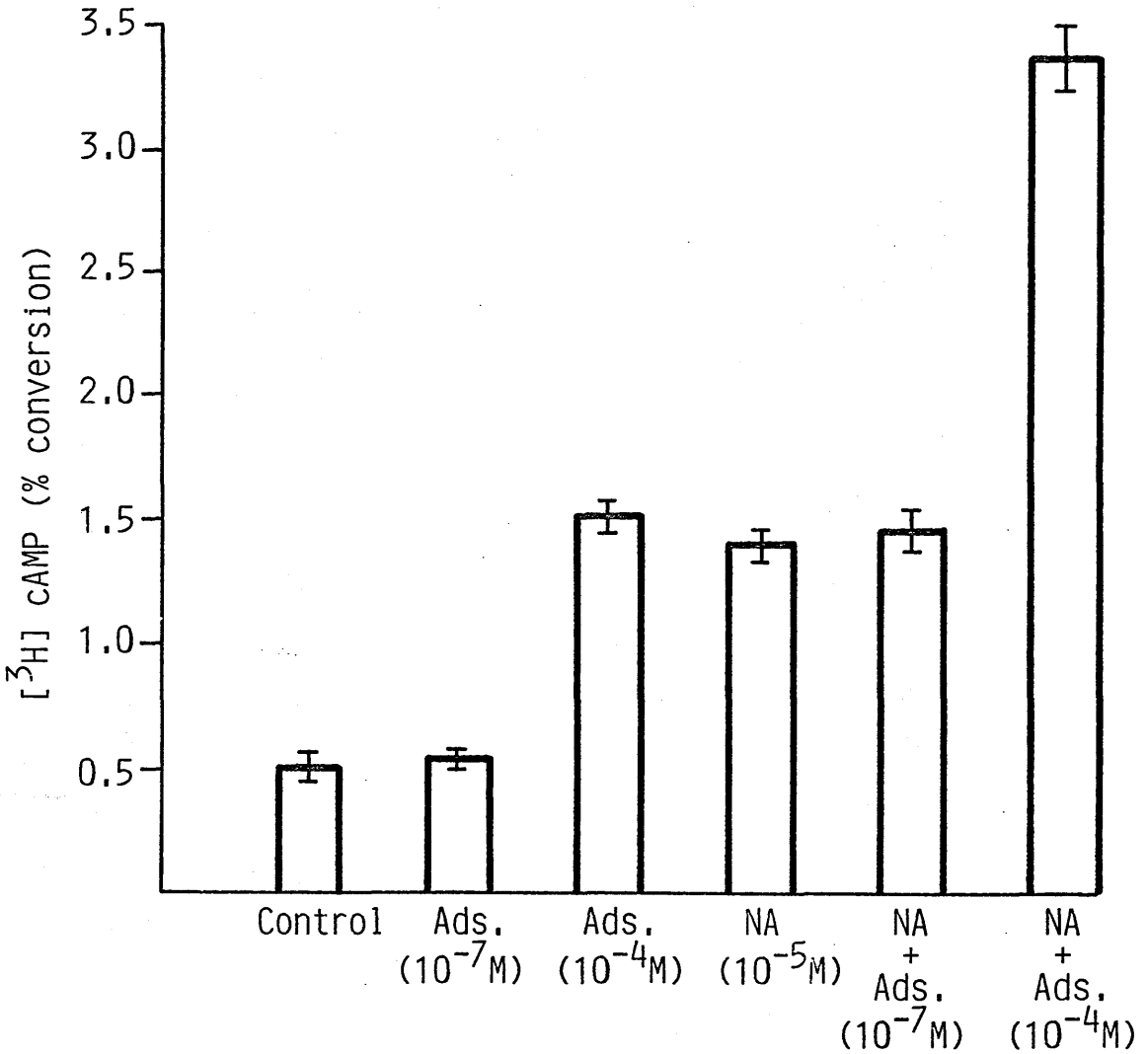


Figure 37 Effect of adenosine on NA stimulation of cAMP formation in O.C. tissue chops

Tissue chops were labelled with [^{3}H] adenine then incubated with adenosine (10^{-7}M; 10^{-4}M) or NA (10^{-5}M) or both for 10 min.

Results are means ± S.E.M. of 4 experiments.

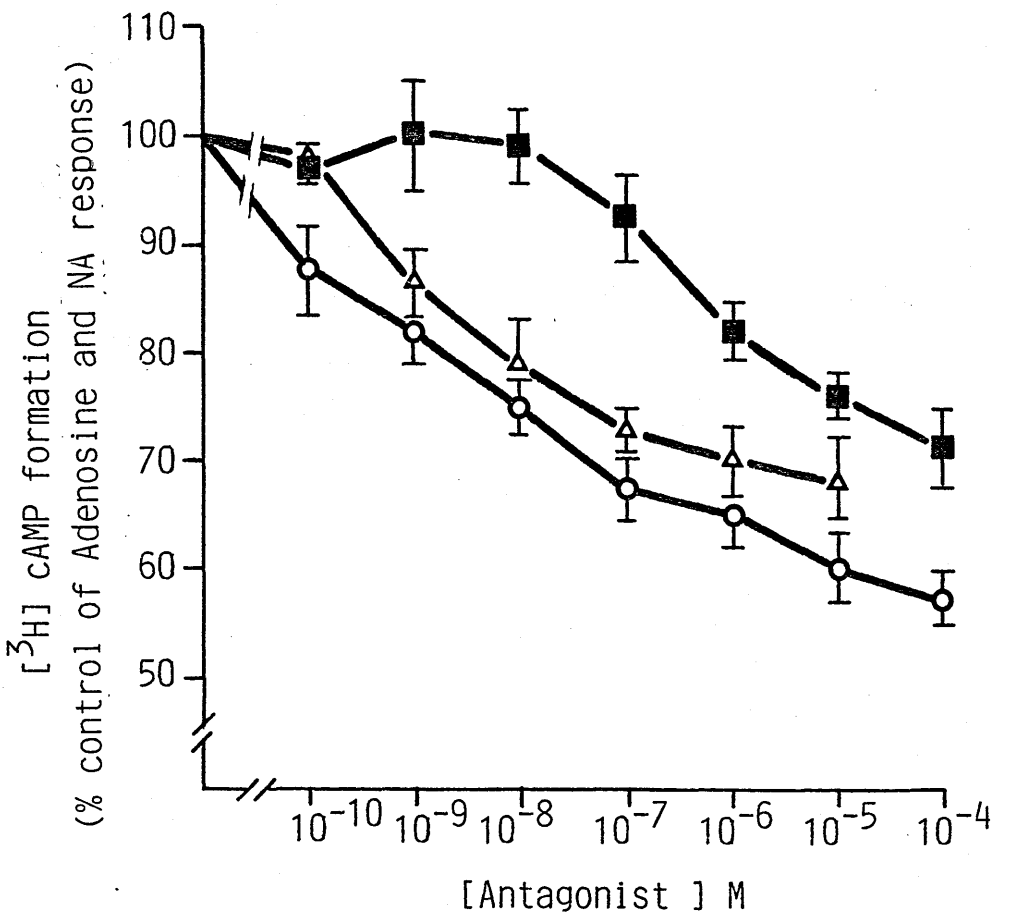


Figure 38 Effect of α- and β-adrenergic antagonists on adenosine and NA stimulation of cAMP formation

Tissue chops labelled with [^{3}H] adenine were preincubated with different concentrations of propanolol (■), prazosin (Δ) and yohimbine (O) for 10 min in the presence of adenosine (10^{-4}M) combined with NA (10^{-5}M) for 10 min. The basal level of cAMP formation was 0.55% conversion, while adenosine combined with NA stimulated cAMP formation to 3.55% conversion. Therefore, adenosine combined with NA increased cAMP formation by 3.0% conversion, which is expressed as 100% control on the figures. Results are means ± S.E.M. of 5 experiments.

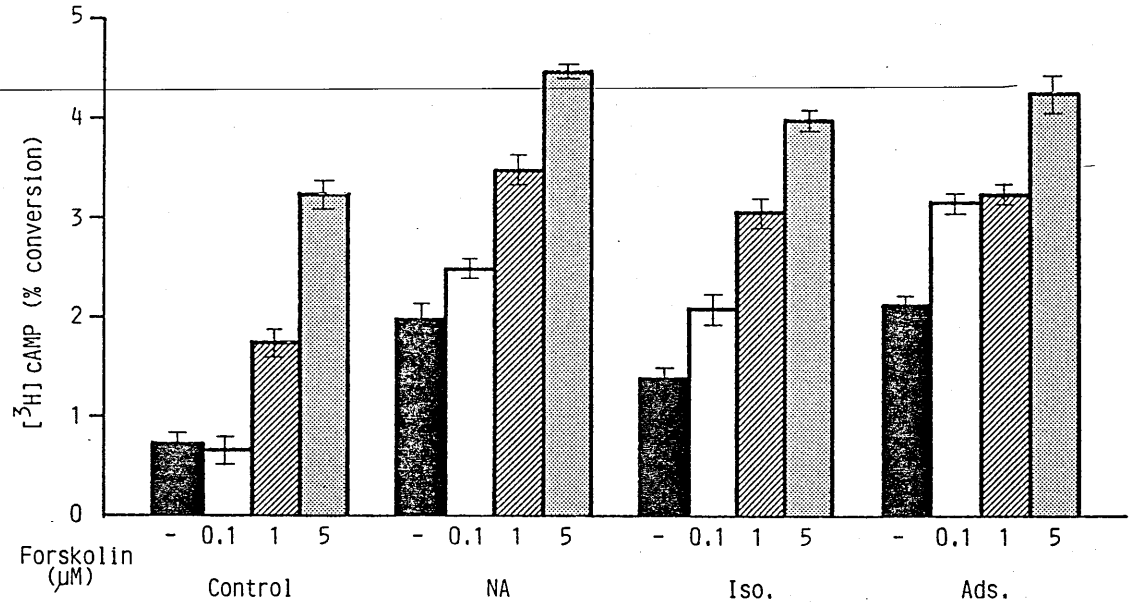


Figure 39 Effect of forskolin on NA, isoprenaline and adenosine stimulation of cAMP formation in O.C. tissue chops

Tissue chops were labelled with [^{3}H] adenine then incubated with 10μM NA, 1μM isoprenaline (Iso) or 100μM adenosine (Ads) either in the absence (■) or presence of 0.1μM (□), 1μM (▨) or 5μM (▩) forskolin for 10 min. Results are means ± S.E.M. of 3 experiments.

3.3.2 K^+ stimulation of cAMP formation in the absence or presence of IBMX, RO 20-1724 or DMI

Potassium-stimulated cAMP formation in O.C. tissue chops (Fig. 40). Maximum increased in cAMP formation of 2.2 fold was achieved by 40mM K^+ and remained effectively constant until 80mM. Increasing K^+ above 80mM led to a decrease in cAMP formation. There is no evidence that IBMX or RO 20-1724 enhanced K^+ stimulation of cAMP formation when compared to their respective control (Fig. 41).

DMI was included in the release experiments to prevent re-uptake of release of [^{3}H]NA (Fig. 13), thus it was important to see if it had any effect on cAMP formation. However, no evidence was found that DMI (10^{-6} M) modified cAMP formation under depolarizing condition (Fig. 42).

3.3.2.1 Influence of forskolin on K^+ stimulation of cAMP formation

Forskolin (10^{-4} M) in the presence of 5mM and 20mM K^+ stimulated cAMP formation by about 9 fold. However, in the presence of 40mM and 60mM K^+, cAMP formation due to forskolin (10^{-4} M) was reduced by 50% and 100% respectively (Fig. 43). RO 20-1724 increased the basal level (5mM K^+) as well as 40mM K^+ stimulation of cAMP formation in a concentration-dependent manner (Fig. 44). In the presence of RO 20-1724 (10^{-3} M), forskolin (10^{-5} M) stimulation of cAMP formation was not reduced to half with 40mM K^+ depolarization (Fig. 44). This suggests that the decrease in cAMP formation observed with forskolin in the presence of 40mM K^+ and 60mM K^+ may due to the stimulation of Ca^{2+}-dependent PDE, activated by the influx of Ca^{2+} into tissue chops following K^+ depolarization.

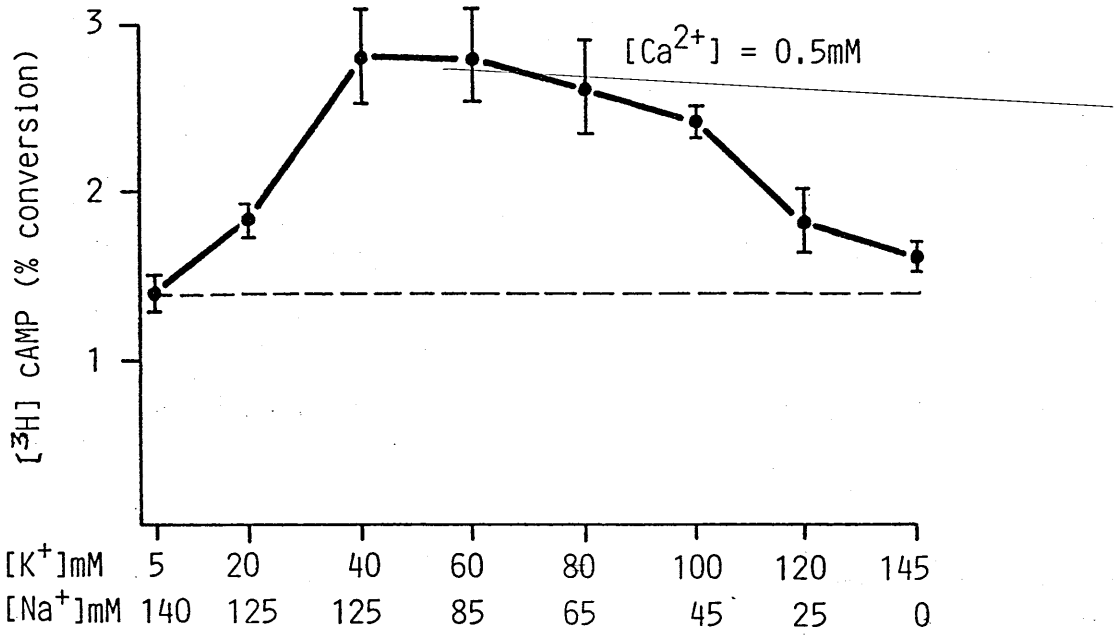


Figure 40 K^+ stimulation of cAMP formation in O.C. tissue chops

Tissue chops were labelled with [^{3}H] adenine for half an hour and then incubated with different K^+ concentrations for 10 min. Results are means ± of 4 experiments.

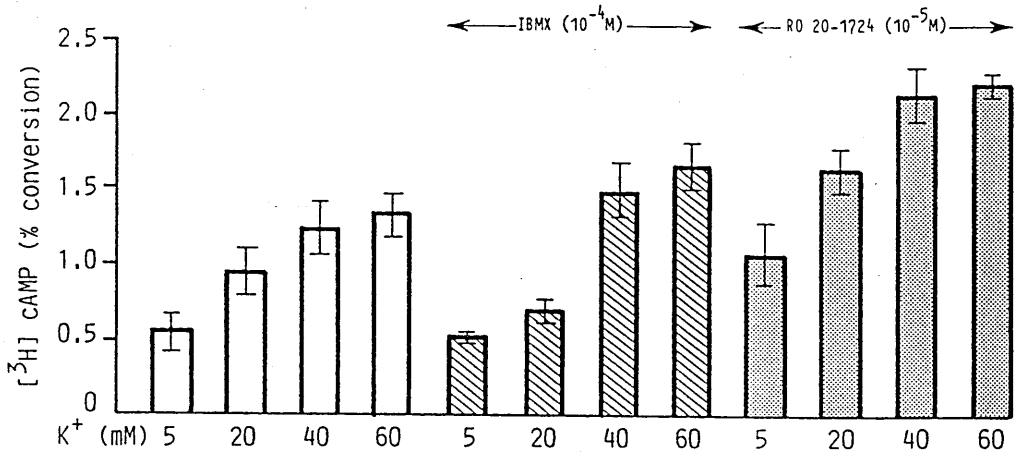


Figure 41 Effect of IBMX and RO 20-1724 on K^+ stimulation of cAMP formation in O.C. tissue chops

Tissue chops were labelled with [^{3}H] adenine then incubated with different K^+ concentrations either alone (□) or in the presence of 10^{-4}M IBMX (▨) or 10^{-5}M RO 20-1724 (▩) for 10 min. Results are means ± S.E.M. of 4 experiments.

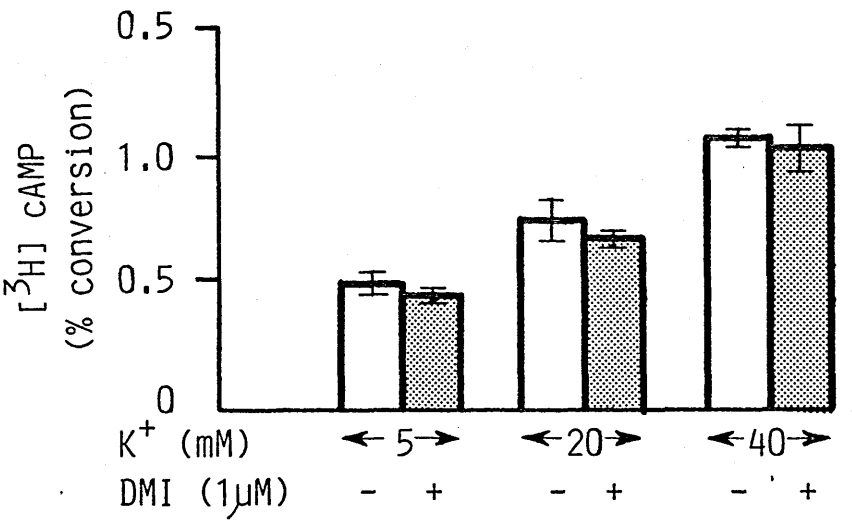


Figure 42 Effect of DMI on K^+-stimulation of cAMP formation in O.C. tissue chops

Tissue chops were labelled with [^{3}H] adenine then incubated with different K^+ concentrations either in the presence (□) or absence (▨) or 10^{-6} M DMI for 10 min. Results are means ± S.E.M. of 3 experiments.

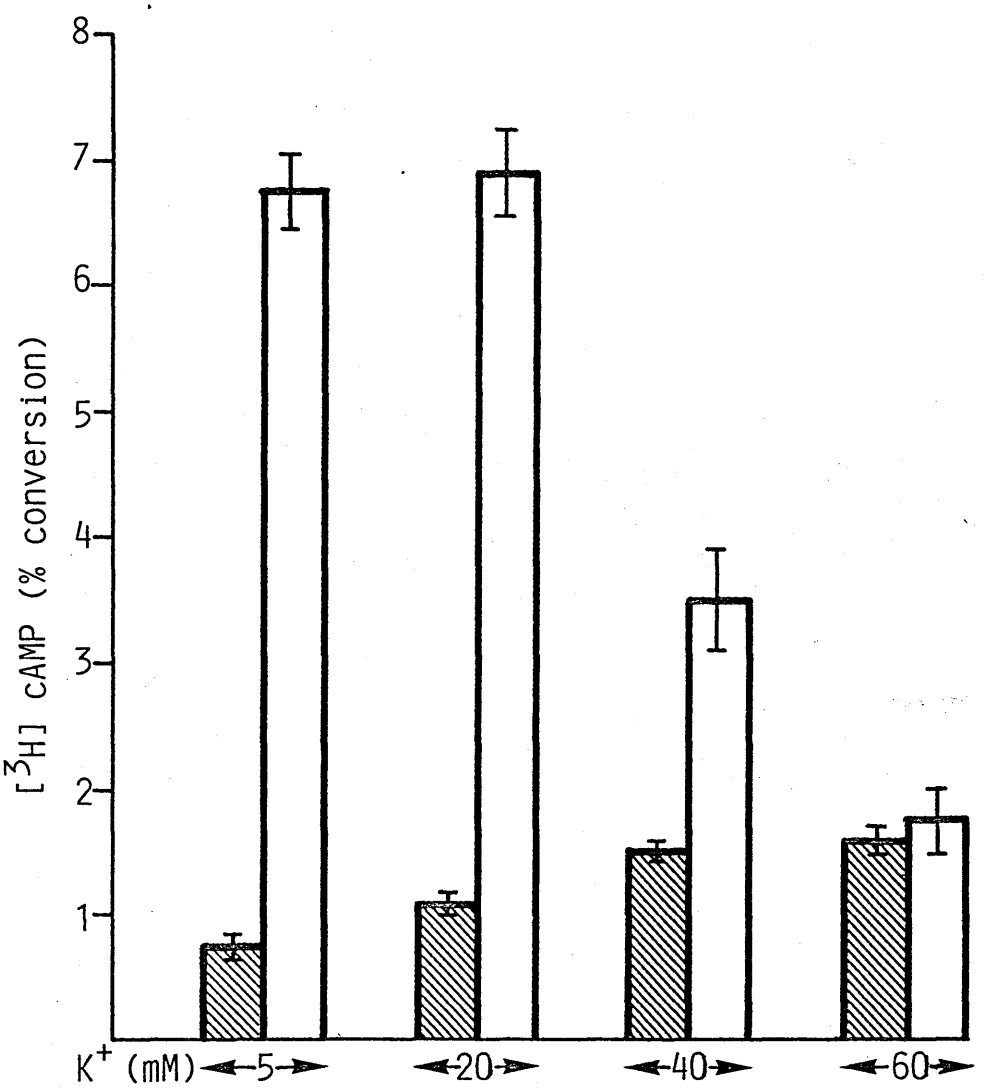


Figure 43 Influence of forskolin on K^+ stimulation of cAMP formation in O.C. tissue chops

Tissue chops were labelled with [^{3}H] adenine then incubated with different K^+ concentration either alone (▨) or in the presence of 10^{-4}M forskolin (□) for 10 min. Results are means ± S.E.M. of 3 experiments.

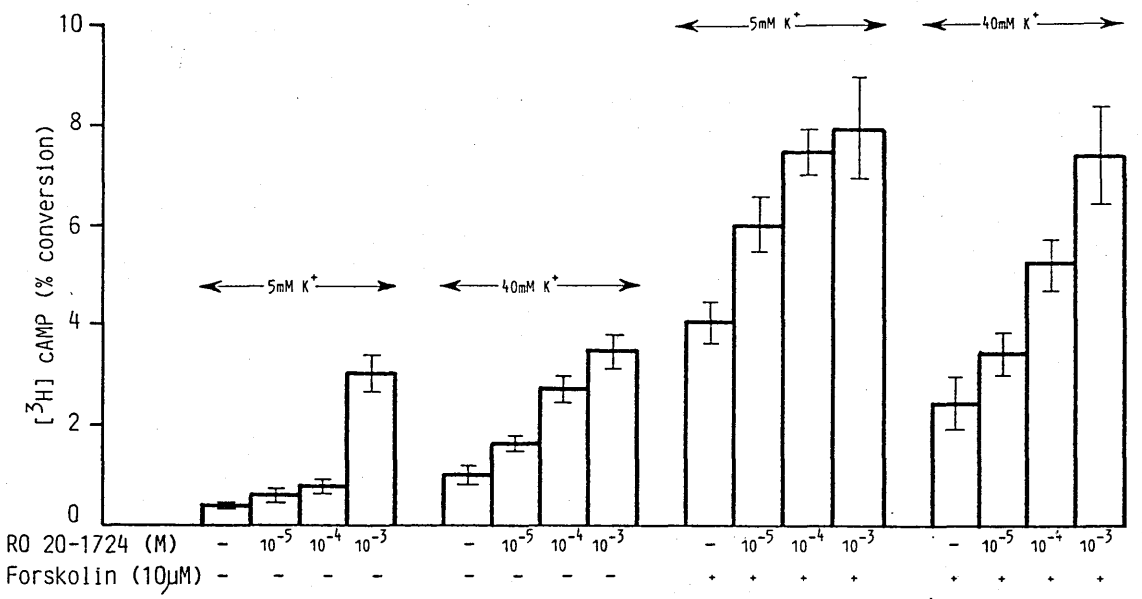


Figure 44 Effect of RO 20-1724 and forskolin on K^+ stimulation of cAMP formation in O.C. tissue chops

Tissue chops were labelled with [^{3}H] adenine then incubated with RO 20-1724 and forskolin in either 5mM or 40mM K^+ for 10 min. Results are means ± S.E.M. of 3 experiments.

3.3.3 Effect of α_2-adrenergic agonists in the presence of various cAMP stimulating agents:

3.3.3.1 Forskolin

Forskolin (10^{-5}M) stimulated cAMP formation 3-5 fold. Increasing concentrations of the α_2-adrenergic agonist, UK 14304-18, had no effect on forskolin stimulation of cAMP formation (Fig. 45). This is further supported by the observation that neither UK 14304-18 (10^{-5}M; Fig. 46) nor clonidine (10^{-6}M; Fig. 47) affected the forskolin dose response curve.

3.3.3.2 Noradrenaline (NA)

α_2-Adrenergic agonists, BHT-920, clonidine and UK 14304-18 inhibited NA (10^{-5}M) stimulation of cAMP formation in a biphasic and concentration dependent manner (Fig. 48) with IC_{50} values of 5×10^{-7}M, 10^{-8}M and 6×10^{-9}M respectively. This suggests a potency order of UK 14304-18 > clonidine > BHT-920. At concentration of 10^{-5}M, BHT-920, clonidine and UK 14304-18 maximally inhibited the NA (10^{-5}M) stimulation of cAMP formation by 22%, 30% and 55% respectively (Fig. 48). The results indicate that UK 14304-18 is the most potent and effective α_2-adrenergic agonist. This is further supported by the evidence that UK 14304-18 (10^{-5}M) shifted the dose response curve of NA to the right (Fig. 49). On the contrary, the α_1-adrenergic agonist, phenylephrine (10^{-5}M) did not affect the dose response curve of NA (Fig. 49). This suggests that only the α_2-adrenergic agonist inhibits NA stimulation of cAMP formation.

UK 14304-18 (10^{-5}M) did not affect the dose-response curve of isoprenaline (Fig. 50). On the contrary, phenylephrine (10^{-5}M)

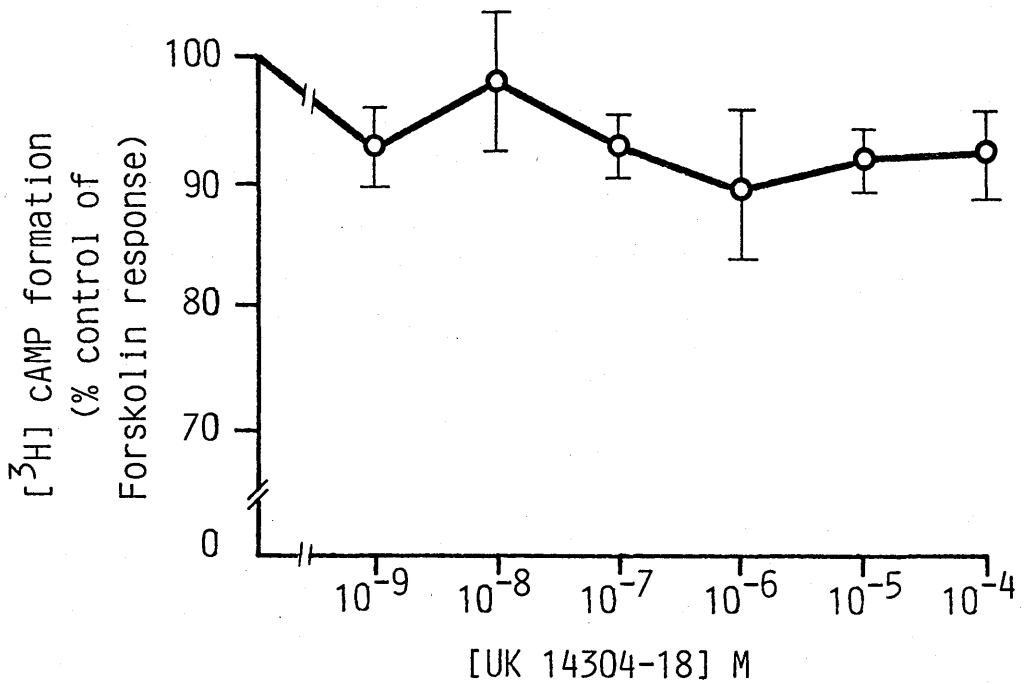


Figure 45 Dose response of UK 14304-18 on forskolin stimulation of cAMP formation in O.C. tissue chops

Tissue chops were labelled with [^{3}H] adenine then preincubated with different concentrations of UK 14304-18 for 5 min and then further incubated in the presence of 10^{-5}M forskolin for 10 min. Basal level of cAMP formation was 0.85 ± 0.06% conversion, and 10^{-5}M forskolin stimulated cAMP formation 4.8 ± 0.08% conversion. Therefore, 10^{-5}M forskolin stimulation increased cAMP formation by 3.95% conversion, which is expressed as 100% on the figure. Result is the mean ± S.E.M. of 3 experiments.

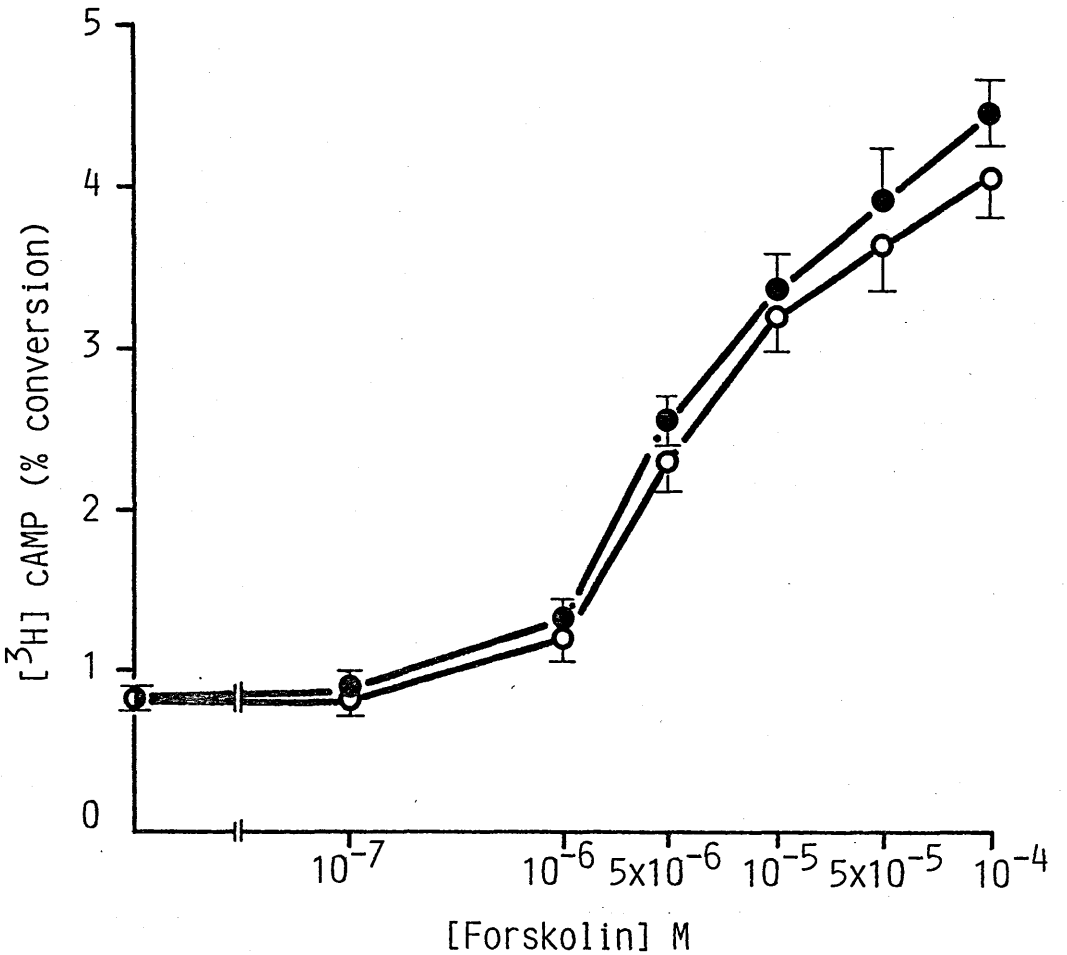


Figure 46 Effect of UK 14303-18 on forskolin dose response stimulation of cAMP formation in O.C. tissue chops

Tissue chops were labelled with [^{3}H] adenine then preincubated in the absence (●) or presence (O) of 10^{-5}M UK 14304-18 for 5 min and then further incubated in the presence of different concentrations of forskolin for 10 min. Results are means ± S.E.M. of 4 experiments.

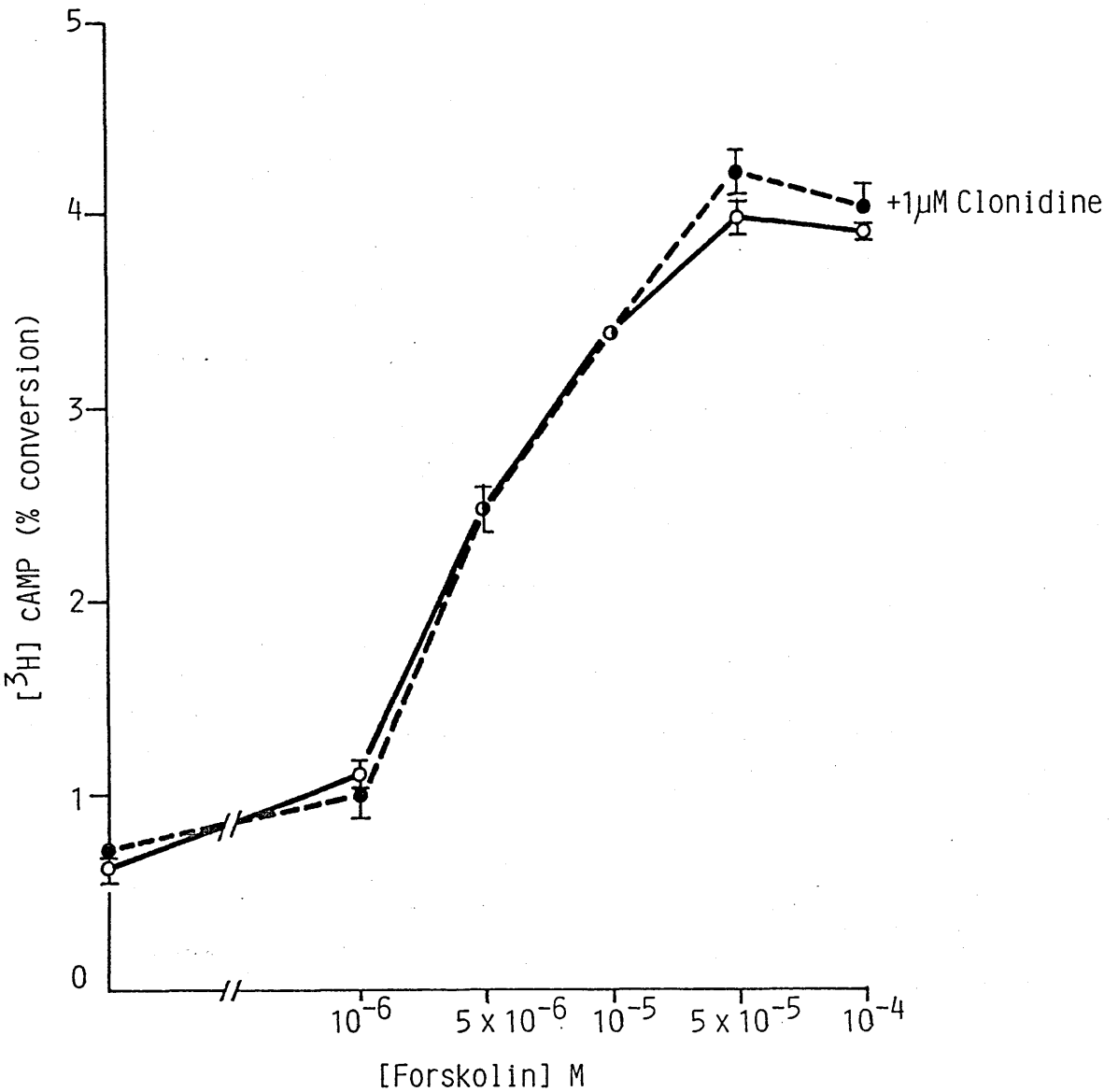


Figure 47 Effect of clonidine on forskolin dose response stimulation of cAMP formation in O.C. tissue chops

Tissue chops were labelled with [^{3}H] adenine then preincubated in the presence (O) or absence (●) of 10^{-5} M clonidine for 5 min and then further incubated in the presence of different concentrations of forskolin for 10 min. Results are means ± S.E.M. of 3 experiments.

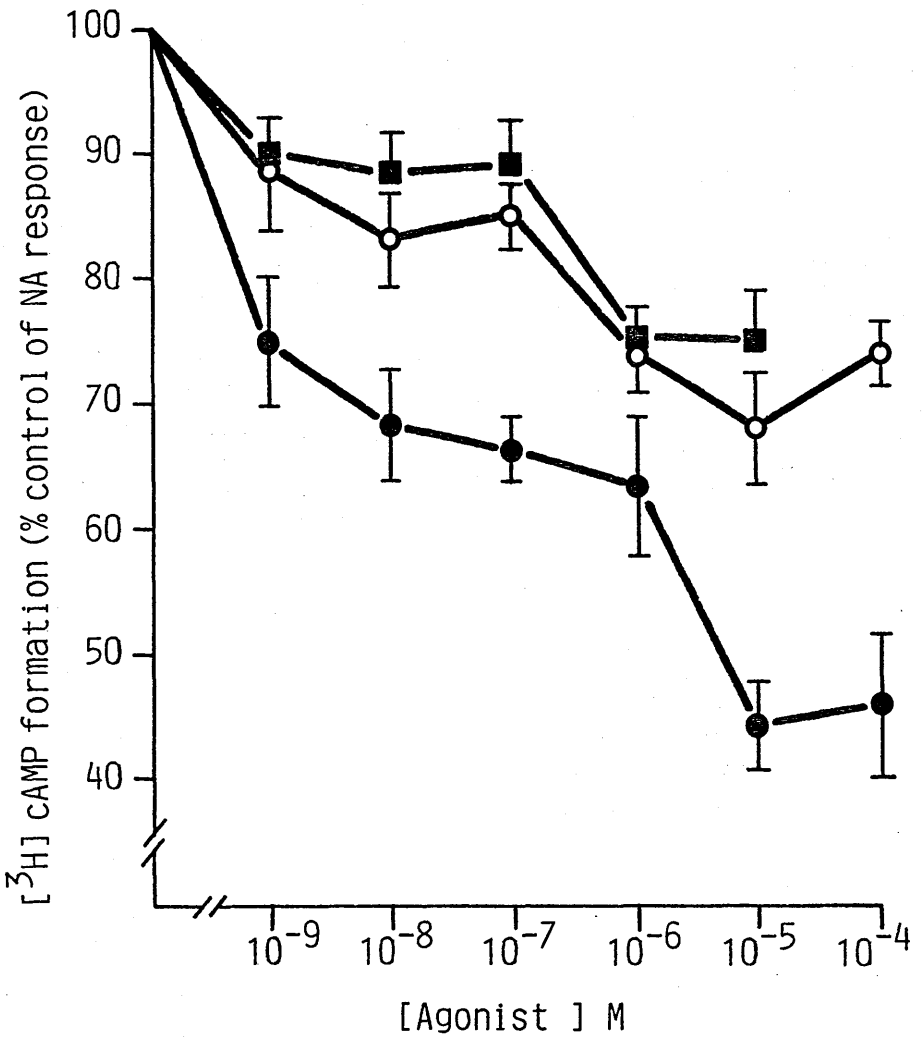


Figure 48 Effects of BHT-920, clonidine and UK 14304-18 on NA stimulation of cAMP formation in O.C. tissue chops

Tissue chops were labelled with [^{3}H] adenine then preincubated with different concentrations of BHT-920 (■), clonidine (O) and UK 14304-18 (●) for 5 min, and then further incubated for 10 min in the presence of 10^{-5} M NA. The basal level of cAMP formation was 0.53% conversion, while 10^{-4} M NA stimulated cAMP formation to 1.5% conversion. Therefore, NA stimulation increased cAMP formation by 0.97% conversion, which is expressed as 100% control on the figure. Results are means ± S.E.M. of 6 experiments.

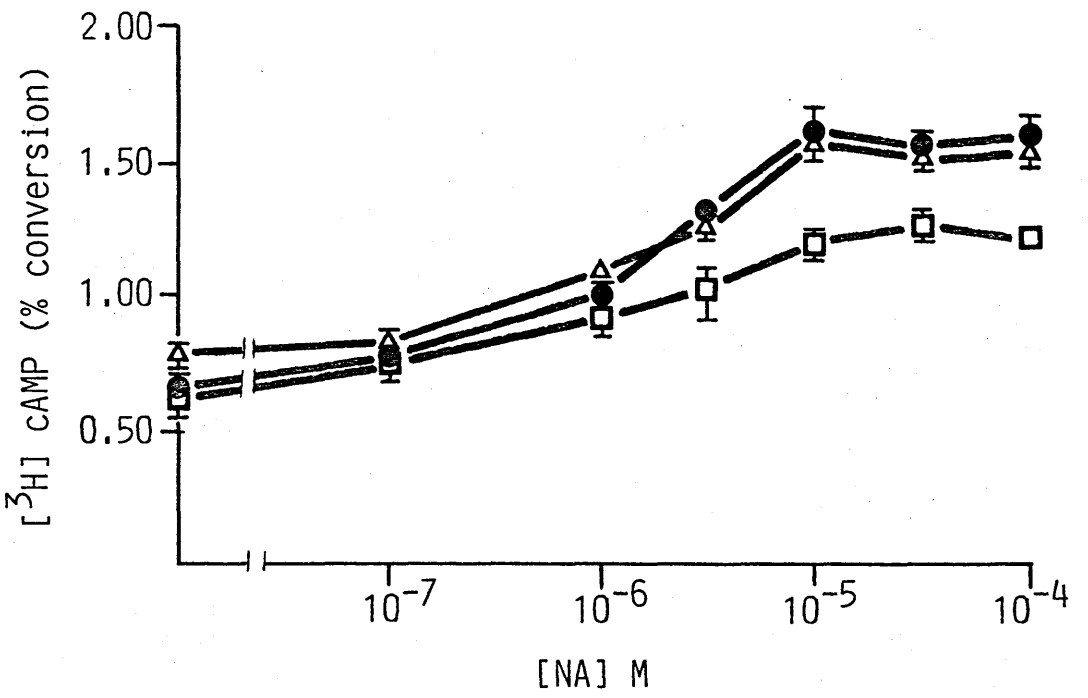


Figure 49 Effect of phenylephrine or UK 14304-18 on NA stimulation of cAMP formation in O.C. tissue chops

Tissue chops were labelled with [^{3}H] adenine then preincubated with 10^{-5}M phenylephrine (△) or 10^{-5}M UK 14304-18 (□) for 5 min and then further incubated with increasing concentrations of NA in the presence of respective drugs mentioned above or alone (●) for 10 min. Results are means ± S.E.M. of 4 experiments.

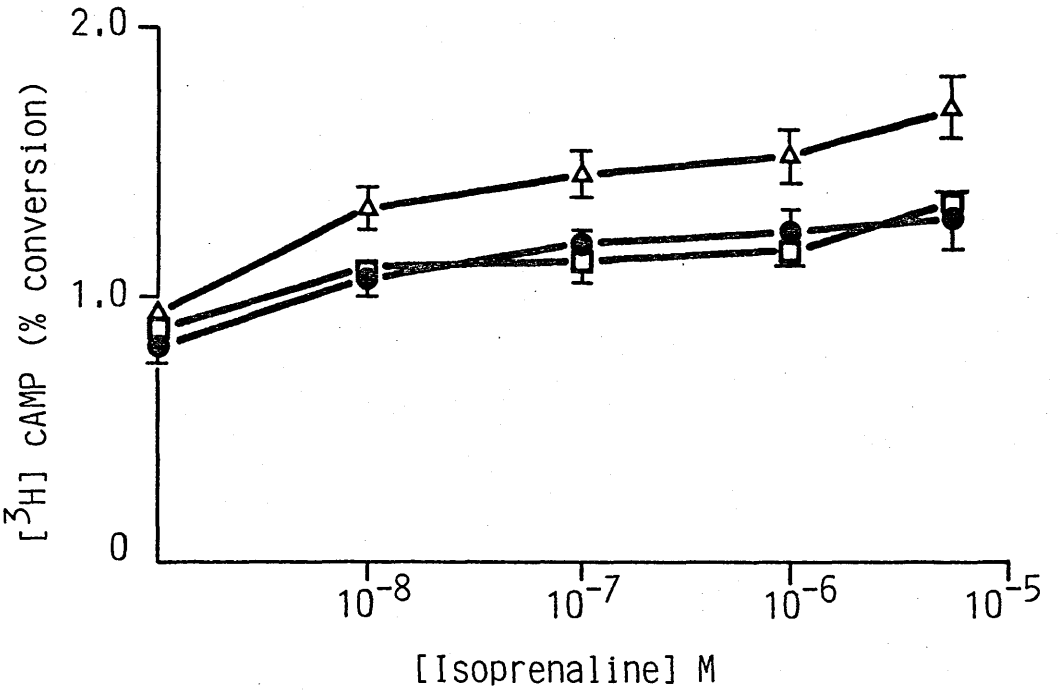


Figure 50 Effect of phenylephrine or UK 14304-18 on isoprenaline stimulation of cAMP formation in O.C. tissue chops

Tissue chops labelled with [^{3}H] adenine were preincubated with 10^{-5}M phenylephrine (△) or 10^{-5}M UK 14304-18 (□) for 5 min and then further incubated with increasing concentration of isoprenaline in the presence of respective drugs mentioned above or alone (●) for 10 min. Results are means ± S.E.M. of 3 experiments.

potentiated the dose-response curve of isoprenaline (Fig. 50). The results show that the α_1-adrenergic agonist was able to potentiate the β-adrenergic agonist stimulation of cAMP formation.

3.3.3.3 Adenosine

UK 14304-18 (10^{-5}M) slightly potentiated the stimulation of cAMP formation by 10^{-5}M adenosine (Fig. 51). However, a greater potentiation (0.9-fold) was observed at higher concentrations of adenosine (10^{-3}M). The EC_{50} value for adenosine stimulation of cAMP formation was not altered by UK 14304-18 (10^{-5}M).

3.3.3.4 K^+

UK 14304-18 (10^{-6}M) did not affect K^+ stimulation of cAMP formation in the absence or presence of RO 20-1724 (10^{-5}M) (Fig. 52). The apparent slight inhibition observed with 10^{-5}M UK 14304-18 and 20mM K^+ was statistically insignificant.

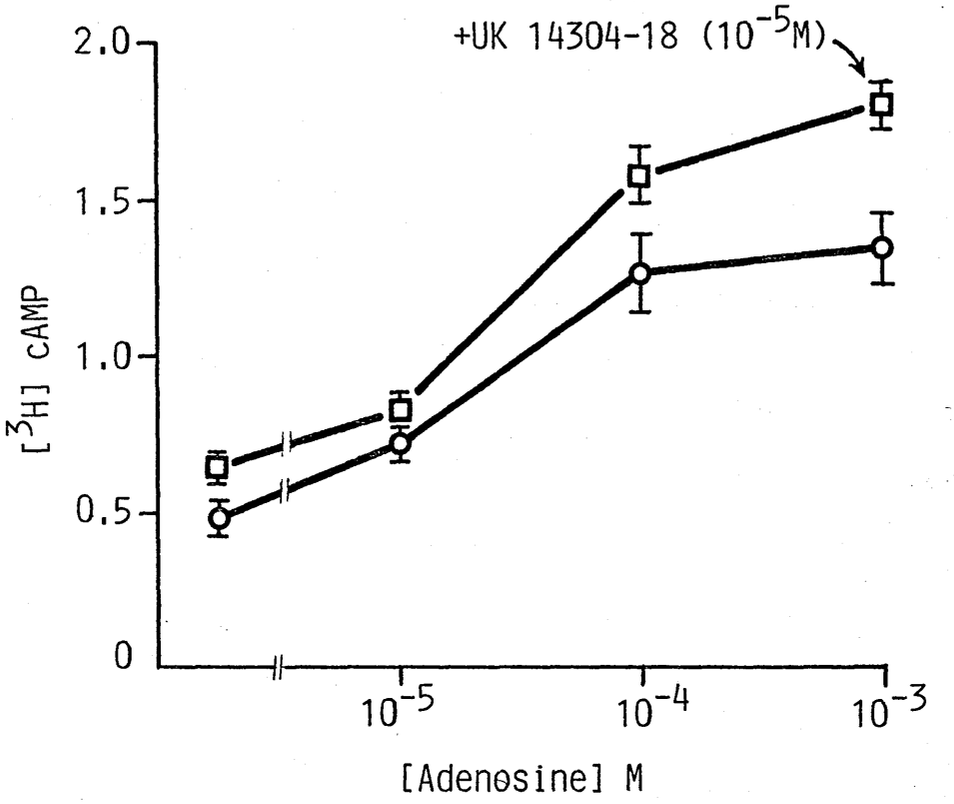


Figure 51 Effect of UK 14304-18 on adenosine stimulation of cAMP formation in O.C. tissue chops

Tissue chops labelled with [^{3}H] adenine were preincubated in the absence (○) or presence (□) of 10^{-5}M UK 14304-18 for 5 min, and then further incubated with different concentrations of adenosine for 10 min. Results are means ± S.E.M. of 4 experiments.

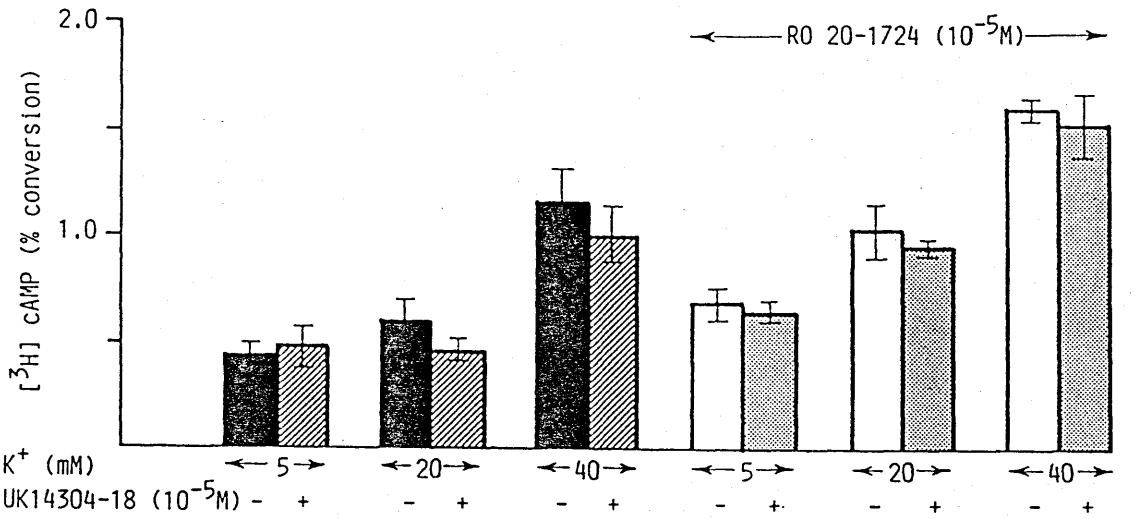


Figure 52 Effect of UK 14304 on K^+ (in the absence or presence of RO 20-1724) stimulation of cAMP formation in O.C. tissue chops

Tissue chops labelled with [^{3}H] adenine were preincubated in the absence or presence of UK 14304-18 indicated on the figure for 5 min, and then further incubated with different [K^+] in the absence (■) or presence (▨) of UK 14304-18, or with different [K^+] combined with RO 20-1724 in the absence (□) or presence (▩) of UK 14304-18 for 10 min. Results are means ± S.E.M. of 3 experiments.

4. DISCUSSION

A large number of studies have provided evidence that receptors located on nerve endings regulate the release of neurotransmitters. The presynaptic regulation of neurotransmitter release was initially studied in the peripheral nervous system (Langer, 1977, 1981) but has since been shown to occur in the central nervous system (CNS)(Taube et al., 1977; Chesselet, 1984; Middlemiss, 1988). For example, an α_2-adrenergic agonist such as clonidine, has been shown to regulate the release of NA (Reichenbacher et al., 1982; Schoffelmeer and Mulder, 1983) and serotonin (Frankhuyzen and Mulder, 1982; Ellison and Campbell, 1986) from the CNS. However, although a considerable amount of work has been carried out on the presynaptic regulation of neurotransmitter release, little information is available about the second messenger mediating this regulation. Thus, the aim of this project was to examine the hypothesis that the α_2-adrenergic regulation of NA release from O.C. and hypothalamic tissue chops was associated with a decrease in cAMP formation. The results presented in this thesis will be discussed under three main headings -

(1) The use of tissue chops and incubation assay to study the release of NA,

(2) α_2-Adrenergic regulation of NA release, and

(3) The possible role of cAMP in mediating the α_2-adrenergic regulation of NA release.

4.1 The use of rat brain tissue chops and incubation assay to study NA release

4.1.1 Choice of brain region

In this study, the NA concentration found in the hypothalamus was 1404 ± 251ng/g. This is in agreement with literature values of 1370 ± 50ng/g (Holtzman, 1974) and 1622 ± 182ng/g (Oke et al., 1978). The DA concentration of the hypothalamus in this study was found to be 220 ± 16 ng/g. This is in agreement with the findings of Shellenberger, (1971) who reported a value of 260 ± 20ng/g. The NA and DA concentrations found in the O.C. were 502 ± 347ng/g and 59 ± 18ng/g respectively, which agree well with the values of 380 ± 40ng/g and 65.9 ± 0.2ng/g reported by Westerink and Mulder (1981). Thus, the NA content of the brain regions chosen for the present study agreed with the literature values. This provides confirmation that the correct brain regions were identified and that they contain noradrenergic nerve endings.

4.1.2 Comparison between tissue chops and synaptosomes preparations

The two preparations frequently used for the in vitro study of biochemistry in the CNS are: (i) chopped brain slices and (ii) synaptosomes. The advantage of using chopped brain slices is that they are a more intact preparation in which to study biochemical mechanism associated with neuronal function, without the influence of blood brain barrier. In particular, most studies on presynaptic regulation of nerve terminals have used a more intact system that is provided by synaptosomes (Middlemiss, 1988). One problem to consider when working with brain slices is that thick slices may become anoxic (Palfrey and Mobley, 1987), which will disrupt the energy metabolism of the neurons.

In addition, anoxia increases cAMP levels (Gross and Ferrendelli, 1980), thereby affecting neurotransmitter release. On the other hand, thin slices may not contain enough nerve endings making typical synaptic contacts (Palfrey and Mobley, 1987). The thickness of brain slices routinely used to study presynaptic regulation of neurotransmitter release and cAMP formation range between 0.2mm to 0.3mm (Carmichael and Israel, 1973; Gothert, 1979; Schoffelmeer et al., 1986) and 0.26mm respectively (Perkin and Moore, 1973; Karbon and Enna, 1985). Because of this, tissue chops of 0.26mm in thickness were used in the present study.

Synaptosomes are pinched off nerve terminals containing mitchondria and small vesicles filled with neurotransmitters (Gray and Whittaker, 1962; Gordon-Weeks, 1987). Thus, they have the capacity to synthesize, store, release and metabolise neurotransmitters (Deutsch et al., 1981). One advantage of synaptosomes is that they are less heterogenous than tissue chops. Most preparations of synaptosomes, however, still contained significant numbers of other particles such as fragmented membranes, myelin sheath and free mitochondria (Gordon-Weeks, 1987). Besides this, they usually contain a heterogenous population of nerve endings, and so do not, in this sense, represent a purer preparation than tissue chops. Furthermore, synaptosomes possess high osmotic sensitivity (Marchbanks, 1967; Keen and White, 1971) thereby giving rise to "spurious release" of endogenous neurotransmitter. In addition, some researchers (Lane and Aprison, 1977; Mulder et al., 1978; De Langen et al., 1979) find that it is difficult to demonstrate consistent regulation of neurotransmitter release by presynaptic receptors with synaptosomes preparations. Thus, they concluded that synaptosomes are not ideal preparations for a study on presynaptic

regulation of neurotransmitter release. In view of this, chopped brain slices were selected for the study of α_2-adrenergic regulation of NA release in the occipital cortex (O.C.) and hypothalamus of rat brain.

4.1.3 Comparison between superfusion and incubation assays

Neurotransmitter release from brain slices or synaptosomes is usually studied by preloading the preparations with radioactive neurotransmitter. Depolarization-stimulated release is then followed by measuring the efflux of labelled triated [^{3}H] neurotransmitter into the superfusion or incubation fluids. Advantages and disadvantages of both the assays are discussed below.

(a) Superfusion assay: In this assay, preloaded brain slices or synaptosomes are placed on a filter, and the superfusion fluids are pumped over them at a regular flow rate, the superfusates are collected in separate tubes. The release, expressed as a percentage of total tissue tritium, is usually calculated as the ratio of a second stimulation, S_2 to the first stimulation, S_1.

The advantage of superfusion technique is that the brain slices or synaptosomes are continuously exposed to fresh superfusion fluids, reducing the problem of re-uptake and feedback regulation effects. One disadvantage of superfusion is that clogging of filter may occur, which may then affect the maintenance of regular flow rates. Furthermore, relatively large volumes of buffer are needed, which can lead to large use of expensive chemicals.

(b) Centrifugation assay: In this assay, preloaded brain slices are distributed in a small volume into different Eppendorf tubes. Here, only a small volume (i.e. 0.5ml) is required. Stimulus evoked release of neurotransmitters is studied by incubating the brain slices in the

Eppendorf tubes containing high K^+ with regular shaking for a short period of time. Release is terminated by rapidly centrifuging the Eppendorf tubes. The supernatants are removed and collected in different vials. The remaining tritium in the pellet is extracted with PCA. The release of tritiated noradrenergic neurotransmitter ($[^3H]NA$) expressed as a percentage of total tissue tritium is calculated as below:

$$\% \text{ release of } [^3H]NA = \frac{\text{cpm(supernatant)}}{\text{cpm (pellet) + cmp (supernatant)}} \times 100$$

The advantage of using centrifugation assay is that small amounts of buffer are required, with a more economical use of agonists and antagonists. In addition, more concentrated solutions of released neurotransmitters can be achieved. Another advantage of using centrifugation assay is that it provided a convenient technique in which to compare the effect of α_2-adrenergic agonists on both NA release and cAMP formation under comparable conditions. However, the main disadvantages of this assay are that the re-uptake and feedback inhibition (autoinhibition) of the release of NA at the nerve terminals may be more pronounced than in the superfusion system. An important stage in the present study therefore, was to establish that $[^3H]NA$ release from rat brain tissue chops using incubation assay gave results consistent with those reported in the literature for the superfusion technique. The use of incubation assay has also been reported in the study of endogenous NA and DA release from rat brain slices (Lane and Aprison, 1977; Kant and Meyerhoff, 1978) as well as the neurotransmitter release from cells in monolayer (Marriott et al., 1988).

4.1.4 Influence of uptake inhibitors on the regulation of NA release

The tricyclic antidepressant imipramine and its metabolites desipramine (DMI), 2-hydroxyimipramine and 2-hydroxydesipramine (Javid et al., 1978) as well as narcotic analgesics including codeine, cocaine, morphine, naloxone and methadone (Carmichael and Israel, 1973) have been shown to inhibit the uptake of NA. In the present study, DMI (10^{-6} M) inhibited the uptake of [^{3}H]NA by 90% (Fig. 13). In the absence of DMI, the basal release of [^{3}H]NA has beeen shown to consist of only 10% [^{3}H]NA while the remaining 90% was [^{3}H] metabolites of NA (Taube et al., 1977). In the presence of DMI, however not only is the basal release of [^{3}H] metabolites is strongly reduced (Taube et al., 1977), but also in the presence of uptake inhibitors including cocaine and DMI, electrical as well as K^+-stimulated release of [^{3}H]NA consisted mainly of [^{3}H]NA and not its metabolites (Langer et al., 1976; Taube et al., 1977). Two possibilities are that uptake inhibitors are either acting as monoamine oxidase inhibitors, or else they are increasing the uptake into storage vesicles which thus protects NA from metabolism. Thus, in the present study, DMI (10^{-6} M) was added in the incubation medium of centrifugation assay in the study of NA release experiments.

4.1.5 K^+ stimulated Ca^{2+} dependent release of [^{3}H]NA

Release of [^{3}H]NA from brain slices or synaptosomes is usually stimulated electrically or by exposing the tissue preparation to high K^+ concentration (Schoffelmeer, 1981; Daniel and Leslie, 1986). It is generally accepted that depolarization whether achieved electrically or by increasing K^+ concentration activates the voltage sensitive Ca^{2+} channels and that neurotransmitter release is induced by the consequent rise in intracellular Ca^{2+}. Other depolarizing agents

including veratrine and scorpion venom have also been shown to increase the $^{45}Ca^{2+}$ uptake in rat brain synaptosomes (Blaustein, 1975).

The present result demonstrates that 20mM or 40mM K^+ stimulation of [^{3}H]NA release from O.C. tissue chops is dependent on Ca^{2+} (Fig. 16) while basal (5mM) release of [^{3}H]NA is not affected by Ca^{2+} throughout the range 0.5mM - 2.0mM (Fig. 16). Similarly, non-depolarized induced release of endogenous NA using synaptosomes prepared from rat hypothalamus, brainstem and cerebellum brain regions is not dependent on Ca^{2+} (Daniel and Leslie, 1986). The release of [^{3}H]NA has been shown to be directly proportional to the membrane potential developed by different K^+ concentration throughout the range 13 - 120mM K^+ (Dismukes et al., 1977). This suggests that K^+ provides a suitable stimulus for studying release from rat brain tissue chops. K^+-stimulated Ca^{2+}-dependent release is also observed in other neurotransmitter systems including GABA (Cotman et al., 1976), serotonin (Klein and Kendal, 1980) acetylcholine (Suszkiw and O'Leary, 1983; Tanaka et al., 1985) and dopamine (Leslie et al., 1985).

Schoffelmeer et al. (1981) found that 20mM K^+-stimulated release of [^{3}H]NA reached a maximum value between 0.6mM - 0.9mM Ca^{2+} followed by a slight decrease at 2mM Ca^{2+}. They also found that high Ca^{2+} concentrations (2-5mM) decreased $^{45}Ca^{2+}$ release from rat brain slices. The decrease in [^{3}H]NA release at high Ca^{2+} concentrations suggest that Ca^{2+} inhibits its own transport through Ca^{2+} channels in the nerve terminals. In contrast, no inhibition of 20mM K^+ or 40mM K^+-stimulated release of [^{3}H]NA is observed at 2mM Ca^{2+} in the present study (Fig. 16). This is in agreement with Cotman et al. (1979) who found no inhibition of K^+ stimulated release of γ-aminobutynic acid (GABA) and NA using superfusion method by Ca^{2+} concentrations above 2mM.

Orrego (1979) reported that Ca^{2+} independent release of neurotransmitter originating from extravesicular cytoplasmic pools was increased under strongly depolarizing conditions. Therefore, mildly depolarizing K^+ concentration (20mM) was used in the present study to decrease the contribution of NA release from non-vesicular pools.

The brain consists of several cell types in addition to neurons. Electrophysiological studies show that one of these cell types, glia, express K^+ channels (Bevan and Raff, 1985). Furthermore primary cultures of glial cells have been shown to accumulate neurotransmitters (Kimelberg and Katz, 1986). Thus, the contribution of glia to the release of neurotransmitter should be considered. Sugino et al. (1984) found that stimulation of cultured rat astrocytoma cells, C6 BU-1 with 50mM K^+ did not increase the intracellular Ca^{2+} concentrations. Also, when neurotransmitters such as NA, DA and acetylcholine are applied to glia cell cultures in the presence of the Ca^{2+} indicator, Quin 2, no change in fluorescence is observed, indicating that there is no change in intracellular Ca^{2+} (Sugino et al., 1984). This implies that glial cells do not express voltage-dependent Ca^{2+} channels. Thus, K^+-stimulated release of [^{3}H]NA from chopped brain slices in the study is mainly of neuronal origin.

4.1.6 Conditions for α_2-adrenergic regulation of [^{3}H]NA release

Noradrenaline regulates its own release via α_2-adrenergic receptors located on noradrenergic nerve endings (Starke, 1979; Langer, 1981). These receptors are described as the 'presynatic autoreceptors', the first term referring to their location on nerve terminals and the second to their sensitivity to the neurotransmitter released by the neurons themselves (Chesselet, 1984).

The present study shows that the α_2-adrenergic agonist, clonidine, inhibition of 30mM K^+-stimulated release of [^{3}H]NA from O.C. tissue chops does not depend on pretreatment of tissue chops (Figs. 17, 18). Thus clonidine inhibition of 30mM K^+ stimulation of [^{3}H]NA release is the same whether clonidine is preincubated for up to 30 min (Fig. 18) or added immediately (Fig. 18) before evoking release with K^+. This suggests that the effect of clonidine is very rapid. On the contrary, in superfusion studies, tissues are normally superfused with drugs for 20 - 30 min. (Wemer et al., 1981; Ueda et al., 1983) as it is generally presumed to obtain a better effect of the drugs on the regulation of neurotransmitter release.

Clonidine inhibition of NA release is dependent on the extent of K^+ depolarization, since clonidine inhibited 40mM, 30mM and 20mM K^+ stimulation of [^{3}H]NA release by 17.0%, 37% and 50% respectively (Fig. 19). The results obtained in the present study is in agreement with the findings of Wemer et al. (1981) using another α_2-adrenergic agonist, oxymetazoline who report that at low depolarizing stimuli a greater proportion of NA release is subject to α_2-adrenergic agonist inhibition. One possible explanation is that α_2-adrenergic agonists may increase K^+ permeability during depolarization, thereby leading to hyperpolarization which is more pronounced at low K^+ concentrations. This could then lead to a decrease in NA release as shown in the peripheral nervous system (Alberts et al., 1981; Zimanyi et al., 1988). Another alternative explanation provided is that α_2-adrenergic agonists inhibit the voltage-sensitive Ca^{2+} channels (Gothert et al., 1979; Langer, 1981), and that this effect is more clearly observed at suboptimum depolarization conditions when Ca^{2+} influx might be expected to be rate limiting. In contrast,

Schoffelmeer and Mulder (1984) demonstrated that α_2-adrenergic agonists, NA and clonidine inhibition of [^{3}H]NA release from rat brain cortex slices is independent of the degree of depolarization when the release was effected by 15mM or 56mM K^+. Instead the activation of these presynaptic receptors causes a decrease in Ca^{2+} availability or the utilization of Ca^{2+} by the secretion process upon invasion of an action potential.

4.1.7 Influence of oxygenation on the effect of K^+ and clonidine on [^{3}H]NA release

The present study demonstrates that K^+-stimulated as well as basal release of [^{3}H]NA from rat O.C. tissue chops is higher in non oxygenated (Figs. 14, 19) than in oxygenated HEPES buffer (Figs. 14, 19). Furthermore, clonidine is less effective at inhibiting NA release in non-oxygenated than in oxygenated HEPES buffer (Fig. 19). One possible explanation would be that anoxia increases cAMP formation (Gross and Ferrendelli, 1980), thus leading to the enhanced K^+-stimulated release of [^{3}H]NA, thereby overcoming the α_2-adrenergic inhibition of K^+-stimulated release. Alternatively, since anoxia also effects glucose metabolism of the cell, thus altering the phosphorylation state of regulatory proteins and this could affect the release of neurotransmitters (Gross and Ferrendelli, 1980). Thus, all release experiments were carried out with the HEPES buffer equilibrated with 95% O_2 - 5% CO_2 and then adjusting the pH to 7.4 before use. Using this procedure, the degree of inhibition observed with clonidine is comparable with that obtained by the superfusion technique (Taube et al., 1977; Wemer et al., 1979).

4.2 α_2-Adrenergic regulation of [^{3}H]NA release from rat O.C. and hypothalamic tissue chops

Autoradiographic study shows that both α_1 and α_2-adrenoceptors are widely distributed in the intact rat brain tissue (Young and Kuhar, 1979; 1980). Based on a pharmacological characterization study α-adrenoceptors in rat brain cortex are found to be exclusively of α_2-type (Hedler et al., 1981). However, a 6-hydroxydopamine-induced destruction of noradrenergic nerve terminals increases rather than decreases the number of receptor binding sites of α_2-ligand, clonidine (U'Prichard and Snyder, 1979), thus suggesting a pre- and post-synaptic location of α_2-adrenoceptors.

The present study compared the effect of the α_2-adrenergic agonists, clonidine, BHT-920 and UK 14304-18 with NA on the K^+ stimulation of [^{3}H]NA release from O.C. tissue chops. It was found that α_2-adrenergic agonists inhibit 20mM K^+-stimulated release of [^{3}H]NA with the following order of potency (IC_{50} values in parenthesis), UK 14304-18 (1×10^{-7} M) > clonidine (3.5×10^{-7} M) > BHT-920 (5.5×10^{-7} M) > NA (8.5×10^{-7} M) and a maximum inhibition of 70%, 53%, 37% and 84% respectively achieved at 10^{-5} M concentration of these agonists (Fig. 20). This shows that although UK 14304-18 is the most potent α_2-adrenergic agonist, it is not as effective as NA. UK 14304-18 has also been shown to be more potent than clonidine and NA at regulating [^{3}H]NA release from ileum and pulmonary artery (Cambridge, 1981; Van Meel et al., 1981). Ligand-binding studies in rat brain membranes showed that UK 14304-18 is highly selective for α_2-adrenoceptors as UK 14304-18 (10^{-9} M) displaced bound α_2-adrenergic agonist, [^{3}H] clonidine by 50% whereas a concentration of 10^{-6} M was needed to displace the α_1-adrenergic antagonist, [^{3}H] prazosin by an equivalent amount (Cambridge, 1981).

This confirms that UK 14304-18 is both a selective and potent α_2-adrenergic agonist. Since clonidine (Medgett et al., 1978) and BHT-920 (Lues and Schumann, 1984) have been shown to be partial adrenergic agonist in the smooth muscle, it then suggests that the lower effectiveness of both clonidine and BHT-920 may possibly be due to the pre- and post-synaptic effect of both drugs. In contrast, Chicini and Singer,(1987) showed that BHT-920 acts mainly as presynaptic α_2-adrenergic agonist on the electrical stimulated release of [^{3}H]NA from rat brain cortex and hypothalamus.

The hypothalamic tissue chops, NA and clonidine at submicromolar concentrations (10^{-7}M - 10^{-6}M) inhibited 30mM K^+-stimulated release of [^{3}H]NA to the same extent (Fig. 21). At higher concentrations, however, NA (10^{-4}M) is shown to be more effective than clonidine (10^{-4}M) at inhibiting the 30mM K^+-stimulated release of [^{3}H]NA (Fig. 21). This finding is in agreement with more effective inhibition of 20mM K^+-stimulated release of [^{3}H]NA from the O.C. tissue chops by NA than clonidine. Using superfusion techniques, other researchers have also shown that α_2-adrenergic agonists including NA, clonidine, phenoxybenzamine and oxymetazoline inhibited K^+ and electrical stimulation of [^{3}H]NA release in rat brain slices (Taube et al., 1977; Wemer et al., 1982; Reichenbacher et al., 1982; Chesselet, 1984; Middlemiss, 1988) and synaptosomes (Lane and Aprison, 1977; De Langen et al., 1979). The results presented in this thesis show that the method used to examine α_2-adrenergic agonists inhibition of [^{3}H]NA release gives comparable results to those reported in the literature. The α_2-adrenergic antagonist, yohimbine, not only reversed the inhibition caused by α_2-adrenergic agonists but also (Figs. 22, 25) enhanced the K^+-stimulated release of

[^{3}H]NA from O.C. and hypothalamic tissue chops by 25% and 10% at 10^{-6} M and 10^{-5} concentrations respectively (Figs. 20, 21). This suggests that endogenous NA is able to inhibit the K^+-stimulated release of [^{3}H]NA from O.C. and hypothalamic tissue chops by acting at α_2-adrenoceptors. Similarly, using superfusion technique Wemer et al. (1979) found that α_2-adrenergic antagonists, yohimbine and phentolamine enhanced the K^+-stimulated release of [^{3}H]NA by 60% and 90% at 10^{-5} M concentration respectively from rat frontal cortex brain slices. Thus they concluded that presynaptic α-adrenoceptors are partially activated by released endogenous NA thereby reducing the K^+-evoked release of [^{3}H]NA. This view is supported by the observation that phentolamine does not enhance the release of [^{3}H]NA from synaptosomes since with this preparation endogenous NA is more effectively removed by superfusion medium than from brain slices (De Langen et al., 1979). It is interesting that less inhibition of release appears to take place with the assay used in this study than in the perfusion system used by Wemer et al. (1979). Thus endogenous NA appears to have less effect on release in this study than in the work reported by Wemer et al. (1979).

In contrast to the effect of α_2-adrenergic antagonist yohimbine, in O.C. tissue chops, the α_1-adrenergic antagonist, prazosin (Fig. 24) and β-adrenergic antagonist, propranolol (Fig. 23) did not reverse the NA inhibitory effect of K^+-stimulated release of [^{3}H]NA. The present results are, in agreement with the findings of Taube et al. (1977) and Reichenbacher et al. (1982) who also show that propranolol and prazosin had no effect on the stimulated release of [^{3}H]NA from both rat and rabbit cortical slices. This suggests that in O.C. tissue chops, only the α_2-adrenoceptors are involved in the

regulation of K^+-stimulated release of [^{3}H]NA. Furthermore, in O.C. tissue chops, propranolol increased the inhibitory effect of NA when the concentration of NA increased from 10^{-6}M to 10^{-5}M (Fig. 23). Similarly, in the peripheral nervous system, NA inhibition of K^+-stimulated release of [^{3}H]NA increased when the concentration of propranolol was increased (see Langer, 1981 for review). One possible explanation is that propranolol which block the β-adrenoceptors increases the effective concentrations of NA in the vicinity of the nerve terminals, thereby allowing more NA to activate α_2-adrenoceptors which leads to a greater inhibition of [^{3}H]NA release. This can be depicted diagrammatically as shown in Figure 53. Receptor binding studies showed that the density of β-adrenoceptors in rat cerebral cortex is about 50fmol/mg protein (Dibner et al., 1979).

Unlike O.C. tissue chops, Ueda et al. (1983) demonstrates that the release of endogenous NA in the hypothalamus may be regulated via presynaptic α_2 as well as β_1 and β_2-adrenoceptors. Thus, they found that not only yohimbine but also isoprenaline enhanced electrical stimulation (2HZ) of endogenous NA release. In contrast, the α_1-adrenergic antagonist, prazosin only weakly enhanced NA release. Furthermore, the stimulation of release by β-adrenergic agonist, isoprenaline is antagonised by the β_1-adrenergic antagonist, atenolol and the β_2-adrenergic antagonist, butoxamine. These studies of Ueda et al. (1983) suggest the a role for β_1 and β_2-adrenoceptors in the regulation of [^{3}H]NA release.

It is generally accepted that during depolarization, adenosine (Kuroda, Y. and McIlwain, H., 1979; Fredholm and Hedqvist, 1980) and ATP, ADP and AMP (McIlwain, 1977; Burnstock, 1981) released from the

nerve terminals might themselves function as neurotransmitters and cause presynaptic inhibition or regulate the release of other neurotransmitter systems such as noradrenaline (Harms et al., 1979; Jonzon and Fredholm, 1984; Allgaier et al., 1987), dopamine (Harms et al., 1979; Javis and Williams, 1987) and acetylcholine (Pedata et al., 1983; Corradetti et al., 1984).

The present study demonstrates that the adenosine A_1 agonist, CHA (Fredholm and Dunwiddie, 1988) inhibited the 20mM K^+-stimulated release of [^{3}H]NA more potently than adenosine (Fig. 26) from the O.C. tissue chops. Additionally, adenosine inhibition of K^+-stimulated release is reversed by IBMX (Fig. 27) but not by the PDE-inhibitor RO 20-1724 (Fig. 28) suggesting that IBMX acts as an adenosine antagonist as well as a PDE inhibitor. Furthermore, the fact that IBMX is able to further enhance the K^+-stimulated release of [^{3}H]NA in the presence of 10^{-6}M adenosine by 20% (Fig. 27) suggests the possible involvement of released endogenous adenosine in the regulation of K^+ stimulation of [^{3}H]NA release. The above findings are in agreement with those reported by other researchers which show that adenosine and ATP inhibition of depolarization stimulated release of [^{3}H]NA can be antagonised by methylxanthines including theophylline, IBMX and caffeine (Phillis et al., 1979; Fredholm, 1980).

This view is supported by the observation that the selective adenosine A_1 antagonist, CPDPX (Martison et al., 1987) not only more potently reverses the adenosine inhibitory effect, but at concentrations greater than 10^{-5}M further enhances the 20mM K^+ stimulated release of [^{3}H]NA by 10% (Fig. 29). However, the finding that IBMX which reverses the adenosine inhibition of 20mM K^+-stimulated release of [^{3}H]NA does not reverse the α_2-adrenergic agonist, NA inhibition of 20mM K^+-stimulated release of [^{3}H]NA (Fig. 30) suggests that endogenous adenosine does inhibition [^{3}H]NA release.

4.3 The possible role of cAMP in mediating the α_2-adrenergic regulation of NA release

α_2-Adrenoceptors have been shown to inhibit cAMP formation in adipocytes (Aktories et al., 1980; Burns et al., 1982), neuronal cell NG 108/15 (Sabol and Nirenberg, 1979; Kahn et al., 1982), platelets (Jakobs et al., 1976) and rat pancreatic islets (Yamazaki et al., 1982). In view of this, a likely mechanism for α_2-adrenergic agonist inhibition of NA release is that it is mediated by decreasing cAMP levels. However, G protein linked to the inhibition of voltage sensitive Ca^{2+} channels and G_K protein linked to the regulation of K^+ channels have recently been described (Sasaki and Sato, 1987; Logothetis et al., 1987) Allagier et al. (1985) demonstrated that pretreatment of rabbit hippocampus brain slices with pertussis toxin (IAP) which ADP-riboxylates the G-protein of the cell membrane or N-ethylmaleimide (NEM) (Allagier et al., 1986) which inactivates G-protein reduces the effect of both α_2-adrenergic agonist, clonidine and α_2-adrenergic antagonist, phentolamine response on the electrical stimulated release of [^{3}H]NA. This raises the possible role of cAMP in the regulation of NA release. Thus, the present study sought to provide evidence for a role of cAMP by studying the effect of (a) conditions that increase cAMP i.e. of cAMP analogue db-cAMP, PDE-inhibitors to prevent the catabolism of cAMP or by direct activation of the catalytic unit of adenylate cyclase by forskolin on α_2-adrenergic inhibition of NA release and (b) a comparison between the conditions under which α_2-adrenergic agonists inhibit NA release and cAMP formation. Thus, if α_2-adrenergic agonists inhibit NA release by inhibiting cAMP formation, then a correlation between these two actions of α_2-adrenergic agonists would be predicted.

4.3.1 Influence of cAMP on α_2-adrenoceptors regulated release of [^{3}H]NA from O.C. and hypothalamic tissue chops

The present study shows that forskolin, db-cAMP and IBMX enhance the 20mM K^+-stimulated release of [^{3}H]NA from O.C. tissue chops by 20.8%, 29.0% and 19.0% respectively (Table 5) but had no effect on the basal release of [^{3}H]NA. In addition, when forskolin or db-cAMP is combined with IBMX, the release of [^{3}H]NA stimulated by 20mM K^+ is further increased by 10% (Table 6). Similarly, in hypothalamic tissue chops, dbcAMP or forskolin when combined with IBMX enhance the 25mM but not the 30mM K^+-stimulated release of [^{3}H]NA by 25.5% and 23.6% respectively (Table 8A and B). This suggests that cAMP may be involved in the regulation of depolarization-evoked release of [^{3}H]NA from rat O.C. and hypothalamic tissue chops. These results are in agreement with the finding that substances which increase intracellular cAMP formation such as cAMP analogues including db-cAMP and 8-Br-cAMP as well as PDE-inhibitors including IBMX, 7-benzyl-IBMX, ZK 62771 and RO 20-1724, or forskolin enchanced the electrical-stimulated release of [^{3}H]NA from rat brain slices (Markstein et al., 1984; Schoffelmeer et al., 1985, 1986) and synaptosomes (Schoffelmeer et al., 1985). On the contrary, veratrine or 13mM K^+-induced release of [^{3}H]NA from rat neocortical slices, is enhanced only by cAMP analogues and forskolin while PDE inhibitors including IBMX, ZK 62771 and RO 20-1724 inhibited the stimulated release of [^{3}H]NA (Schoffelmeer and Mulder, 1983b).

The difference between the effect of PDE inhibitors on electrical stimulation and K^+ or veratrine-induced release of [^{3}H]NA may be related to the differences in the degree of stimulation. An alternative explanation for the effect of IBMX on K^+-stimulated release of [^{3}H]NA between the present study and that of Schoffelmeer

et al. (1985), is that IBMX may penetrate better into the neocortical rat brain slices used in the study of Schoffelmeer et al. (1985) compared to the cortical brain slices obtained from adult rats used in the present study. In support of this although IBMX inhibited the 13mM K^+-stimulated release of [^{3}H]NA, theophylline, an adenosine antagonist, which does not normally appreciably penetrate cell membranes enhances the stimulated release of [^{3}H]NA (Schoffelmeer et al., 1985). The present study suggests that IBMX enhances the 20mM K^+-stimulated release of [^{3}H]NA by acting as an adenosine antagonist. Furthermore, in agreement with the findings of Schoffelmeer et al. (1983b), RO 20-1724 which inhibits cAMP-PDE selectively (Daly, 1977) but does not act as adenosine antagonist, is found to inhibit the K^+-stimulated release of [^{3}H]NA by about 20% (Table 7). The result suggests that RO 20-1724 may affect the K^+-stimulated release of [^{3}H]NA possibly via other unknown release mechanisms.

If α_2-adrenergic agonists inhibit the K^+-stimulated release of [^{3}H]NA by decreasing the intracellular cAMP formation, it would then be anticipated that the increase in intracellular cAMP by db-cAMP or forskolin could counteract the inhibitory effect of the agonist. However, the present study demonstrates that α_2-adrenergic agonist inhibition of 20mM K^+-stimulated release of [^{3}H]NA from O.C. tissue chops is partially reversed by db-cAMP either in the absence (Table 5) or presence (Table 6) of IBMX, but unexpectedly, not by forskolin either in the absence (Table 5) or presence (Table 6) of IBMX. In hypothalamic tissue chops α_2-adrenergic agonist inhibition of 25mM K^+-stimulated release of [^{3}H]NA is not reversed by either db-cAMP (Fig. 8) or forskolin (Fig. 8) combined with IBMX suggesting that unlike O.C. tissue chops changes in intracellular cAMP

levels by db-cAMP do not affect the α_2-adrenergic agonist inhibitory effect on the 25mM K^+-stimulated release of [^{3}H]NA from hypothalamic tissue chops.

Rabe et al. (1982) demonstrate that unstimulated release of [^{3}H]NA from clonal pheochromocytoma cell line, PC 12 is not affected by any concentration of forskolin. However, forskolin up to 10μM concentration is able to enhance the 50mM K^+-stimulated release of [^{3}H]NA, while higher concentration (10^{-4} M) of forskolin tends to inhibit [^{3}H]NA release. Their results thus suggest a modulatory role of forskolin on [^{3}H]NA release. This may provide an explanation for the failure of forskolin to reverse the α_2-adrenergic agonists inhibition of K^+-stimulated release of [^{3}H]NA observed in the present study.

The inhibition of α_2-adrenergic agonists on K^+ stimulated release of [^{3}H]NA from O.C. tissue chops is very rapid (Figs. 17, 18). The fact that a minimum incubation period of 30 minutes with very high concentration of db-cAMP (10^{-3} M) is required to partially reverse the α_2-adrenergic agonists inhibition of K^+-stimulated release of [^{3}H]NA suggest that other mechanisms may be involved (Table 5). Electrophysiological studies show that neurotransmitters including NA, 5-HT and GABA which do not have any effect on Na^+ and K^+ voltage-sensitive channels, inhibits the voltage sensitive Ca^{2+} channels in chick sensory neurons (Dunlap and Fishback, 1981). Furthermore, db-cAMP has been shown to cause a prolonged hyperpolarization of neuronal membranes, and hence a prolongation of Ca^{2+} influx, thereby preventing the inhibitory effect of these neurotransmitters on voltage sensitive Ca^{2+} channels (Reuter, 1983). This may be an alternative suggestion for the present study to explain

why db-cAMP was able to partially reverse the α_2-adrenergic agonists inhibitory effect on K^+-stimulated release of [^{3}H]NA from the O.C. tissue chops but not forskolin. One interpretation of the data obtained in the present study is that α_2-adrenergic agonists may inhibit the K^+-stimulated release of [^{3}H]NA via G-protein linked to voltage sensitive Ca^{2+} channels.

4.3.2 Stimulation of cAMP formation

4.3.2.1 Stimulation of β-adrenergic agonist isoprenaline, α and β-adrenergic agonist, NA and adenosine

The present study demonstrates that isoprenaline stimulated cAMP formation 1.5 fold (Fig. 31), whereas NA stimulated cAMP formation about 3.0 fold (Fig. 31). Furthermore, isoprenaline stimulation of cAMP formation is potentiated by α_1-adrenergic agonist, phenylephrine (Fig. 50) but not by α_2-adrenergic antagonist, UK 14304-18 (Fig. 50). This is in agreement with the findings of Etgen et al. (1987) who also show that isoprenaline stimulation of cAMP formation is enhanced by phenylephrine but not clonidine. Thus, the results suggest that the greater stimulation of cAMP formation observed with NA compared with isoprenaline is due to the synergistic effect of α_1 and β -adrenergic agonist.

From the present study, NA stimulation of cAMP formation is not affected by phenylephrine (Fig. 49) but is inhibited by α_2-adrenergic agonists in a biphasic manner with the following order of potency (IC_{50} values in parenthesis) UK 14304-18 (6×10^{-9} M) > clonidine (10^{-8} M) > BHT-920 (5×10^{-7} M). This suggests that there could be two subclasses of α_2-adrenoceptors blocking the response of NA-stimulation of cAMP formation.

NA stimulation of cAMP formation is more potently blocked by α_1-adrenergic antagonist, prazosin than α_2-adrenergic antagonist, yohimbine and β-adrenergic antagonist, propranolol (Fig. 34). The result is supported by the findings of Perkin and Moore (1973), Davis et al. (1978), Daly et al. (1980) and Johnson and Minneman (1986) who also reported that α_1-adrenergic antagonist is more potent than α_2-adrenergic antagonist at inhibiting the NA stimulation of cAMP formation in rat brain slices. Thus, the failure of α_2-adrenergic antagonist to enhance the NA stimulation of cAMP formation argues against the involvement of α_2-adrenoceptors in NA stimulatory effect of cAMP formation. This further confirms the present suggestion that NA stimulation is due to the synergistic effect of α_1 and β-adrenergic agonist.

Evidence of a potentiation of β-stimulation by α_1-adrenoceptor is also observed in membrane particulate fractions prepared from guinea pig cortex (Daly et al., 1980). In contrast, cultures of astrocytes isolated from perinatal mouse brain (Van Calker et al., 1978; 1979) or rat cerebral cortical tissue (McCarty and De Vellis, 1978) do not show α_1 augmentation of β-adrenergic agonist stimulation of cAMP formation since a greater increase in cAMP formation is observed with isoprenaline alone. Furthermore, isoprenaline stimulation of cAMP formation is inhibited by NA, and the effect of which can be reversed by α_2-adrenergic antagonists such as phentolamine and phenoxybenzamine (Van Calker et al., 1978). In addition, NA stimulation of cAMP formation can be further enhanced by phentolamine (McCarthy and De Vellic, 1978). Unlike brain slices, these results suggest α_2-adrenoceptors in astrocytes cultures are linked to the adenylate cyclase.

In contrast, Pilc and Enna (1986) reported that isoprenaline stimulation of cAMP formation is not potentiated by phenylephrine but by UK 14304-18. Furthermore, they reported that isoprenaline and α-adrenergic agonist (6-fluoronorepinephrine) stimulation of cAMP formation is more potently blocked by yohimbine than by prazosin. The inhibitor results of the present study taken together with those of other researchers (Perkin and Moore, 1973; Davis et al., 1978; Daly et al., 1980; Daly et al., 1981; Johnson and Minneman, 1986; Etgen et al., 1987) do not support the results obtained by Pilc and Enna, (1986) which suggest that NA stimulation of cAMP formation is due to the synergistic effect of β and α_2-adrenergic agonist.

Since cAMP formation in brain slices may continuously be catabolised by PDE, the effect of PDE on isoprenaline and NA stimulation of cAMP formation were examined with PDE inhibitors IBMX (Well and Kramer, 1981) and RO 20-1724 (Schwabe et al., 1978). The results show that surprisingly IBMX inhibited the isoprenaline and NA (Fig. 32) stimulation of cAMP formation, whereas RO 20-1724 although raising the basal level of cAMP did not alter either the EC_{50} values i.e. 4 x 10^{-6}M and 55 x 10^{-9}M or the overall stimulation i.e. 1.5 fold and 3 fold of cAMP formation stimulated by isoprenaline and NA respectively (Fig. 33). Since IBMX can act as a potent adenosine antagonists (Stiles, 1986), the abolition by IBMX of NA and isoprenaline stimulation of cAMP formation suggests that endogenous adenosine potentiates the action of β-adrenergic agonist on cAMP formation in rat brain slices. The above observations are in agreement with Daly et al. (1980) who show that NA, isoprenaline, histamine, dopamine, serotonin and adenosine stimulation of cAMP formation in guinea pig cerebral cortex particulate preparation is inhibited by adenosine antagonists, theophylline and

IBMX, but not potentiated by PDE inhibitor ZK 62771. Furthermore, Daly et al. (1980) in rat cerebral cortical slices also found that adenosine is necessary for a α-response.

In the present study, IBMX inhibited adenosine stimulation of cAMP formation, causing a shift to the right of adenosine concentration curve (Fig. 16), while RO 20-1724, although raising the basal level of cAMP formation does not affect the EC_{50} value (6×10^{-5} M) of adenosine (Fig. 36). Indeed, NA stimulation of cAMP formation is further potentiated by 0.9 fold in the presence of adenosine (Fig. 37). This agrees with the hypothesis that adenosine potentiates NA effects in rat brain.

Interestingly when NA stimulation of cAMP formation is potentiated by adenosine, adrenergic antagonists have a different order of potency and efficacy (Fig. 38) when compared with their action of NA stimulation along (Fig. 34). Thus (IC_{50} values in parenthesis) yohimbine (8×10^{-9} M) > prazosin (2.5×10^{-9} M) > propranolol (6×10^{-7} M) and inhibited cAMP formation by 40%, 30% and 22% respectively at 10^{-5} M concentration (Fig. 38). This suggests that yohimbine is the most effective and potent at inhibiting the adenosine combined with NA stimulation of cAMP formation. This is supported by the findings of Schultz and Kleefeld (1979) who show that adenosine combined with NA stimulation of cAMP formation is inhibited more by phentolamine than by propranolol. In addition, the present study also demonstrates that adenosine stimulation of cAMP formation is potentiated by UK 14304-18 (Fig. 51). On the contrary, in the absence of adenosine, NA stimulation of cAMP formation is most effectively blocked by propranolol. Thus, the results suggest that adenosine potentiates NA stimulation of cAMP formation by modulating the cell surface receptors.

4.3.2.2 Stimulation of cAMP formation by forskolin

Forskolin which is known to increase cAMP formation by directly activating the catalytic unit of the adenylate cyclase (Seamon and Daly, 1981, 1983; Daly et al., 1982; Bender et al., 1984) stimulated cAMP formation in a concentration-dependent manner (Fig. 31) with EC_{50} value of 5×10^{-6} M and a maximum stimulation of 6 to 8 fold cAMP formation achieved at 10^{-4} M either alone or in the presence of PDE inhibitors including IBMX (Fig. 32) and RO 20-1724 (Fig. 33). Therefore this suggests that in brain slices PDE activity is low compared to cell culture where PDE-inhibitors are found to enhance cAMP formation (Van Calker et al., 1978; Wojcik and Neff, 1984). In addition, from the above study, it can be concluded that failure of α_2-adrenergic agonist to inhibit the K^+-stimulation of [^{3}H]NA release could not be due to the failure of forskolin to stimulate cAMP formation in the O.C. tissue chops used in the present study, since there are considerable increasedin cAMP formation with the concentrations of forskolin used to try and reverse α_2-adrenergic inhibition. Forskolin (10^{-6} M and 5×10^{-6} M) which itself stimulated cAMP formation, is only able to increase NA, isoprenaline and adenosine stimulation of cAMP formation in an additive manner (Fig. 39). On the contrary, at submicromolar concentration (10^{-7} M), forskolin, which itself does not have any effect on cAMP formation potentiated NA (10^{-5} M), isoprenaline (10^{-6} M) and adenosine (10^{-4} M) stimulation of cAMP formation by 0.7, 0.9 and 1.5 fold respectively (Fig. 39). Forskolin (10^{-6} M) has also been shown to potentiate cAMP formation by histamine, serotonin, NA, isoprenaline, adenosine, prostaglandin E2 and vasoactive intestinal peptide in rat and guinea pig cerebral cortical slices as well as in rat striatal slices (Daly et al., 1982; Seamon and

Daly, 1983). It has been proposed that at low concentration, forskolin acts synergistically with the high affinity sites of Gs, which then potentiate the receptors effect on cAMP formation (Daly et al., 1982; Seamon and Day, 1983; Seamon and Wetzel, 1984; Barovsky et al., 1984, 1985).

Forskolin stimulation of cAMP formation in human adipocytes (Burns et al., 1982, 1987), human platelets (Insel et al., 1982) and in rat cerebral cortical membranes (Kitamura et al., 1985) have been shown to be inhibited by α_2-adrenergic agonists such as clonidine, NA and adrenaline. Furthermore, using rat neonatal cerebral cortical slices, Duman and Enna, (1986) reported that α_2-adrenergic agonists including 6-fluoronorepinephrine, clonidine and UK 14304 inhibited forskolin stimulation of cAMP formation in a concentration dependent manner, while α_1-adrenergic agonist, phenylephrine slightly potentiated the forskolin response. Thus all the above findings suggest that α_2-adrenergic agonist is directly linked to Gi and thus to the catalytic unit of adenylate cyclase.

In contrast, the present study found that the α_2-adrenergic agonist, clonidine (Fig. 47) and UK 14304-18 (Fig. 46) were unable to inhibit forskolin stimulation of cAMP formation in O.C. rat brain slices. This is a surprising observation in view of reports that (a) forskolin stimulation is inhibited by α_2-adrenergic agonist and (b) there are plenty of α_2-adrenoceptors in cortex (Rouot et al., 1980; Loftus et al., 1984; Boyajian et al., 1987; Boyajian and Leslie, 1987). This suggests that not all α_2-adrenergic receptors are linked to adenylate cyclase via Gi.

4.3.2.3 Effect of depolarizing agents on cAMP formation

Electrical stimulation (Kakinchi et al., 1968a, b; Zanella and Rall, 1973), K^+ depolarisation (Shimiju and Daly, 1972) as well as

other depolarizing agents including batrochotoxin, veratine and ouabain (Shimizu et al., 1970) have been shown to stimulate cAMP formation in rat brain slices.

The present study demonstrates that K^+ depolarization (40 - 80mM) stimulated cAMP formation maximally between 2.2 and 2.5 fold (Fig. 40) in rat O.C. tissue chops. High K^+ concentrations (100-140mM) decreases cAMP formation (Fig. 40) possibly due to influx of Ca^{2+} which activates the calcium/calmodulin dependent PDE (Hemmings et al., 1986).

In contrast, in guinea pig cerebral cortex slices, K^+ depolarization (140mM) stimulated cAMP formation by 48 fold, while maximum K^+ stimulation (150mM) in the absence of Na^+ only slightly reduced the K^+ response (Shimizu et al., 1972). This could simply be due to the difference of Ca^{2+} sensitivity of PDE between different animal slices. In addition, K^+, batrochotoxin, ouabain, veratine stimulation of cAMP formation are dependent on Ca^{2+} (Shimizu et al., 1970; Zanella et al., 1973; Ferrendelli et al., 1976; Schwabe et al., 1978). An alternative explanation is that low Ca^{2+} activates while high Ca^{2+} inhibit adenylate cyclase activity (Brostrom et al., 1977).

Forskolin (10^{-6}M) stimulated cAMP formation by 6 fold under non depolarizing (5mM K^+) and mild depolarizingf (20mM K^+) conditions (Fig. 43). However, when combined with 40mM and 60mM K^+, its response on cAMP formation is reduced to 50% and 100% respectively (Fig. 43). This suggests that the influx of Ca^{2+} during depolarization may activate the calcium/calmodulin dependent PDE (Hemmings et al., 1986), thereby leading to a decrease in cAMP formation. This is confirmed by the finding that RO 20-1724 (10^{-3}M)

completely reverses the effect of forskolin combined with 40mM K^+ stimulation of cAMP formation (Fig. 44).

Cocaine, a potent NA uptake inhibitor (Carmichael and Israel, 1973) has been shown to inhibit K^+, ouabain, veratine, NA, histamine and adenosine stimulation of cAMP formation (Shimizu et al., 1972). Since DMI, which is used as NA uptake inhibitor in the study of [^{3}H]NA release, its effect on K^+ stimulation of cAMP formation in the O.C. tissue chops is examined. The present study demonstrates that 20mM and 40mM K^+ stimulation of cAMP formation is not affected by DMI (1uM) (Fig. 42).

The present study demonstrates that UK 14304-18 does not inhibit the K^+ stimulation of cAMP formation either alone or in the presence of RO 20-1724 (Fig. 41). Since depolarization is mainly due to the neuronal event rather than glial, and the proportion of neurons to glia population is not determined, it may therefore be difficult to pick up a small α_2-effect situated on the nerve terminals during K^+-stimulation of cAMP formation.

In conclusion, the findings that activation of α_2-adrenoceptors by α_2-adrenergic agonist leads to large inhibition of K^+-stimulated release of [^{3}H]NA, and the failure of the agonist to inhibit K^+-stimulation of cAMP formation under parallel experimental conditions suggests that there is little correlation between cAMP inhibition and α_2-adrenergic inhibition on [^{3}H]NA release.

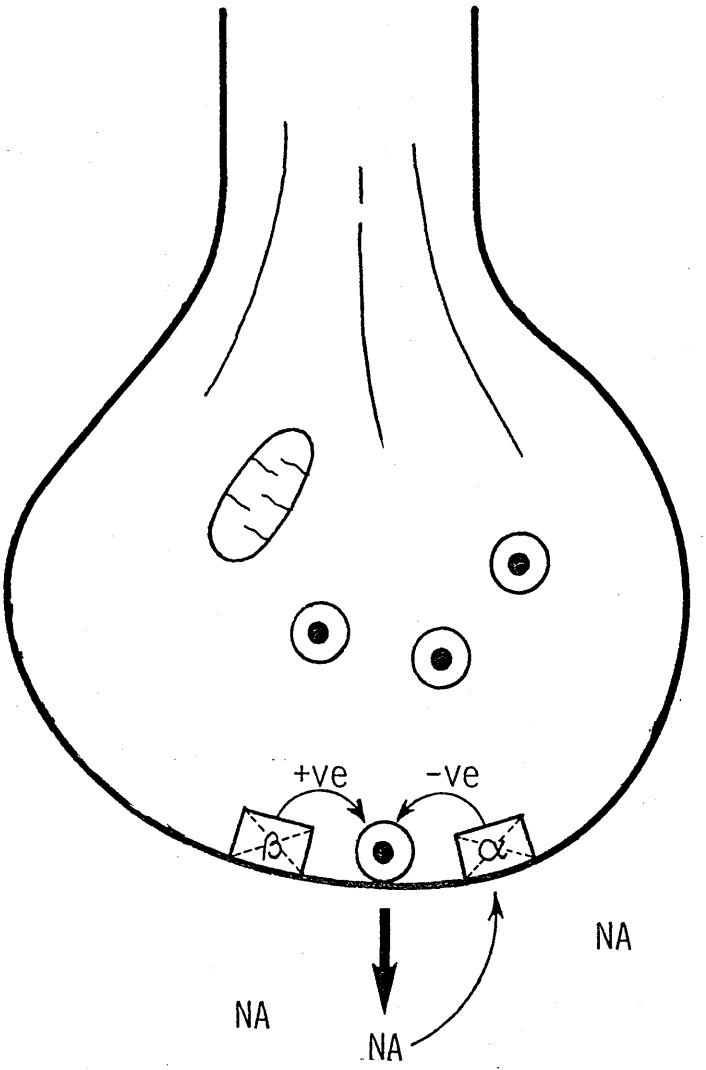


Figure 53 Schematic representation of presynaptic autoinhibition

α_2-Adrenoceptors acted upon by NA in the presence of β-adrenergic antagonist, propranolol on the noradrenergic nerve terminals in the CNS.

5 CONCLUSION

The ability of forskolin and db-cAMP to enhance K^+-stimulated release of [^{3}H]NA suggests a role for cAMP in synaptic transmission. There is no evidence that α_2-adrenergic agonists inhibit K^+-stimulated release of [^{3}H]NA by inhibiting cAMP formation, since forskolin does not reverse the α_2-adrenergic inhibitory effect. α_2-Adrenergic agonist inhibition of K^+-stimulated release of [^{3}H]NA is very rapid. However, the fact that db-cAMP is only able to partially reverse the α_2-adrenergic agonist inhibitory effect over a period of 30 minutes incubation at a high concentration suggests that other mechanisms may be involved. Furthermore, the failure of α_2-adrenergic agonists to inhibit forskolin, isoprenaline, adenosine and K^+ stimulation of cAMP formation under parallel experimental conditions indicates that there is no correlation between cAMP and α_2-adrenergic agonists inhibitory effect on [^{3}H]NA release. In contrast, α_2-adrenergic agonists are found to inhibit the NA stimulation of cAMP formation in a biphasic manner. This suggests that different pools of α_2-adrenoceptors are present. However, the fact that α_2-adrenergic antagonists do not enhance the NA stimulation of cAMP formation suggests that different mechanisms may be involved.

My results however support the increasing evidence in the literature which suggests that α_2-adrenoceptors may be directly linked to voltage-sensitive Ca^{2+} channels via G-proteins, since α_2-adrenergic inhibitory effects on [^{3}H]NA release can only be observed during depolarization.

REFERENCES

Adler-Graschinsky, E. and Langer, S.Z. (1975) Br. J. Pharmacolol. 53, 43-50.

Adolfsson, R., Gootfries, C.G., Roose, B.E. and Winblad, B. (1979) Br. J. Psychiatry 135, 216-223.

Aghajanian, G.K. and Van der Maalen, C.P. (1982) Science 215, 1394-1396.

Ahlquist, R.P. (1948) Am. J. Physiol. 153, 586-600.

Aktories, K., Schultz, G. and Jakobs, K.H. (1980) Naunyn-Schmiedeberg's Arch. Pharmacol. 312, 167-173.

Alberts, P., Bartfai, T. and Stjarne, L. (1981) J. Physiol. (Lond.) 312, 297-334.

Allgaier, C., Feuerstein, T.J., Jackisch, R. and Hertting, G. (1985) Naunyn-Schmiedeberg's Arch. Pharmacol. 331, 235-239.

Allgaier, C., Feuerstein, T.J. and Hertting, G. (1986) Naunyn-Schmiedeberg's Arch. Pharmacol. 333, 104-109.

Allgaier, C., Hertting, G. and Kugelgen, O.V. (1987) Br. J. Pharmacol. 90, 403-412.

Arnold, E.B., Molinoff, P.B. and Rutledge, C.O. (1977) J. Pharmacol. Exp. Ther. 202, 544-557.

Baker, P.F., Meves, H. and Ridgway, E.B. (1973) J. Physiol. (Lond.) 231, 527-548.

Barovsky, K., Pedone, C. and Brooker, G. (1984) Mol. Pharmacol. 25, 256-260.

Barovsky, K. and Brooker, G. (1985) Mol. Pharmacol. 28, 502-507.

Bartschat, D.K. and Blaustein, M.P. (1985) J. Physiol. (Lond.) 361, 419-440.

Bender, J.L., Wolf, L.G. and Neer, E.J. (1984) Advances in Cyclic Nucleotide and Protein Phosphorylation Research 17, 101-109.

Bennett, B.A. and Sundberg, D.K. (1981) Life Sci. 28, 2811-2817.

Berridge, M.J. and Irvine, R.F. (1984) Nature 312, 315-321.

Berthelsen, S. and Pettinger, A. (1977) Life Sci. 21, 595-606.

Bevan, S. and Raff, M. (1985) Nature 315, 229-230.

Bhat, S.V., Bajwa, B.S., Dornauer, H. and de Souza, N.J. (1977) Tetrahydron Lett. 19, 1669-1672.

Bianchi, B.R. and Takimoto, G.S. (1984) Life Sci. 34, 607-615.

Biel, J.H. and Lum, B.B. (1966) Prog. Drug. Res. 10, 46P.

Blatz, A.L. and Magleby, k.L. (1986) Nature 323, 442-444.

Blaustein, M.P. (1975) J. Physiol. (Lond.) 247, 617-655.

Blaustein, M.P. (1979) (ed. Paton, D.M.) pp 39-58, Permagon Press, Oxford.

Blinks, J.R., Prendergast, F.G. and Allen, D.G. (1976) Pharmacol. Rev. 28, 1-93.

Bloom, F.E. (1975) Rev. Physiol. Biochem. Pharmacol. 74, 1-103.

Bowman, W.C. and Rand, M.J. (1980a) Textbook of Pharmacol., 2nd edition, Blackwell Scientific Publication, PP5.22-5.23.

Bowman, W.C. and Rand, M.J. (1980b) Textbook of Pharmacol., 2nd edition, Blackwell Scientific Publication, PP9.10-9.14.

Boyajian, C.L., Loughlin, S.E. and Leslie, F.M. (1987a) J. Pharmacol. Exp. ther. 241, 1079-1091.

Boyajian, C.L. and Leslie, F.M. (1987b) J. Pharmacol. Exp. Ther. 241, 1092-1098.

Bradford, H.F. (1986a) Chemical Neurobiology, Freeman, W.H. and Company, N.Y., 195P.

Bradford, H.F. (1986b) Chemical Neurobiology, Freeman, W.H. and Company, N.Y. PP265-302.

Brooker, G. Pedone, C. and Barovsky, K. (1983) Science 220, 1169-1170.

Brostrom, C.O., Brostrom, M.A. and Wolff, D.J. (1977) J. Biol. Chem. 252(16), 5677-5685.

Burnstock, G. (1981) J. Physiol. (Lond.) 313, 1-35.

Burns, T.W., Langley, P.E., Terry, B.E., Bylund, D.B. and Forte, L.R. (1982) Life Sci. 31, 815-821.

Burns, T.W., Langley, P.E., Terry, B.E., Bylund, D.B. and Forte Jr., L.R. (1987) Life Sci. 40, 145-154.

Bylund, D.B. and Snyder, S.H. (1976) Mol. Pharmacol. 12, 568-580.

Bylund, D.B.(1985) Pharmacol. Biochem. Behav. 22, 835-843.

Bylund, D.B. (1988) Trends Pharmacol. Sci. 9, 356-361.

Cambridge, D. (1981) Eur. J. Pharmacol. 72, 413-415.

Cambridge, D., Davey, M.J. and Massingham, R. (1977) Br. J. Pharmacol. 59, 514-515.

Cambridge, D. and Davey, M.J. (1980) Br. J. Pharmacol. 69, 345-346.

Carmichael, F.J. and Israel, Y. (1973) J. Pharmacol. Exp. Ther. 186, 253-260.

Cedarbaum, J.M. and Aghajanian, G.K. (1976) Brain Res. 112, 413-419.

Cedarbaum, J.M. and Aghajanian, G.K. (1977) Eur. J. Pharmacol. 44, 375-385.

Celuch, S.M., Dubocovich, M.L. and Langer, S.Z. (1978) Br. J. Pharmacol. 63, 97-108.

Cherubini, E., North, R.A. and Surprenant, A. (1984) Br. J. Pharmacol. 83, 3-5.

Chesselet, M.J. (1984) Neurosci. 12(2), 347-357.

Cheung, W.Y. (1980) Science 207, 19-27.

Cichini, G. and Singer, E.A. (1987) Naunyn-Schmiedeberg's Arch. Pharmacol. 335(6), 613-617.

Clonet, D.H. and Ratner, M. (1970) Science 168, 854-856.

Cooper, D.M.F., Londos, C. and Rodbell, M. (1980) Mol. Pharmacol. 18, 598-601.

Corradetti, R., Lo Conte, G., Moroni, F., Passani, M.B. and Pepeu, G. (1984) Eur. J. Pharmacol. 104, 19-26.

Cotman, C.W., Haycock, J.W. and White, W.F. (1976) J. Physiol. (Lond.) 254, 475-505.

Cox, J.A., Malnoe, A. and Stein, E.A. (1981) J. Biol. Chem. 256, 3218-3223.

Cox, J.A. (1988) J. Biol. Chem. 249, 621-629.

Cross, A.J., Crow, T.J., Perry, E.K. and Perry, R.H. (1981) Br. Med. J. 282, 93-94.

Dahlof, C., Ljung, B. and Ablad, B. (1978) Eur. J. Pharmacol. 50, 75-78.

Daly, J.W. (1977) Int. Rev. Neurobiol. 20, 105-168.

Daly, J.W., McNeal, E., Partington, C., Neuwirth, M. and Creveling, C.R. (1980) J. Neurochem. 32(2), 326-337.

Daly, J.W., Padgett, W., Nirnikitpaisan, Y., Creveling, C.R., Cantacuzene, D. and Kirk, K.L. (1980) J. Pharmacol. Exp. Ther. 212, 382-389.

Daly, J.W., Padgett, D. and Seamon, K.B. (1982) J. Neurochem. 38, 532-544.

Daly, J.W. (1984) Advances in Cyclic Nucleotide and Protein Phosphorylation Res. 17, (ed. Greengard, P. et al.), Raven Press, N.Y.

Daniell, L.C. and Leslie, S.W. (1986) J. Neurochem. 46, 249-256.

Daum, P.R., Hill, S.J. and Young, J.M. (1982) Br. J. Pharmzcol. 77, 347-357.

Davis, J.N., Arnett, C.D., Hoyler, E., Stalvey, L.P., Daly, J.W. and Skolnick, P. (1978) Brain Res. 159, 125--135.

De Belleroche, J.S., Bradford, H.F. and Jones, D.G. (1976) J. Neurochem. 28, 561-571.

De Champlain, J., Krakoff, L.R. and Axelrod, J. (1967) Circulation Res. 20, 136-145.

De Langen, C.D.J., Hogenboom, F. and Mulder, A.H. (1979) Eur. J. Pharmacol. 60, 79-89.

De Riemer, S.A., Strong, A.J., Albert, K.A., Greenfard, P. and Kaczmarek, L.K. (1985) Nature 313, 313-316.

Deutsch, C., Drown, C., Rafalowska, U. and Silver, I.A. (1981) J. Neurochem. 36, 2063-2072.

Dibner, K.D. and Molinoff, P.B. (1979) J. Pharmacol. Exp. Ther. 210(3), 433-439.

Dismukes, K., De Boer, A.A. and Mulder, A.H. (1977) Naunyn-Schmiedeberg's Arch. Pharmacol. 299, 115-122.

Dolphin, A.C. and Archer, E.R. (1983) Neurosci. Lett. 43, 49-54.

Doxey, J.C., Smith, C.F.C. and Walker, J.M. (1977) Eur. J. Pharmacol. 36, 313 - 320.

Drew, G.M. (1978) Br. J. Pharmacol. 64, 293-300.

Dubocovich, M.L. and Langer, S.Z. (1976) J. Pharmacol. Exp. Ther. 198, 83-101.

Dubocovitch, M.J. (1979) PP29-36, Permogon Press, Oxford.

Duman, R.S. and Enna, S.J. (1986) Brain Res. 384, 391-394.

Duman, R.S., Karbon, E.W., Harrington, C. and Enna, S.J. (1986) J. Neurochem. 47, 800-810.

Dunlap, K. and Fischbach, G.C. (1981) J. Physiol. (Lond.) 317, 519-535.

Dunwiddie, T.V., Hoffer, B.J. and Fredholm, B.B. (1981) Naunyn-Schmiedeberg's Arch. Pharmacol. 316, 326-330.

Eckstein, F., Cassel, D., Levkovitz, H., Lowe, M. and Selinger, Z. (1979) J. Biol. Chem. 254, 9829-9834.

Ellison, D.W. and Campbell, I.C. (1986) J. Neurochem. 46, 218-223.

Ernsberger, P., Meeley, M.P., Mann, J.J. and Reis, D.J. (1987) Eur. J. Pharmacol. 134, 1-13.

Etgen, A.M. and Petitti, N. (1987) J. Neurochem. 49, 1732-1739.

Ewald, D.A., William, A. and Levitan, I.B. (1985) Nature 315, 503-506.

Fain, J.N. and Garcia-Sainz, J.A. (1980) Life Sci. 26, 1183-1194.

Falk, B., Hillarp, N.A., Thieme, G. and Torp, A. (1962) J. Histochem. Cytochem. 10, 348-354.

Falloon, J., Malech, H., Milligan, G., Uson, C., Kahn, R., Goldsmith, P. and Spiegel, A (1986) FEBS Lett. 209, 351-356.

Felice, L.J., Felice, J.D. and Kissinger, P.T. (1978) J. Neurochem. 31, 1461-1465.

Ferrendelli, J.A., Rubin, E.H. and Kinscherf, D.A. (1976) J. Neurochem. 26, 741-748.

Forn, J. and Krishna, G. (1971) Pharmacol. 5, 193-204.

Forn, J., Krueger, B.K. and Greengard, P. (1974) Science 186, 1119-1120.

Frankhuyzen, A.L. and Mulder, A.H. (1982) Eur. J. Pharmacol. 81, 97-106.

Fredholm, B.B. (1980) Trends Pharmacol. Sci. 1, 124-132.

Fredholm, B.B. and Dunwiddie, T.V. (1988) Trends Pharmacol. Sci. 9, 130-134.

Fredholm, B.B. and Hedquist, P. (1980) Biochem. Pharmacol. 29, 1635-1643.

Fredholm, B.B. and Lindgren, E. (1987) Acta. Physiol. Scand. 130, 95-105.

Fuxe, K. (1965) Acta. Physiol. Scand. 64, 247-249.

Garcia-Sainz, J.A., Hoffman, B.B., Li, S.Y., Lefkowitz, R.J. and Fain, J. (1980) Life Sci. 27, 953-961.

Gilman, A.G. (1984) Cell 36, 577-579.

Glowinski, J. and Iversen, L.L. (1966) J. Neurochem. 13, 655-669.

Goldberg, A.L. and Singer, J.J. (1969) Proc. Natl. Acad. Sci. USA, 64, 134-141.

Gordon-Weeks, P.R. (1987) Neurochem. a practical approach (ed. Turnder, A.J. and Bachelard, H.S.) IRL Press, Oxford, Washington D.C. PP1-26.

Gothert, M. (1979) Naunyn-Schmiedeberg's Arch. Pharmacol. 307, 29-37.

Gothert, M., Phol., I.M. and Wehking, E. (1979) Naunyn-Schmiedeberg's Arch. Pharmacol. 307, 21-27.

Grant, J.A. and Scrutton, M.C. (1980) Br. J. Pharmacol. 71, 121-134.

Gray. R. and Johnston, D. (1987) Nature (Lond.) 327, 620-622.

Gray, E.G. and Wittaker, V.P. (1962) J. Anatomy 96(1), 79-88.

Graziano, M.P. and Alfred, G.G. (1987) Trends Pharmacol. Sci. 8, 478-481.

Griffin, M.T., Law, P.Y. and Loh, H.H. (1985) J. Neurochem. 45, 1585-1589.

Gross, R.A. and Ferrendelli, J.A. (1980) J. Neurochem. 34(5), 1309-1318.

Harms, H.H., Wardeh, G. and Mulder, A.H. (1978) Eur. J. Pharmacol. 49, 305-308.

Harms, H.H. Wardeh, G. and Mulder, A.H. (1979) Neuropharmacol. 18, 577-580.

Harris, J. and Roth, R. (1970) Mol. Pharmacol. 7, 593-604.

Hedler, H., Stamm, G., Weitzell, R. and Starke, K. (1981) Eur. J. Pharmacol. 70, 43-52.

Heffener, T.G. and Seiden, L.S. (1980) Brain Res. 183, 403-419.

Hemmings Jr., H.C., Nairn, A.C. and Greengard, P. (1986) Raven Press, N.Y. (ed. Martin, J.B. and Barchas, J.D.) PP47-69.

Hollins, C. and Stone, T.W. (1980) Br. J. Pharmacol. 69, 107-112.

Holtzman, S.G. (1974) Biochem. Pharmacol. 23, 3029-3035.

Holtz, W. G.G., Rane, S.G. and Dunlap, K. (1968) Nature 319, 670-672.

Horns, J.P. and McAfee, D.J. (1980) J. Physiol. (Lond.) 301, 191-204.

Huang, M., Shimizu, H. and Daly, J.W. (1971) Mol. Pharmacol. 7, 155-162.

Huang, M., Ho, A.K.S. and Daly, J.W. (1973) Mol. Pharmacol. 9, 711-717.

Insel, P.A., Stengel, D., Ferry, N. and Hanoune, J. (1982) J. Biol. Chem. 257, 7485-7490.

Iversen, L.L. and Glowinski, J. (1966) Nature 210, 1006-1008.

Jackish, R., Werle, E. and Herting, G. (1984) Neuropharmacol. 23, 1363-1371.

Jackish, R., Strittmatter, H., Kasakov, L. and Hertting, G. (1984) Naunyn-Schmiedeberg's Arch. Pharmacol. 327, 319-325.

Jackisch, R., Fehr, R. and Hertting, G. (1985) Neuropharmacol. 24(6), 499-507.

Jakobs, K.H., Saur, W. and Schultz, G. (1976) J. Cyclic Nucleotide Res. 2, 381-392.

Jarvis, M.J. and Williams, M. (1987) Trends Pharmacol. Sci. 8, 330-331.

Javid, J.I., Perel, J.M. and Davis, J.M. (1979) Life Sci. 24, 21-28.

Johnson, R.D. and Minneman, K.P. (1986) Eur. J. Pharmacol. 129, 293-305.

Jonzon , B. and Fredholm, B.B. (1984) Life Sci. 35, 1971-1979.

Kahn, D.J., Mitrius, J.C. and U'Prichard, D.C. (1982) Mol. Pharmacol. 21, 17-26.

Kakiuchi, S. and Rall, T.W. (1968a) Mol. Pharmacol. 4, 367-378.

Kakiuchi, S. and Rall, T.W. (1968b) Mol. Pharmacol. 4, 379-398.

Kakiuchi, S. and Rall, T.W. and McIlwain, H. (1969) J. Neurochem. 16, 485-491.

Kalisker, A., Rutledge, C.O. and Perkins, J.P. (1973) Mol. Pharmacol. 9, 619-629.

Kant, G.J. and Meyerhoff, J.L. (1978) Life Sci. 23, 2111-2118.

Karbon, E.W. and Enna, S.J. (1984) Mol. Pharmacol. 27, 53-59.

Karbon, E.W. and Enna, S.J. (1986) Nature 323, 829P.

Katz, B. and Miledi, R. (1967) J. Physiol (Lond.) 192, 407-436.

Katz, B. and Mildei, R. (1969) J. Physiol (Lond.) 203, 459-487.

Kawahara, R.S. and Bylund, D.B. (1985) J. Pharmacol. Exp. Ther. 233, 603-610.

Keen, P. and White, T.D. (1971) J. Neurochem. 18, 1097-1103.

Keller, R., Oke, A., Mefford, I. and Adams, R.N. (1976) Life Sci. 19, 995-1004.

Keller, H.H., Bartholini, G. and Pletscher, A. (1974) Nature (Lond.) 248, 528-529.

Kimelberg, H.K. and Katz, D.M. (1986) J. Neurochem. 47, 1647-1652.

Kitamura, Y., Nomura, Y. and Segawa, T. (1985) J. Neurochem. 45, 1504-1508.

Klainer, L.M., Ch, Y.M., Freidberg, S.L., Rall, T.W. and Sutherland, E.W. (1962) J. Biol. Biochem. 237, 1239-1243.

Klein, M. and Kandel, E.R. (1978) Proc. Natl. Acad. Sci. USA, 75, 3512-3516.

Klein, M. and Kandel, E.R. (1980) Proc. Natl. Acad. Sci. USA, 77(11), 6912-6916.

Kelin, R.L. (1982) Neurotransmitter vesicles (ed. by Kelin R.L., Langer, H.C. and Zimmerman, H.) PP134-174, Academic Press, N.Y.

Kobilka, B.K., Matsui, H., Kobilka, T.S., Yang-Feng, T.L., Francke, U., Caron, M.G., Lefkowitz, R.J. and Regan, J.W. (1987) Science 238, 650-656.

Kojima, I., Shibata, H. and Ogata, E. (1986) FEBS Lett. 204, 347-351.

Korf, J. and Sebens, J.B. (1979) J. Neurochem. 32, 463-468.

Kreutzberg, G.W., Reddington, M., Lee, K.S. and Schubert, P. (1983) J. Neural. Trans. 18, 113-115.

Krishna, G., Weiss, B. and Brodie, B.B. (1968) J. Pharmacol. Exp. Ther. 163(2), 379-385.

Kuroda, Y. and McIlwain, H. (1979) J. Neurochem. 22, 691-699.

Lands, A.M., Arnold, A., McAuliff, J.P., Luduena, F.P. and Brown, T.C. (1967) Nature 214, 597-598.

Lane, J.D. and Aprison, M.H. (1977) Life Sci. 20, 665-672.

Langer, S.Z. (1977) Br. J. Pharmacol. 60, 481-497.

Langer, S.Z. (1974) Biochem. Pharmacol. 23, 1793-1800.

Langer, S.Z. (1981) Pharmac. Rev. 32, 337-362.

Lasch, P. and Jaboks, K.H. (1979) Naunyn-Schmiedeberg's Arch. Pharmacol. 306, 119-125.

Lees, A.J. and Smith, E. (1983) Brain 106, 257-270.

Leslie, S.W., Woodward, J.J. and Wilcox, R.E. (1985) Brain Res. 325, 99-105.

Levitzki, A. (1987) Trends Pharmacol. Sci. 8, 299-303.

Litosch, I., Wallis, C. and Fain, J.N. (1985) J. Biol. Chem. 260, 5464-5471.

Litosch, I. and Fain, J.N. (1986) Life Sci. 39, 187-194.

Llinas, R., Blinks, J.R. and Nicholson, C. (1972) Science 176, 1127-1129.

Llinas, R. and Nicholson C. (1975) Proc. Natl. Acad. Sci USA, 72(1), 187-190.

Llinas, R.R. and Heuser, J.E. (1977) Prog. Bull. 15, 557-687.

Loftus, D.J., Stolk, J.M. and U'Prichard, D.C. (1984) Life Sci. 35, 61-69.

Logothetis, D.E., Kuarchi, Y., Galper, J., Neer, E.J. and Clapham, D.E. (1987) Nature 325, 321-326.

Londos, C. and Wolff, J. (1977) Proc. Natl. Acad. Sci., USA, 74, 5482-5486.

Londos, C., Cooper, D.M.F. and Wolff, J. (1980) Proc. Natl. Acad. Sci. USA, 77(5), 2551-2554.

Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. (1951) J. Biol. Chem. 193, 265-275.

Lues, I. and Schumann, J.H. (1984) Naunyn-Schmiedeberg's Arch. Pharmacol. 325, 42-46.

Marchbanks, R.M. (1967) J. Biol. Chem. 104, 148-157.

Marriot, D., Adams, M. and Boarder, M.R. (1988) J. Neurochem. 50, 616-623.

Martinson, E.A., Johnson, R.A. and Wells, J.N. (1987) Mol. Pharmacol. 31, 247-252.

Markstein, R., Digges, K., Marshall, N.R. and Starke, K. (1984) Naunyn-Schmiedeberg's Arch. Pharmacol. 325, 17-24.

McCarthy, K.D. and de Vellis, J. (1978) J. Cyclic Nuc. Res. 4, 15-26.

McCarthy, K.D. and de Vellis, J. (1979) Life Sci. 24, 639-650.

McGeer, E.G., Gibson, S. and McGeer, P.L. (1967) Can. J. Biochem. 45, 1557-1560.

McIlwain, H. (1977) Neurosci. 2, 357-372.

Medgett, I.C., McCulloch, M.W. and Rand, M.J. (1978) Naunyn-Schmiedeberg's Arch. Pharmacol. 304, 215-221.

Mestikawy, S.E., Glowinski, J. and Hamon, M. (1983) Nature (lond.) 302, 820-832.

Meves, H. and Pichon, Y. (1977) J. Physiol. 268, 511-532.

Miach, P.J., Dausse, J.P., Cardot, A. and Meyer, P. (1980) Naunyn-Schmiedeberg's Arch. Pharmacol. 312, 23-26.

Middlemiss, D.N. (1988) Trends Pharmacol. Sci. 9, 83-84.

Milligan, G., Gierschik, P., Spiegel, A.M. and Klee, W.A. (1986) FEBS Lett. 195, 225-230.

Milligan, G., Streaky, R.A., Gierschik, P., Spiegel, A.M. and Klee, W.A. (1987) J. Biol. Chem. 262(18), 8628-8630.

Milligan, G. (1988) Biochem. J. 255, 1-13.

Mitchell, T., Winslow, J.W., Smith, J.A., Seidman, J.C. and Neer, E.J. (1986) Proc. Natl. Acad. Sci. USA, 83, 7663-7667.

Minneman, K.P. and Molinoff, P.B. (1980) Biochem. Pharmacol. 29, 1317-1323.

Moran, N.C. (1967) Ann. N.Y. Acad. Sci. 139, 648-652.

Moskowitz, N.S., Puszkin, S. and Schook, W. (1983) J. Neurochem. 41, 1576-1586.

Moskowitz, N., Schook, W. and Puszkin, S. (1984) Brain Res. 290, 273-280.

Mulder, A.H., De Langen, C.D.J., De Regt, V. and Hogenboom, F. (1978) Naunyn-Schmiedeberg's Arch. Pharmacol. 303, 193-196.

Muscholl, E. (1978) (ed. Paton, D.M.) 87-110, Pergamon Press, Oxford.

Nachshen, D.A. and Blaustein, M.P. (1980) J. Gen. Physiol. 76, 709-728.

Nachshen, D.A. and Blaustein, M.P. (1982) J. Gen. Physiol. 79, 1065-1087.

Nakamura, T. and Ui, M. (1984) FEBS Lett. 173, 414-418.

Neer, E.J., Lok, J.M. and Wolf, L.G. (1984) J. Biol. Chem. 259, 14222-14229.

Nestler, E.J. and Greengard, P. (1983) Nature 305, 583-588.

Nichols, R.A., Haycock, J.W., Wang, J.K.T. and Greengard, P. (1987) J. Neurochem. 48, 615-621.

Neioullon, A., Cheramy, A. and Glowinski, J. (1977) J. Neurochem. 28, 819-828.

Oke, A., Keller, K. and Adams, R.N. (1978) Brain Res. 148, 245-250.

Orrego, F. (1979) Neurosci. 4, 1037-1057.

Palfrey, H.C. and Mobley, P. (1987) Neurochem. a practical approach (ed. Turner, A.J. and Bachelard, H.S.) PP161-191, IRL Press, Oxford, Washington DC.

Patel, J., Maranges, P.J., Stivers, J. and Goodwin, F.K. (1982) Brain Res. 237, 203-214.

Partington, C.R., Edwards, M.W. and Daly, J.W. (1980) Proc. Natl. Acad. Sci. USA, 77, 3024-3028.

Paupardin-Tritsch, D., Hammond, C., Gerschenfeld, H.M., Nairn, A.C. and Greengard, P. (1986) Nature, 323, 312-314.

Pedata, F. Pepeu, G. and Spignoli, G. (1983) Br. J. Pharmacol. 80, 471-478.

Perkins, J.P. and Moore, M.M. (1973) J. Pharmacol. Exp. Ther. 185, 371-378.

Phillis, J.W., Edstrom, J.P., Kostopoulos, G.K. and Kirkpatrick, J.R. (1979) Can. J. Physiol. Pharmacol. 57, 1289-1312.

Pilc, A. and Enna, S.J. (1986) J. Pharmacol. Exp. Ther. 237(3), 725-730.

Pollard, J.H. (1977) "A handbook of numerical and statistical techniques" Cambridge University Press.

Powel, C.E. and Slater, I.H. (1958) J. Pharmacol. Exp. Ther. 122, 480P.

Przuntek, H., Heyd, G. and Burger, A. (1971) Eur. J. Pharmacol. 15, 200-208.

Rabe, C.S., Schneider, J. and McGee, R. (1982) J. Cyclic Nucleotide Res. 8, 371-384.

Raiteri, M., Marchi, M. and Maura, G. (1983) Eur. J. Pharmacol. 91, 141-143.

Raiteri, M., Maura, G. and Versace, P. (1983) J. Pharmacol. Exp. Ther. 224(3), 679-684.

Reichenbacher, D., Reiman, W. and Starke, K. (1982) Naunyn-Schmiedeberg's Arch. Pharmacol. 319, 71-77.

Reimann, W., Zumstein, A., Starke, K. and Herting, G. (1979) Naunyn-Schmiedeberg's Arch. Pharmacol. 319, 71-77.

Reuter, H. (1983) Nature 301, 569-574.

Robins, T.W. (1988) Nature 336, 207-208.

Rosenthal, W. and Schultz, G. (1987) Trends Pharmacol. Sci. 8, 351-354.

Rouot, B.M., U'Prichard, D.C. and Snyder, S.H. (1980) J. Neurochem. 34(2), 374-384.

Sabol, S.K. and Nirenberg, M. (1979) J. Biol. Chem. 254, 1913-1920.

Santos, J.N., Hempstead, K.W., Kopp, L.E. and Miech, R.P. (1968) J. Neurochem. 15, 367-376.

Sarantos-Laksa, C., Majenski, H., McCulloch, M.W. and Rand, M.J. (1980) Archs. Int. Pharmacody. Ther. 247, 294-304.

Sasaki, K. and Sato, M. (1987) Nature, 325, 259-262.

Sattin, A. and Rall, T.W. (1970) Mol. Pharmacol. 6, 13-23.

Schlicker, E., Gothert, M. and Clansing, R. (1982) Naunyn-Schmiedeberg's Arch. Pharmacol. 320, 38-44.

Schmidt, M.J., Schmidt, D.E. and Robinson, G.A. (1971) Science 173, 1142-1143.

Schmidt, M.J., Hopkins, J.T., Schmidt, D.E. and Robinson, G.A. (1972) Brain Res. 42, 465-477.

Schaepp, D.D., Knepper, S.M. and Rutledge, C.O. (1984) J. Neurochem. 43, 1758-1761.

Schoffelmeer, A.N.M., Werner, J. and Mulder, A.H. (1981) Neurochem. Int. 3(2), 129-136.

Schoffelmeer, A.N.M. and Mulder, A.H. (1982) Naunyn-Schmiedeberg's Arch. Pharmacol. 318, 173-180.

Schoffelmeer, A.N.M. and Mulder, A.H. (1983) Naunyn-Schmiedeberg's Arch. Pharmacol. 323, 188-192.

Schoffelmeer, A.N.M. and Mulder, A.H. (1983b) Naunyn-Schmiedeberg's Arch. Pharamcol. 323, 188-192.

Schoffelmeer, A.N.M. and Mulder, A.H. (1984) Eur. J. Pharmacol. 105, 129-135.

Schoffelmeer, A.N.M., Wardeh, G. and Mulder, A.H. (1985) Naunyn-Schmiedeberg's Arch. Pharmacol. 330, 74-76.

Schoffelmeer, A.N.M., Hogenboom, F. and Mulder, A.H. (1985) J. Neurosci. 5(10), 2685-2689.

Schoffelmeer, A.N.M., Wierenga, E.A. and Mulder, A.H. (1986) J. Neurochem. 46, 1711-1717.

Schramm, M. and Selinger, Z. (1984) Science 225, 1350-1356.

Schultz, J.E. (1978) FEBS 54, 81-91.

Schultz, J. and Daly, J.W. (1973) J. Neurochem. 21, 1319-1326.

Schultz, J. and Daly, J.W. (1973a) J. Biol. Chem. 248, 843-852.

Schultz, J. and Kleefeld, G. (1979) Pharmacol. 18, 162-167.

Schwabe, U., Ohga, Y. and Daly, J.W. (1978) Naunyn-Schmiedeberg's Arch. Pharmacol. 302, 141-151.

Seamon, K.B. and Daly, J.W. (1980) J. Biol. Chem. 256, 9799-9801.

Seamon, K.B., Padgett, W.L. and Daly, J.W. (1981) J. Cyclic Nucleotide Res. 7, 201-224.

Seamon, K.B. and Daly, J.W. (1983) Trends Pharmacol. Sci. 4, 120-123.

Seamon, K.B. and Wetzel, B. (1984) Advances in Cyclic Nucleotide and Protein Phosphorlyation Res. 17, 91-99.

Shellengerger, M.K. (1971) Neuropharmacol. 10, 347-357.

Shimizu, H., Daly, J.W. and Creveling, C.R. (1969) J. Neurochem. 16, 1609-1619.

Shimizu, H., Creveling, C.R. and Daly, J.W. (1970) Mol. Pharmacol. 6, 184-188.

Shimizu, H. and Daly, J.W. (1972) Eur. J. Pharmacol. 17, 240-252.

Shimizu, H., Takenoshita, M., Huang, M. and Daly, J.W. (1973) J. Neurochem. 20, 91-95.

Shimizu, H., Ichishita, H. and Odagiri, H. (1974) J. Biol. Chem. 249, 5952-5955.

Siegelbaum, S., Camardo, J.S. and Kandel, E.R. (1982) Nature 299, 413-417.

Silinsky, E.M. (1986) Trends Pharmacol. Sci. 7, 180-185

Solomon, Y., Londos, C. and Rodbell, M. (1974) Anal. Biochem. 58, 541-548.

Solomon, Y. (1979) Advances in Cyclic Nucleotide Res. Vol. 10 (ed. Brooker, G., Greengard, P. and Robinson, G.A.) Raven Press, NY.

Starke, K., Montel, H., Gay, K.W. and Merker, R. (1974) Naunyn-Schmiedeberg's Arch. Pharmacol. 285, 133-150.

Starke, K., Borowski, E. and Endo, J. (1975) Eur. J. Pharmacol. 34, 385-388.

Starke, K. (1979) (ed. Paton, D.M.) PP143-183, Pergamon Press, Oxford.

Sternweis, P.C. and Robishaw, J.D. (1984) J. Biol. Chem. 259, 13806-13813.

Stiles, G.L. (1986) Trends Pharmacol. Sci. 7, 486-490.

Sugina, H., Oguar, A., Kuda, Y. and Amano, T. (1984) Brain Res. 322, 127-130.

Suszkiw, J.B. and O'Leary, M.E. (1983) J. Neurochem. 41, 868-873.

Sutherland, E.W. and Rall, T.W. (1958) J. Biol. Chem. 232, 1077-1080.

Sutherland, E., Rall, T.W. and Menon, T. (1962) J. Biol. Chem. 237, 1220-1227.

Svensson, T.H., Bunney, B.S. and Aghajanian, G.K. (1975) Brain Res. 92, 291-306.

Snyder, S.H. and Goodmann, R.R. (1980) J. Neurochem. 35, 5-15.

Tanaka, C., Fujiwara, H. and Fujii, Y. (1985) FEBS 195, 129-134.

Taube, H.D., Starke, K. and Borowski, E. (1977) Naunyn-Schmiedeberg's Arch. Pharmacol. 299, 123-141.

Taylor, C.W. (1987) Trends Pharmacol. Sci. 8, 79-80.

Taylor, C.W. and Merritt, J.E. (1986) Trends Pharmacol. Sci. 7, 238-242.

Thomas, J.M. and Hoffman, B.B. (1987) Trends Pharmacol. Sci. 8, 308-311.

Ueda, H., Goshima, Y. and Misu, Y. (1983) Life Sci. 33, 371-376.

Ui, M. (1984) Trends Pharmacol. Sci. 5, 277-279.

U'Prichard, D.C. and Snyder, S.H. (1979) Life Sci. 24, 79-88.

U'Prichard, D.C., Greenberg, D.A. and Snynder, S.H. (1979) Mol. Pharmacol. 13, 454-473.

Van Calker, D., Muller, M. and Hamprecht, B. (1978) J. Neurochem. 30, 713-718.

Van Calker, D., Muller, M. and Hamprecht, B. (1978) J. Neurochem. 33, 999-1005.

Van Meel, J.C.A., de Jonge, A., Timmermans, P.B.M.W.M. and Van Zwieten, P.A. (1981) J. Pharmacol. Exp. Ther. 219, 760-767.

Van Orden, L.S., Bensch, K.G. and Giarman, N.J. (1967) J. Pharmacol. Exp. Ther. 155, 428-439.

Versteag, D.H., Van der Gugten, J., De Jong, W. and Palkovits, M. (1976) Brain Res. 113, 563-574.

Voigt, K. and Krishna, G. (1967) Pharmacol. 9, 311-313.

Watterson, D.M. and Vincenzi, F.F. (1980) Ann. NY. Acad. Sci. 356, P466.

Weimer, N. and Jardetsky, O. (1964) Naunyn-Schmiedeberg's Arch. Exp. 248, 308-318.

Weinshilboum, R.M., Thoa, N.B., Johnson, D.B., Kopin, I.J. and Axelrod, J. (1971) Science 174, 1349-1351.

Weiss, B. and Costa, E. (1968) Biochem. Pharmacol. 17, 2107-2110.

Well, J.N. and Kramer, G.L. (1981) Mol. Cell. Endocrin. 23, 1-9.

Werner, J., Van der Lugt, J.C., De Langen, C.D.J. and Mulder, A.H. (1979) J. Pharmacol. Exp. Ther. 211, 445-451.

Werner, J., Schoffelmeer, A.N.M. and Mulder, A.H. (1982) J. Neurochem. 39, 349-356.

Westerink, B.H.C. and Mulder, T.B.A. (1981) J. Neurochem. 36, 1449-1462.

Westfall, T.C., Kitay, D. and Wahl, G. (1976) J. Pharmacol. Exp. Ther. 199, 149-159.

Wikberg, (1978) Acta. Physiol. Scand. 103, 225-239.

Williams, L.T. and Lefkowitz, R.J. (1979) P157, Raven Press, NY.

Williams, M. and Risley, E.A. (1980) Eur. J. Pharmacol. 64, 369-370.

Williams, M. and Risely, E.A. (1980) Proc. Natl. Acad. Sci. USA, 77, 6892-6896.

Wojcik, W.J. and Neff, N.H. (1984) Mol. Pharmacol. 25, 24-28.

Wolff, D.J. and Brostrom, C.O. (1979) Adv. Cyclic Nucleotide Res. 11, 27-88.

Wood, C.L. Arnett, C.D., Clarke, W.R., Tsai, B.S. and Lefkowitz, R.J. (1979) Biochem. Pharmacol. 28, 1277-1282.

Wooten, G.F., Thoa, N.B., Kopin, I.J. and Axelrod, J. (1973) Mol. Pharmacol. 9, 178-183.

Wu, Ph., Phillis, J.W., Balls, K. and Rinaldi, B. (1980) Can J. Physiol. Pharmacol. 58, 576-579.

Wu, P.H., Phillis, J.W. and Thierry, D.L. (1982) J. Neurochem. 39, 700-708.

Yamazaki, S., Kanatada, T. and Ui, M. (1982) Mol. Pharmacol. 21, 648-653.

Yatani, A., Tsuda, Y., Akaibe, N. and Brown, A.M. (1982) Nature 296, 169-171.

Young, W.S. III and Kuhar, M.J. (1979) Eur. J. Pharmacol. 59, 317-319.

Young, W.S. III and Kuhar, M.J. (1980) Proc. Natl. Acad. Sci. USA, 77, 1696-1700.

Zanella, J. Jr. and Rall, T.W. (1973) J. Pharmacol. Exp. Ther. 186(2), 241-252.

Zimanyi, I., Folly, G. and Vizi, E.S. (1988) J. Neurosci. Res. 20, 102-108.