Interaction of some Antihypertensive Drugs with Central Monaminergic and Cholinergic Receptors

(SUMMARY)

A DISSERTATION SUBMITTED TO THE ALIGARH MUSLIM UNIVERSITY ALIGARH FOR THE DEGREE OF DOCTOR OF PHILOSOPHY IN ZOOLOGY

BY

Ghazala Hussain
M. Phil. (Zoology)

DIVISION OF PHARMACOLOGY CENTRAL DRUG RESEARCH INSTITUTE LUCKNOW-226001
SUMMARY
Hypertension is a frequently occurring disease of the developed as well as the developing countries. Although a persistent elevation of blood pressure beyond the normal range may be devoid of clinical symptoms but the complications resulting from this disorder are severe. Peripheral vascular resistance, vascular reactivity, cardiac output, the autonomic nervous control, blood viscosity etc. may all be involved in a very complex and interdependent manner.

The life span of hypertensive patients can be increased by many years if appropriate therapeutic measures bring about a reduction in blood pressure to near normal level. The main thrust of antihypertensive therapy consists of treatment with drugs. Usually the treatment is symptomatic regardless of the type of hypertension. However, the cause should be treated as soon as possible, for which, reasonable knowledge of the cause of hypertensive disease and the mechanism of action of antihypertensive drugs is important. Many potential sites and mechanisms exist through which a drug may exert its antihypertensive effect.

The first attempt at modern pharmacotherapy of hypertension began around 1955 with the availability of drugs like reserpine, hydralazine, veratrum alkaloids, thiazide diuretics and guanethidine. Although, these drugs made the first real impact on the morbidity and mortality associated with the complications of hypertension, the side
effects of these agents remained unacceptable to many patients. As a result some of these compounds were discarded or had to be used in combination with other drugs. Attention was subsequently drawn towards the influence of the central nervous system in the development of hypertensive disease. Thus clonidine was developed, which decreased blood pressure by acting mainly on the central nervous system. The brain stem has been shown to be an important site of action.

The etiology of primary hypertension is obscure and hence the choice of drug treatment cannot be wholly adequate. Moreover, the pharmacotherapy remains the most useful tool in the management of primary hypertension. The treatment has to be often life long and a high standard of efficacy and safety is required of the drugs used. The antihypertensive drugs can be mainly classified as (1) centrally acting antihypertensives, mainly adrenergic agonists (2) peripherally acting antihypertensives, which can be antiadrenergic, mineralocorticoids, diuretics and direct acting drugs. We have selected clonidine a centrally acting drug, hydralazine a peripherally acting antihypertensive, reserpine which acts by releasing catecholamines and centhaquin a new centrally acting antihypertensive drug.

1. Radioreceptor binding studies: Two types of binding sites exist, one which is specific to the type of receptor under investigation and the other is nonspecific binding to the biopolymers of the tissue. Since the number of specific receptors sites are limited, and the nonspecific sites are unlimited, the binding experiment was repeated in the presence of excess amount of non-radiolabelled ligand. The various ligands used for
Radioreceptor binding to different neurotransmitter receptors are as follows:

<table>
<thead>
<tr>
<th>Receptor</th>
<th>Hot ligand</th>
<th>Cold ligand</th>
</tr>
</thead>
<tbody>
<tr>
<td>a-adrenergic</td>
<td>$^3$H-Dihydroergocryptine</td>
<td>Phentolamine (1 $\mu$M)</td>
</tr>
<tr>
<td></td>
<td>0.25-12 nM (Amersham)</td>
<td></td>
</tr>
<tr>
<td>b-adrenergic</td>
<td>$^3$H-Dihydroalprenolol</td>
<td>Propranolol (1 $\mu$M)</td>
</tr>
<tr>
<td></td>
<td>0.12-4 nM (Amersham)</td>
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</tr>
<tr>
<td>Muscarinic</td>
<td>$^3$H-Quinuclidinyl benzylate 0.1-2 nM (NEN)</td>
<td>Atropine (1 $\mu$M)</td>
</tr>
<tr>
<td>Dopamine</td>
<td>$^3$H-Spiperone</td>
<td>Haloperidol (1 $\mu$M)</td>
</tr>
<tr>
<td></td>
<td>0.1-5 nM (NEN)</td>
<td></td>
</tr>
<tr>
<td>5-hydroxytryptamine ($5HT_1$)</td>
<td>$^3$H-5-hydroxytryptamine 0.25-15 nM (NEN)</td>
<td>5-hydroxytryptamine ($5HT_1$) (1 $\mu$M)</td>
</tr>
<tr>
<td>5-hydroxytryptamine ($5HT_2$)</td>
<td>$^3$H-Spiperone 0.15-10 nM (NEN)</td>
<td>Ketanserin (1 $\mu$M)</td>
</tr>
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Radioreceptor binding studies were carried out with these ligands for the specific receptors in corpus striatum, cortex, hypothalamus and medullary regions of the rat brain. The changes in receptor affinity and density was studied.

2. Effects of centhaquin on blood pressure and heart rate in normal and cervical sectioned rats was recorded on a Grass Model 7 Polygraph.

3. Microinjection studies were performed in cats by placing a cannula in the nucleus tractus solitarius (NTS). Drugs like centhaquin, clonidine, phenolamine, yohimbine and prazosin were injected by the help of a microsyringe.
4. Studies on thermoregulation and blood pressure. Rats were cannulated at the lateral cerebral ventricle (icv) and allowed to recover. Then they were chronically treated with the oral administration of four antihypertensive drugs selected in this study. The rats were then tested for their responses to noradrenaline, dopamine, acetylcholine and 5-HT. Temperature measurements were done by measuring the core temperature of the albino rats with a thermistor probe inserted 2 cm into the rectum and connected to a programmed apple computer. The temperature was recorded before the drug administration and immediately after it at regular intervals of 10 minutes for 5 hours. Blood pressure was recorded by the IITC in 6 instrument which measures the systolic as well as the mean blood pressure. The blood pressure before administration of the drug was compared to the change in blood pressure after the drug injection.

Till now, not much attention has been given to the status of central neurotransmitter receptors in hypertension, and the effect of antihypertensive drugs on central neurotransmitter receptors. It was thought that if, antihypertensive drugs, like centhaquin, clonidine (which are centrally acting), hydralazine (which acts through a peripheral mechanism) and reserpine (which has a complex mechanism of action, but mainly peripheral) are administered chronically, they are likely to produce different actions on various central neurotransmitter receptors. Broadly centrally acting antihypertensives will have direct effect on the CNS receptors, while peripherally acting antihypertensives are not likely to produce any change in central receptors. If peripherally acting antihypertensives do produce alteration in central receptors, it could
be due to a secondary (feedback) effect i.e. due to decreased blood pressure the central receptors are altered. Since medulla and hypothalamus are the main areas of brain involved in blood pressure regulation, alteration of receptor characteristics in these areas will be of direct relevance to regulation of blood pressure. Other brain regions like striatum and cortex are less involved in cardiovascular regulation.

Receptor binding studies show that clonidine and centhaquin increase both the affinity and density of $\alpha$-adrenoceptors in hypothalamus and medulla of rat brain, while peripherally acting antihypertensive drugs, hydralazine and reserpine decrease the density of $\alpha$-adrenoceptors in all four brain regions. Since the affinity of clonidine for presynaptic $\alpha$-adrenoceptors is at least ten times higher than its affinity for postsynaptic $\alpha$-adrenoceptors, it could be possible that repeated administration of clonidine decreases the release of norepinephrine in hypothalamus and medulla by acting on presynaptic receptors. Thus the decreased concentration of norepinephrine in the synapse will result in increase in the density of post synaptic $\alpha$-adrenoceptors, as observed in the present study. The mean blood pressure response to noradrenaline in hydralazine and reserpine treated rats was similar to the control rats, while it was opposite in clonidine and centhaquin treated rats. This shows a clear differentiation between responses of noradrenaline in centrally and peripherally acting antihypertensive drugs and also strengthens our contention that besides clonidine, centhaquin a newly developed antihypertensive drug acts on central adrenergic receptors, which in all probability could be $\alpha$ adrenergic receptors. Centhaquin and
clonidine did not affect the density or affinity of β adrenergic receptors. Further supporting the fact that β adrenergic receptors are not involved in the action of centrally acting antihypertensive drugs. In the present study both biochemical and pharmacological evidences have been provided indicating an alteration in central adrenergic receptors.

In hypothalamus and medulla clonidine and centhaquin did not affect either the density or the affinity of 3H-spiperone binding. Some non specific changes was observed in hydralazine and reserpine treated rats. Dopamine (50 µg, ICV) produced no significant change in either colonic temperature or mean blood pressure in control, clonidine and reserpine treated rats. In hydralazine treated rats a significant decrease in blood pressure and increase in colonic temperature were observed. In centhaquin treated rat no change in blood pressure occurred, however a significant increase in colonic temperature was observed. It is clear from the above results which provide both biochemical and pharmacological evidence that central dopaminergic receptors are not involved in blood pressure regulation or in the action of centrally acting antihypertensive drugs.

Centhaquin treatment produced no change in the density and affinity of 3H-quinuclidinyl benzylate binding. Clonidine treatment produced a decrease in the density and affinity of the muscarinic receptors. No change was observed in the density and affinity of the muscarinic receptors by hydralazine and reserpine treatment. Acetylcholine (10 µg, ICV) produced a decrease in colonic temperature in control rats. Peripherally
acting antihypertensive drugs, hydralazine and reserpine treated rats showed no change in colonic temperature. Centrally acting antihypertensive drug, centhaquin treated rats showed a decrease in colonic temperature, as control rats. However, clonidine treated rats showed a significant increase in colonic temperature in response to acetylcholine. Blood pressure was decreased by acetylcholine (10 μg, ICV) in control rats as well as in centhaquin, hydralazine and reserpine treated rats. However clonidine treated rats showed a significantly greater decrease in blood pressure in comparison to other groups of rats. These results indicate that clonidine does act on cholinergic receptors and might be producing some of antihypertensive effect through these receptors.

The evidences for the involvement of serotonergic system in the regulation of blood pressure are very strong. These results of the present study indicate both biochemically and pharmacologically that 5-HT receptors of the brain might be involved in the action of centrally acting antihypertensive drugs.

Centhaquin when administered intravenously produced hypotension and bradycardia in normal rats. In cervical sectioned rats it did not produce any effect on blood pressure or heart rate. This suggests that centhaquin produces hypotension and bradycardia by acting directly in the central nervous system. Centhaquin given intrathecally did not produce any effect on blood pressure and heart rate. This showed that the site of action of centhaquin was above the spinal cord.

Microinjection of centhaquin in the NTS produced mainly bradycardia with little effect on blood pressure. In order to determine the nature of receptors involved several
antagonists were administered prior to the administration of centhaquin. It was found that phentolamine, which blocks both $\alpha_1$ and $\alpha_2$ adrenergic receptors could block the effect of centhaquin. In order to determine which subtype of receptor is involved $\alpha_1$ adrenergic receptor antagonist prazosin was given prior to the administration of centhaquin. The effect of centhaquin was blocked by prazosin. Similarly $\alpha_2$ adrenergic receptor antagonist yohimbine blocked the effect of centhaquin. It became difficult to comment which subtype of receptor is involved. It could be possible that in NTS the $\alpha$ adrenergic receptors are of undifferentiated type and are responsive to both prazosin and yohimbine.
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CERTIFICATE

This is to certify that the work embodied in this thesis, entitled "Interaction of some antihypertensive drugs with central monoaminergic and cholinergic receptors" has been carried out by Miss GHAZALA HUSSAIN M Phil, under our supervision. She has fulfilled the requirements for degree of Doctor of Philosophy in zoology, faculty of life sciences, Aligarh Muslim University, Aligarh, regarding the nature and prescribed period of investigational work. The work included in this thesis is original unless stated otherwise and has not been submitted for any other degree.

Prof. Ather H Siddiqi
Ph.D. (Alig), Ph.D. (Purdue)
Chairman Dept. of Zoology &
Dean, Faculty of Life Sciences
Aligarh Muslim University,
ALIGARH.

Dr. R.C. Simal
Deputy Director & Head
Pharmacology Division.
Central Drug Research
Institute,
LUCKNOW.

Dr. Anil Gulati
Scientist
Central Drug Research Institute,
LUCKNOW.
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(GHAZALA HUSSAIN)
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INTRODUCTION
Arterial hypertension, is a frequently occurring disease all over the world. Although persistent elevation of blood pressure beyond the normal range may be devoid of clinical symptoms but the complications resulting from this disorder are severe. The life span of hypertensive patients can be increased by many years if appropriate therapeutic measures bring about a reduction in blood pressure to near normal level (Abrams, 1974). The treatment of arterial hypertension is based mainly on the administration of drugs lowering blood pressure, although other types of interventions like dietary reduction of sodium, normalization of body weight and in special conditions surgical intervention has been done. Before 1950 virtually no drug therapy was available for the treatment of hypertension. Ganglion blocking agents and α-adrenergic blockers were the first group of compounds used to lower pathologically elevated blood pressure. Inspite of numerous side effects and disadvantages, these compounds made the first real impact on the morbidity and mortality associated with the complications of hypertension. Since then, various groups of effective antihypertensive drugs with less side effects have been introduced into current therapy. The mode of action and the mechanism of side effects of most but not all types of antihypertensive drugs is reasonably well understood. However, the most frequently occurring type of hypertension is the essential hypertension. Lack of knowledge of essential hypertension prevents a complete understanding of the influence of the drug on the hypertensive patient. Several of the newer antihypertensive drugs have proved to be most useful in elucidating the mechanisms involved in the regulation of blood
pressure.

Antihypertensive agents discovered in the early stages of the development of such drugs, may exert a part of their effect by acting through the central nervous system. A centrally mediated hypotensive effect of hydralazine was reported by Ingenito et al. (1969). Although Dontas (1957) showed that intravenous injection of the hydralazine did not reduce splanchnic nerve activity, others concluded that the action of hydralazine was primarily peripheral (Reis and Van Zwieten, 1967). Hydralazine dilates arterial resistance vessels more than venous capacitance vessels. Thus venous return and cardiac output are increased. Effect on cardiac output is further accentuated due to increase in heart rate and myocardial contractility resulting from the autonomic reflex response to lowered blood pressure.

The influence of reserpine on the peripheral sympathetic nervous system is well known. The granular stores of noradrenaline are disrupted leading to its enzymatic degradation and finally to its depletion. The diminished amount of transmitter substance is believed to impair the function of the peripheral sympathetic system thus causing a fall in blood pressure. However the extra-granular stores of noradrenaline are hardly affected by reserpine, so that this part of the transmitter store remains available (Van Zwieten, 1973).

Hypertension is dependent on many factors which are not fully defined. It is generally accepted that the antihypertensive drugs reduce peripheral sympathetic tone, so that a decrease in arterial pressure results. The role of the autonomic nervous...
system in causing or sustaining hypertension has been extensively studied (Hilton and Spyer, 1980). There are many factors that can influence the activity of the autonomic nervous system. The neurotransmitter released from nerve terminals evokes a response by acting on specific receptor sites. The magnitude of the response is mainly dependent upon the concentration of neurotransmitter and the affinity and population of these receptors. Several neurotransmitters like adrenergic, cholinergic, dopaminergic, serotonergic, opioidergic, GABAergic etc have been reported to play a role in the regulation of the cardiovascular system and a change in function of one or more of them may lead to hypertension.

Until recently most studies have been confined to the processes of neurotransmitter synthesis, the frequency of nerve impulses, the process of release of neurotransmitter and its inactivation by uptake mechanism or enzymatic degradation or both. Very little attention has been paid to the status of neurotransmitter receptors in the brain of hypertensive subjects. With the increasing use of ligand binding studies it is possible to precisely determine the type of receptors along with their affinity and density in any particular area of the brain.

Studies have been carried out using the radioligand binding technique to determine the difference in the receptor affinity and population between control and hypertensive rats. It is known that the number of central neurotransmitter receptors alter in an inverse manner to changes in the neuronal activity (Skolnick et al., 1978; U’Pritchard et al., 1979; 1980; Morris et al., 1980). It is expected that a change in
receptors will also reflect the neuronal activity. Most studies have reported an increase in the population of central $a_1$ and $a_2$ adrenoceptors (Gheyouche et al., 1980; Morris et al., 1981; Palmero et al., 1981; Wirz-Justice et al., 1983) while some have reported no change in $a$ adrenoceptors (Hellstrand and Engel, 1980; Cantor et al., 1981) in spontaneously hypertensive rats (SHR). The $a$ adrenoceptors located in the nucleus tractus solitarius (NTS), responsible for the facilitation of transmission of baroreceptor impulses, are of the $a_2$ type. Evidence has also been found that $a_1$ adrenoceptors in NTS have opposite effects (Anden et al., 1978; Mouille et al., 1980; Persson et al., 1981; Huchet et al., 1981; 1982). Increase in the population of central $a_1$ adrenoceptors in spontaneously hypertensive rats may thus be expected to oppose the cardio-inhibitory response to centrally acting sympathomimetics that possibly accompany increase in density of $a_2$ adrenoceptors. On the other hand, $a$ adrenoceptor binding has been reported to be both decreased (Myers et al., 1981) and increased (Wirz-Justice et al., 1983). Observations by Bottiglieri et al. (1988) suggest that increase in $a$ adrenoceptor may be the result of their increased synthesis or decreased degradation. The functional role of $\beta$ adrenoceptors is not clear.

Central dopaminergic system also plays a role in hypertension (Yen et al., 1979; Martin and Quock, 1980; Bhargava, 1983). Evidence of increased $[^3H]$-spiroperidol binding has been obtained in SHR rats (Chiu et al., 1982; Bhargava, 1984). Since, it has been reported that stimulation of central dopaminergic system also results in the inhibition of noradrenergic system (Maj et al., 1970; Seeman, 1980), it could be possible
that the prolonged decrease in brain dopaminergic activity in hypertensive subjects leads to the development of dopamine receptor supersensitivity and an increase in \(\alpha_1\) and \(\alpha_2\) adrenoceptor population.

Serotonergic mechanisms in the brain have been suggested to affect blood pressure but the results are controversial (Antonaccio 1984). There are multiple interactions between the central serotonergic system and sympathetic system with respect to cardiovascular control but their nature is not well understood.

The role of brain acetylcholine in cardiovascular regulation has been considered but not well investigated. Recent work strongly suggests that it may have a role in modulating baroreceptor reflexes and in elevating arterial pressure through increased sympathetic activity (Brezenoff and Coram, 1982).

It is apparent from the available literature that even though a lot of work has been done on the physiological aspect of neurotransmitters but not much has been done to study the influence of repeated administration of antihypertensive drugs on central neurotransmitter receptors. It is important to determine the status of central receptors in animals treated chronically with antihypertensive drugs. The effect of antihypertensive drugs on these receptors will ultimately define the role of these receptors in the antihypertensive action of these drugs and in hypertension.
REVIEW OF LITERATURE
Hypertension is a frequently occurring disease of the developed as well as the developing countries. Although a persistent elevation of blood pressure beyond the normal range may be devoid of clinical symptoms but the complications resulting from this disorder are severe. The genesis of hypertensive disease is complex where many different factors may be involved. Peripheral vascular resistance, vascular reactivity, cardiac output, the autonomic nervous control, blood viscosity etc. may all be involved in a very complex and interdependent manner.

The life span of hypertensive patients can be increased by many years if appropriate therapeutic measures bring about a reduction in blood pressure to near normal level (Abrams 1974). The main thrust of antihypertensive therapy consists of treatment with drugs. Usually the treatment is symptomatic regardless of the type of hypertension. However as quickly as possible the cause should be treated. For this, reasonable knowledge of the cause of hypertensive disease and the mechanism of action of antihypertensive drugs is important. Many potential sites and mechanisms exist through which a drug may exert its antihypertensive effect. The first attempt at modern pharmacotherapy of hypertension began around 1955 with the availability of drugs like reserpine, hydralazine, veratrum alkaloids, thiazide diuretics and guanethidine. Although, these drugs made the first real impact on the morbidity and mortality associated with the complications of hypertension, the side effects of these agents remained unacceptable to many patients. As a result some of these compounds were discarded or had to be used in combination with other drugs. Attention was subsequently drawn
towards the influence of the central nervous system in the development of hypertensive disease. Clonidine (Catapres 155) was developed, which decreased blood pressure by acting mainly on the central nervous system. The brain stem has been shown to be an important site of action of this agent (Schmitt et al., 1968). Reduction of central sympathetic outflow also contributes to the fall in blood pressure produced by this drug and guanabenz (Baun and Shropshire, 1970).

The etiology of primary hypertension is obscure and hence the choice of drug treatment cannot be wholly adequate. Moreover, the pharmacotherapy remains the most useful tool in the management of primary hypertension. The treatment has to be often life long and a high standard of efficacy and safety is required of the drugs used. The antihypertensive drugs can be mainly classified as (1) centrally acting antihypertensives, mainly adrenergic agonists (2) peripherally acting antihypertensives, which can be antiadrenergic, mineralocorticoids, diuretics and direct acting drugs. We have selected clonidine a centrally acting drug, hydralazine a peripherally acting antihypertensive, reserpine which acts by releasing catecholamines and centhaquin a new centrally acting antihypertensive drug for study.

CENTHAQUIN

Centhaquin is an antihypertensive drug developed at the Central Drug Research Institute, Lucknow, India. It is a synthetic, white crystalline compound with poor solubility in water but is soluble in ethanol and propylene glycol. The pharmacological profile of centhaquin shows hypotensive activity in different models of hypertension. It decreases
cardiac output and causes a slight increase in mesenteric blood flow and femoral blood flow showing a little bit of vasodilation. It is a weak central nervous system depressant at the dose at which a hypotensive effect is obtained. Centhaquin shows reasonably good diuretic activity and a wide margin of safety with high LD50 values. The marked hypotensive effect of the compound is retained in decerebrate cats. A small dose of 5 microgram given in the vertebral artery shows a significant fall in blood pressure. The effect of centhaquin on pre and post ganglionic sympathetic nerve stimulation of nictitating membrane is very minimal. The intravenous administration of centhaquin to spinal cat produced a much reduced effect compared to decerebrate unanesthetized cat (Raghubir et al., 1987). Thus centhaquin was found to be a centrally acting antihypertensive agent. The site and mechanism of action of centhaquin are yet to be determined. The nature of neurotransmitters involved in action of centhaquin was also to be determined. Toxicity studies showed this compound to be safe and there was no histopathological, biochemical or physiological abnormality observed after repeated administration of centhaquin in rats, mice or monkeys. The major side effect of centrally acting antihypertensive drugs is sedation and drowsiness. In rats, centhaquin produced a dose dependent decrease in spontaneous motor activity. Doses above 120 mg/kg i.p. produced ataxia, decreased respiration and hypothermia.

CLONIDINE

Clonidine, 2(2,6-dichlorophenylamino)2-imidazoline, also called ST 155 or Catapresan or Catapres or Catapressan, was synthesized by Stahle in 1962. It was
originally developed as a nasal decongestant but during clinical trials clonidine was found to produce bradycardia, hypotension and sedation. Experiments by Graubner and Wolf (1966) confirmed these findings and clonidine was introduced in the clinical practice as an antihypertensive drug.

Clonidine exerts a complex action on the circulation when administered intravenously, it induces biphasic changes in blood pressure, initial hypertension followed by a long lasting hypotensive effect. As the dosage is increased, the intensity and duration of the hypertensive effect becomes greater and hypotension is delayed. In addition, bradycardia and reduction in cardiac output are constant features in animal and man. The initial increase in blood pressure has been suggested to be secondary to vasoconstriction as a result of direct activation of \( \alpha \) adrenoceptors, since clonidine is effective following pretreatment with reserpine (Hoefke and Kobinger, 1967), guanethidine (Razzak et al., 1975) and bretylium. This \( \alpha \) adrenergic effect can be blocked by \( \alpha \) adrenoceptor antagonists such as phentolamine and phenoxybenzamine (Boissier et al., 1968; Constantine and McShane, 1968; Schmitt and Schmitt, 1970). The hypotensive action is caused by a centrally mediated reduction in peripheral sympathetic tone through stimulation of central \( \alpha \) adrenoceptors (Anden et al., 1970; Bock and Van Zwieten, 1971; Haeusler, 1974; Kobinger and Pichler, 1974; Finch et al., 1975; Van Zwieten, 1975) Other possible mechanisms suggested include stimulation of central \( H_2 \)-receptors (Karppanen et al., 1976); involvement of central cholinergic mechanism (Srimal et al., 1977) and central opioid mechanism (Raghubir et
al., 1985). Investigations with spinal animals revealed only increase in blood pressure that was even more distinct than in intact animals (Constantine and McShane, 1968; Kobinger and Walland, 1967a). Administration of clonidine (1 μg/kg) into the cisterna cerebellomedullaris of cat caused a long lasting fall in blood pressure and bradycardia, whereas intravenous injection of the same dose was largely ineffective (Kobinger, 1967; Schmitt et al., 1968). Low concentrations of clonidine infused into one of the vertebral arteries caused a fall in blood pressure and bradycardia, whereas hardly any effect was observed following infusion of the same doses into a peripheral vein (Sattler and Van Zwieten 1967). Clonidine injected intracerebroventricularly (icv) in dogs, cats and rabbits induced a decrease in blood pressure (Sherman et al., 1968; Schmitt, 1969).

According to Haeusler (1982) α2 adrenoceptors in the nucleus tractus solitarius (NTS) are the main site of action of clonidine. Infusion of clonidine into the NTS reduced blood pressure and heart rate (Sinha et al., 1975). Therefore, this area is considered to be the main site of action. However, lesions of this area did not change blood pressure in cat (Antonaccio and Halley, 1976; Laubie et al., 1976), and clonidine continued to reduce blood pressure and heart rate. A small area on the ventral surface of the medulla of cats (Schaffe’s zone) seems to be another site of action of clonidine. Bousquet and Guertzenstein (1973) were able to block the cardiovascular actions of clonidine administration locally in this area with pimozide, thus supporting a dopaminergic action, but piperoxan was also an effective antagonist. Microinjection of
clonidine into the nucleus reticularis lateralis in cats also lead to hypertension and bradycardia (Bousquet et al., 1981). Franz and Madsen (1982) suggested that although the spinal reflex pathway was more sensitive to depression by clonidine than its different descending intraspinal pathway, analysis of the relative depression of transmission at spinal and at brainstem level indicate that the spinal site is more sensitive to clonidine than was considered. The bradycardiac effect is mainly brought about by facilitation of vagal reflex activity (Van Zwieten, 1975).

In the periphery, clonidine has been proposed to interact with presynaptic α-adrenoceptors in the arterial wall and the effect on the cardiac pacemaker seems to be due to stimulation of α-adrenoceptors in adrenergic nerve endings and thus reduction of transmitter release (Starke and Altmann, 1973; Langer, 1977). Kupfer et al. (1980) have reported that the effect of clonidine (0.003 mg/kg, intracisternally) was reversed when the drug was given after the alkaloid. These results suggest possible interactions of the drugs at central GABAergic, glycinergic or cholinergic synapses.

Since the effects of noradrenaline are potentiated after clonidine in pithed rats (Boissier et al., 1968) some peripheral component must be involved. In man, no significant interaction of clonidine with catecholamines was observed (Onesti et al., 1971). In high doses, clonidine reduced the effect of noradrenaline (Boissier et al., 1968). However, Reinhard and Roth (1982) reported that clonidine administration (30 μg/kg) did not alter the levels of noradrenaline, serotonin, dopamine, homovanillic acid or 3,4-dihydroxyphenylacetic acid in the brain. Coupar and Kirby (1972) suggested that
Clonidine may cause venodilation by blocking endogenous release of noradrenaline, an effect which may contribute to the hypotensive action of the drug. Experiments by Anglade et al (1987) demonstrated that clonidine decreases adrenaline release from the adrenal glands through a central and not a peripheral mechanism in dogs, and this action might contribute to its antihypertensive effect.

The concept that a presynaptic \( \alpha \) adrenoceptor stimulation could inhibit noradrenaline release is generally accepted (Starke et al., 1975). Phentolamine increases noradrenaline release and in addition antagonizes the effect of clonidine (Starke et al., 1974). Greenberg and Wilborn (1982) suggested that clonidine may act on the veins or indirectly by inhibiting the release or synthesis of a trophic noradrenergic factor, to reverse the functional and structural changes in the veins. It has been proposed by Lorez et al. (1983) that the effect of clonidine on noradrenaline turnover is most likely the result of a local feedback inhibition through presynaptic \( \alpha \) adrenoceptors. Medgett and Rand (1983) suggested that the NTS and intermediolateral cell column of the spinal cord are the prime centers for the cardiovascular action of clonidine. Since the cardiovascular effects of clonidine can be elicited in the virtual absence of neuronal noradrenaline, the decrease in central noradrenaline turnover and the cardiovascular effects of clonidine are not interrelated phenomena. Observations by Schoener and Pitts (1985) suggested that the dose related depression of mean arterial pressure and heart rate under basal conditions may be related to specific \( \alpha_2 \) adrenoceptor activation of the NTS. Bentley et al. (1986) proposed that clonidine causes a selective reduction in
sympathetic tone to the veins that is mediated at least partly by a central action as well as an expansion of the collateral venous routes. This together with the selective impairment of vasoconstrictor responses to both noradrenaline and adrenaline, may account for the decrease in cardiac output that is most often reported following clonidine induced reduction of mean arterial pressure. Clonidine given peripherally or centrally to normal or spontaneously hypertensive rats is associated with development of tolerance or tachyphylaxis to its cardiovascular response (Mastrianni and Ingenito 1985).

In dogs, intrarenal arterial administration of clonidine not only decreased renal blood flow (RBF) without affecting glomerular filtration (GFR) but increased blood pressure, while intravenous administration decreased RBF without affecting either GFR or blood pressure. This may be explained by the fact that clonidine acts predominantly at post synaptic and central sites in the latter instance (Chrysant and Lavendar, 1975). However, human studies have shown that neither acute nor chronic oral clonidine disturbs RBF and may decrease renal vascular resistance (RVR) in hypertensive patients (Cohen et al., 1969).

Clonidine in hypertensive patients reduced the excretion of urinary catecholamines (Hokfelt et al., 1975). This effect is believed to be due to the decreased sympathetic tone. Clonidine does not change the level of catecholamines in the brain (Hoefke and Kobinger, 1966; Persson and Waldeck, 1970; Bralet and Rochett, 1973). A slight increase in noradrenaline or dopamine levels has been reported after high
doses of clonidine. The increased dopamine level in the corpus striatum could account for the stereotypy seen with high doses of the drug (Persson and Waldeck, 1970). On the contrary Anden and Grabowska (1976) have reported that clonidine produced only a slight decrease of dopamine synthesis and utilization in the striatum. Clonidine has no effect on the firing rate of substantia nigra dopamine neurons.

It did not effect the firing rate of the single substantia nigra dopamine neurons, but it regularized the firing pattern and decreased the burst firing at 2-8 μg/kg, i.v. These effects were antagonized by α₂ adrenoceptor antagonist yohimbine (Grenhoff and Sevensson, 1988).

It has been further suggested that the bradycardiac effect of clonidine results from activation of both central and peripheral α₂ adrenoceptors (Park et al, 1983). Chronic administration of the type-A MAO inhibitor, clorgyline, attenuated the central responses to clonidine through the reduction in sensitivity of brain α adrenoceptors. Pargyline that preferentially inhibits type-B MAO, reduces only the bradycardia induced by clonidine. This indicates a different modulation of the receptors involved in this responses of clonidine. Clonidine also inhibits locus coeruleus (LC) neurones by stimulating α₂ adrenoceptors (Lal and Fielding, 1983). Neurotransmitter concentration in the synaptic cleft may be responsible for the transsynaptic population. The α₂ adrenoceptors which are presynaptically located on the serotonergic terminals but are postsynaptic in relation to the noradrenergic neurons also show increased sensitivity after chronic clonidine treatment (Cerrito et al., 1984). Kawasaki and Takasaki (1985)
suggested that clonidine produces hypertension which is mediated by stimulation of central postsynaptic $\alpha_2$ adrenoceptors. Ketar et al. (1984) have shown by receptor binding studies in the rat cerebral cortex that clonidine is 1,000 times more potent in competing for $\alpha_2$ than for $\alpha_1$ adrenoceptor sites. Stone and Forster (1986) showed that $[^3H]$-clonidine binding was enhanced in vas deferens and it was unchanged or slightly increased in cerebral brain tissue of rat. $[^3H]$-clonidine binds at particulate membrane fractions of human prefrontal cortex, binding showed at least two affinity states (Carlson and Andorn, 1986). Treatment of rat cerebral cortex membranes with 10 mM [(3-cholanidopropyl)-dimethylammonio] 1-propane sulfonate (CHAPS0) solubilized about 30% $[^3H]$-clonidine binding sites in the starting membranes and showed a nonlinear curve, indicating the existence of two distinct binding components (Kitamura et al., 1986). Pharmacological evidence suggests that central $\alpha_2$ adrenoceptor activation is associated with sedation in addition to reduction in blood pressure (Bousquet et al., 1983).

Clonidine in a single dose caused a significant fall in blood pressure and heart rate. These effects were much more pronounced in SHR while being ineffective in normotensive Wistar Kyoto controls (WKY) (Head and De Jong, 1986). Prolonged treatment of SHR with clonidine decreased the mean arterial pressure and heart rate (Pegram et al., 1982). On stopping chronic clonidine treatment, an abrupt rebound of the blood pressure to values higher than those recorded before treatment has been reported by several authors. Repeated treatment with clonidine induces a
functional subsensitivity of central $\alpha_2$ adrenoceptors. This subsensitivity is mainly manifested as attenuation of different effect such as hypothermia (Von Voigtlander et al., 1978; Gorka and Zacny, 1981; Pilc and Vetulani 1982a,b).

Chronic clonidine administration can induce down regulation of not only $\alpha_2$ presynaptic autoreceptors located on noradrenaline terminals but also $\alpha_2$ presynaptic heteroreceptors located on serotonergic terminals (Maura et al., 1985). The increased pressor responsiveness of both SHR and WKY rats to arginine vasopressin following clonidine is an unexpected finding and may be related to peripheral interaction between $\alpha$ adrenoceptor agonist and vasopressin (Datar et al., 1986). Finberg and Kopin (1987) have reported against desensitization of peripheral presynaptic $\alpha_2$ adrenoceptors by chronic administration of $\alpha_2$ adrenoceptor agonist. Sympathetic hyperactivity following withdrawal of chronic clonidine treatment may be mediated by down regulation of postsynaptic $\alpha_2$ adrenoceptors in the central nervous system.

Srimal et al. (1977) suggested that an intact cholinergic link in the brain stem is essential for the hypotensive action of clonidine. In view of demonstrated interaction between clonidine and cholinergic mechanism in some parts of central nervous system (Buccafuso and Spector 1980; Buccafuso et al., 1980; Svesson and Engberg 1980), it is possible that clonidine may express at least a part of its antagonistic action on the carotid arterial occlusion reflex (CAOR) via inhibition of an excitatory cholinergic mechanism in the posterior hypothalamus. Buccafuso et al. (1980) have reported that clonidine significantly reduced turnover of acetylcholine from certain brain regions and
markedly inhibited the pressor response to intravenous administration of physostigmine. Criscione et al. (1983) have proposed that cholinergic mechanism in the NTS tends tonically to lower arterial pressure after clonidine administration and may modulate the baroreceptor reflex without being an integral part of the reflex arc.

Opioid peptides inhibit locus coeruleus neurons by a direct action at post synaptic sites which may be related to increase permeability to one or more ions such as Cl⁻ or K⁺. They cause a stereospecific hyperpolarization of the neuronal membrane which entails an increase in membrane conductance. This effect is reversible by the narcotic antagonist naloxone but not by antagonists of other neurotransmitters, including α₂ adrenergic antagonists (Pepper and Henderson 1980). Due to the fact that naloxone blocked elevation of blood pressure, cardiac output and heart rate in cats, it is evident that the opioid link is present in clonidine induced bradycardia and hypotension (Val’dman et al., 1982; Farsang and Kunos, 1979; Kunos et al., 1987). It was concluded by Chauhan et al. (1983) that the hypertensive effect could be due to an opioidergic mechanism since it was blocked by both naloxone and yohimbine. Although Eriksson and Tuomisto (1983) suggested that opioid mechanism possibly participates in the hypotensive action of clonidine, but its role is less in normotensive than in spontaneously hypertensive animals. In patients of essential hypertension the antihypertensive effect of clonidine could be antagonized by the opioid receptor blocker naloxone. Plasma concentrations or the opioid peptides β-endorphin and Leucine enkephalin which are lower in young hypertensive patients are normalized by stimulation.
of \( \alpha_2 \) adrenoceptors with clonidine (Kraft and Stumpe, 1987). Findings of Kunos et al. (1987) indicate that \( \beta \)-endorphin released from an arcuate NTS pathway acting on opiate receptors in the NTS contributes to the cardiovascular depressor effects of clonidine.

**HYDRAZINO**

Hydralazine (1-hydrazino phthalazine) has been used in the therapy of hypertension for about four decades. The hypotensive effect of hydralazine, which is characterized by its gradual onset, limited degree and long duration, was observed as early as 1945. But the first paper on this blood pressure lowering agent was published five years later (Gross et al., 1950). One of the reasons for the delay was the unpleasant and acute side effects such as headache, nausea, vomiting, flushing, tachycardia and angina. However, in combination with other drugs, hydralazine is very useful for treating chronically hypertensive patients. The role of hydralazine in antihypertensive therapy has expanded, since it was demonstrated that the drug is particularly effective when combined with propranolol or other \( \beta \) adrenergic antagonists (Hansson et al., 1971; Zacest et al., 1972; Gotlieb et al., 1972; Koch-Weser 1974; Person, 1975).

The characteristic action of hydralazine is the lowering of blood pressure, which in all species studied, including man is very similar. The onset of action is gradual, the maximum response is reached after 10 to 20 min, the degree of blood pressure reduction is limited and the duration of action is long (Gross et al., 1950).
In anesthetized and conscious dogs a long lasting reduction in blood pressure was observed (Craver et al., 1951) but this acute hypotensive effect was less pronounced in rats (Bein and Brunner, 1965). In the spinal cat, in which the basal blood pressure is reduced to about 50-60 mm Hg, hydralazine did not induce further fall (Gross et al., 1950).

In acute studies in man, together with the fall in blood pressure an increase in pulse pressure was observed and circulation time was shortened (Schmid and Kellner, 193). Blood flow in the more dilated circulatory beds generally increased unless the fall in arterial pressure was very marked. Provost et al. (1981) observed that the hypotension induced by dihydralazine in normotensive and renal hypertensive rats was caused by decreasing peripheral resistance. Hydralazine caused a rapid fall in blood pressure and peripheral resistance in unrestrained spontaneous hypertensive rats lasting up to 24 hours. Immediately after injections of hydralazine the cardiac output and heart rate increased significantly (Struyker-Boudier et al., 1983). The reduction in peripheral vascular resistance is the result of a general vasodilation of the precapillary resistance vessels (Mellander and Johnson, 1968). Hydralazine affected postcapillary capacitance vessels much less than precapillary resistance vessels (Ablad, 1963). It has no appreciable effects on nonvascular smooth muscle.

Although on the basis of the initial pharmacological studies on hydralazine it was supposed that the drug acted directly on the arteriolar smooth muscle, some data were available which suggested that there was a central site of action (Gross et al., 1950;
Graver et al., 1951; Baun et al., 1972; Fries et al., 1973; Daird et al., 1975; Koch Weser, 1974, 1976). When the isolated brain of cat was perfused in situ, hydralazine, added to the perfusion fluid, produced a fall in blood pressure and tone of tachycardia together with a decrease in hind limb vasculature (Ingenito et al., 1969). However, other investigators reported contradictory results when hydralazine was injected into the cisterna magna or the third ventricle of cats. Only a slight fall in the blood pressure was observed accompanied by a reduced pressor response to intravenously administered noradrenaline (Schmitt and Giguel, 1956). In cats only an enhanced chemoreceptor activity was observed after intracarotid injection, but there was no increase in the splanchnic activity. It was obvious that hydralazine behaved like a slow acting peripheral vasodilator (Dontas, 1957). From extensive studies of Ablad et al. (1963) it was evident that hydralazine exerts its hypotensive effect by means of a direct action on the vascular smooth muscle and has no influence on centers regulating blood pressure. Furthermore, injections of hydralazine into the vertebral artery of cat caused a hypotensive response similar to intravenous injection (Van Zweiten, 1968). In case of a central mechanism of action, the intravertebral injection should induce a more pronounced hypotensive reaction than the intravenous injections.

The site of action of hydralazine appears to be primarily peripheral since it interferes with the hypertensive effect of several peripherally acting pressor substances including serotonin, epinephrine, norepinephrine and pitressin. Bein (1953) has shown that hydrazinophthalazine antagonizes the constrictor effect of ergotamine and
ephedrine after transection of the spinal cord, providing further evidence that hydralazine acts at the blood vessels. Bolt and Saxena (1984) observed that with higher doses of hydralazine the synergistic effect on blood pressure response disappeared due to an increase in cardiac output. Despite effective β adrenoceptor blockade the antihypertensive drugs acted synergistically only when low doses of hydralazine were used.

Hydralazine tends to cause sodium and water retention, expansion of plasma and extracellular fluid volume, weight gain and edema formation (Koch Weser, 1974). These disturbances in electrolyte and fluid balance do not reflect decrease in renal blood flow or glomerular filtration rate, both of which are generally either increased or unchanged during hydralazine therapy (Judson et al, 1956). They are consequences of the reduction in arterial pressure and may be mediated both by the renin-angiotensin-aldosterone system and by a direct renal mechanism. If expansion of plasma and extracellular fluid volume is avoided, tolerance to the antihypertensive action of hydralazine does not develop (Finnerty, 1971). Treatment with hydralazine causes both right ventricular (RV) and left ventricular (LV) hypertrophy and eccentric LV hypertrophy. Intravascular volume expansion associated with redistribution of blood volume to the central compartment, may play a major role in these cardiac effects. Increased renin release but not a generalized increase in sympathetic tone may play a role in the development of tolerance to the antihypertensive effect (Tsopris and Leeman, 1986). The reduction of arterial pressure in hypertensive patients during hydralazine therapy is
accompanied by an increase in peripheral plasma renin activity (Ueda et al., 1968, 1970). In conscious rabbits, the release of catecholamines and renin associated with hydralazine induced hypotension could be blocked by the prostaglandin synthetase inhibitor, indomethacin, the hypotensive response was also enhanced (Graham et al., 1979). The release of renin by hydralazine in dogs could be antagonized by prostaglandin synthetase inhibitor only under conditions of preexisting elevated plasma renin levels, after surgical stress (Wang and Epokes, 1979). In contrast, the hypotension produced by hydralazine in conscious foxhounds was blocked by indomethacin, suggesting that the hypotensive responses are in some way mediated by prostaglandins (Brunner et al., 1965; Slack et al., 1978). Renal vasodilation produced by hydralazine may be mediated by prostaglandins since indomethacin also abolishes this effect (Spokes and Wang, 1976). These experiments suggest the involvement of prostaglandin in the action of hydralazine. A similar suggestion has been made by Chelly et al. (1986). Shepard et al. (1981) have stated that determining the acetylator index before giving hydralazine to the hypertensive patient is useful as the plasma hydralazine levels depend on the acetylator index. Hydralazine caused a slight increase in plasma renin activity and urinary excretion of noradrenaline in patients with essential hypertension (Velasco et al., 1985).

Boenner et al. (1982) have suggested that the increased formation of kinins within the kidney could be involved in the vasodilator and blood pressure lowering effects of dihydralazine.
Several cellular mechanisms for the relaxation of vascular smooth muscle have been suggested. They are: chelation of certain trace metals like copper ions (Perry, 1953); inhibition of dopamine beta-hydroxylase (Lin et al., 1974); interference with the entry of calcium from the extracellular store and the interference with calcium release from intracellular stores (McLean et al., 1978); and activation of beta adrenoceptors in myocardium resulting in elevation of cyclic AMP. This could explain the increase in myocardial contractility. Although it is unlikely that the antihypertensive effects are due to catecholamine depletion, since repeated hydralazine treatment produces only transient change in catecholamine content of the vasculature (Gross, 1977). Some authors have suggested that increase in cyclic nucleotide concentration often found associated with relaxant effect on smooth muscle (Schultz et al., 1977) may not have a direct causal relationship with vascular relaxation (Diamond and Janis, 1978). It was considered that the excitatory effects of hydralazine at toxic doses might be closely associated with the changes in central GABAergic system (Satoh et al., 1981; Hara et al., 1983). Hara et al. (1987) suggested that hydralazine might antagonize the pentylenetetrazol seizures at least partly by modulating the activation of the central serotonergic system.

RESERPINE

The first reports on the antihypertensive properties of Rauwolfia serpentina and its derivatives were published by Bhatia (1942), Gupta et al. (1943), Vakil (1949), Roy (1950) and Wilkins (1953). It was, however, only in early 1950's that Rauwolfia serpentina
preparation like reserpine were introduced for the treatment of mild hypertension. It was soon established that reserpine was useful in reducing aggression also. It was also noted that the blood pressure and pulse rate were lowered (Kline, 1954). By the end of 1960's, reserpine had almost passed out of psychiatric use-being replaced by more efficacious drugs (Luby, 1968). Apart from drowsiness and sedation, reserpine also caused mood changes, especially depression. Depression has been reported in upto 25 percent of patients, treated with reserpine (Aldrich and Anchor, 1973) and even suicidal tendencies have been documented (Lemiex et al, 1956). Nasal stuffiness, which reflects cholinergic stimulation by reserpine can also be troublesome. However, reserpine still has a place in antihypertensive therapy (Freis, 1971).

Reserpine acts on the central nervous system and the peripheral sympathetic system depleting norepinephrine stores in the brain and peripheral adrenergic nerve endings. It does so by inhibiting the uptake of its precursor dopamine, into the storage vesicle which contains the enzyme dopamine β hydroxylase. This action of reserpine also leads to reduced quantities of newly formed dopamine, which is not taken up and stored, and is more rapidly metabolized, primarily by monoamine oxidase (Ruttedge and Weiner, 1967). Thus reserpine is different from the classical centrally acting agents, as it appears to directly inhibit adrenergic mechanisms rather than work through the agonist actions postulated for the other agents. Nevertheless, the end results appear to be similar, that is, sympathetic outflow is decreased and vagal tone is increased. This latter effect may explain the decrease in heart rate seen with reserpine treatment.
Treatment of animals with reserpine resulted in depletion of catecholamines from the sympathetic ganglia, blood vessels, spleen, iris and other tissues with adrenergic innervation (Bertler et al., 1956, Paasonen and Krayr, 1958; Muschol, 1959; Sjostrand, 1962; Berkowits et al., 1971). It also depletes the brain of its content of noradrenaline (Holzbaner and Vogt, 1956) and serotonin (Karki and Paasonen, 1959). The depletion of these two amines in rabbit brain occurs in parallel time courses (Brodie et al., 1957). The depletion of dopamine in rabbit and sheep brain by reserpine takes place more rapidly than noradrenaline (Bertler et al., 1956). In pigeons reserpine causes depletion of serotonin in brain more rapidly than dopamine and noradrenaline (Jurio and Vogt, 1967). Not only are these interspecies differences but there are differences within a species also. Thus reserpine causes a more pronounced depletion of brain catecholamines in infant than in adult rats (Kulkarni and Sheidman, 1966). Dopamine synthesis in rat striatum is increased 3 to 4 times by in vivo treatment with reserpine whereas it doubles in the striatal synaptosomes (Vissari, 1982).

Apart from the depleting effect of reserpine on adrenergic, dopaminergic and serotonergic neurons from their transmitters (Shore, 1972), it may also reduce brain acetylcholine storage capacity (Finberg, 1979). Treatment with reserpine results in an increase in the acetylcholine content of the brain of dog (Melhotra and Mehta, 1966), rat (Malpica et al., 1970) and cat (Malhotra and Prasad, 1968). The effects of reserpine appear to be localized to certain regions, since in the
dog there is an increase in the acetylcholine content of the hypothalamus, but reserpine induced decrease in the hippocampus (Malhotra and Punlik, 1959). These changes in cholinergic systems may be related to the changes in electrical activity of the hippocampus (Killam and Killam, 1967). A cholinergic component has been reported in the response of the nictitating membrane of reserpinized cat (Martin and Cervoni, 1962). It is suggested that there are cholinergic fibers running with the adrenergic fibers in the sympathetic nerve trunk or a cholinergic mechanism is present in the adrenergic nerves (Burn and Rand, 1965).

The administration of reserpine results in reduction of response of effector organs to stimulation of postganglionic adrenergic nerves, even though the effector organ remains responsive to injected noradrenaline (Gaffney et al., 1963). The loss of peripheral adrenergic transmission has a latency and occurs sometime after the appearance of the usual signs of the reserpine syndrome such as sedation, bradycardia, decrease in blood pressure and relaxation of the nictitating membrane. The loss of adrenergic transmission after reserpine treatment is not complete. Thus adrenergic nerve stimulation still produces positive inotropic response in guinea pig atria (Barnett and Benforado, 1966) and chicken heart (Bolton, 1967) but the response is abolished in rabbit (Hukovic, 1959).

Although reserpine can deplete tissues of their catecholamine contents, some signs of increased sympathetic activity in response to release of catecholamines are also found. Reserpine gradually lowers blood pressure without causing an initial pressor
response in animals and man (McQueen et al., 1955; Bein, 1956). Reserpine induced depletion of catecholamines is accompanied by the release of inactive deaminated metabolites. This results in a decrease of active catecholamines at the site of action (Kopin and Gordon., 1962, 1963). A primary hypertensive response to reserpine is observed in spinal animals (Schmitt and Schmitt., 1960) and in animals pretreated with ganglion blocking drugs (Bech et al., 1957). Both these procedures result in considerable enhancement of pressor response to adrenaline and noradrenaline. The hypotensive action of reserpine is greater in hypertensive patients than in normotensive ones, although the incidence of side effects do not differ in the two groups (McQueen et al., 1955). Similar observations have been made in rats (Flückinger., 1969).

Reserpine treated rats, subjected to procedures for producing renal hypertension failed to become hypertensive. The isolated perfused hind quarters of these rats exhibit a lesser vasoconstrictor action of noradrenaline than do those of untreated hypertensive rats (McQueen., 1961). In spontaneously hypertensive rats, reserpine has a more pronounced antihypertensive activity than in renal hypertensive rats (Ebinhera and Martz, 1970).

The antihypertensive action of reserpine is often attributed to the loss of vasomotor and cardiac sympathetic tone consequent to the depletion of noradrenaline from the peripheral adrenergic nerves subserving cardiovascular control (Green., 1962). The depletion of noradrenaline from cardiovascular control centers in the hypothalamus, medulla and from neurons in the lateral horns of the spinal cord which make synaptic
connections with the thoracolumbar sympathetic preganglionic neurons, could also be responsible for the fall in blood pressure.

Reserpine treatment increases both CNS $\alpha_1$ and $\alpha_2$ adrenoceptors (U'Prichard and Snyder, 1978). Bylund and Martinez (1980) have reported the induction of $\alpha_2$ binding sites in the rat salivary gland following reserpinization. Although reserpine depletes catecholamines, it also causes an upregulation of $\beta$ adrenoceptors, which lithium is able to counteract (Treiser and Kellar, 1979) and it probably does this without reversing the reserpine induced catecholamine depletion. In contrast to the ability of lithium to antagonize dopamine antagonist induced supersensitivity, it enhances the behavioral supersensitivity following treatment with reserpine (Friedman et al., 1979). According to Greenberg and Weiss (1979) repeated doses of reserpine to 3 month old rats produced dose related increase in $[^3H]$ dihyroalprenolol (DHA) binding in pineal gland, cerebral cortex and cerebellum. Reserpine increased DHA binding by increasing the density of $\beta$ adrenoceptors. Reserpine induced supersensitivity to isoprenaline does not appear to involve a change in the affinity for $\beta$ adrenoceptor or in receptor number as determined by $[^3H]$ DHA binding (Hawthorn and Broadley, 1982). Senas et al (1986) have suggested a different degree of sensitivity or response of the noradrenaline and adrenaline neurons following reserpine administration.

Chronic treatment with reserpine, which depletes 5-HT stores or p-chlorophenylalanine, 5HT synthesis inhibitor, elicited 20-30 % increase in $[^3H]$ 5-HT binding in numerous brain regions (Bennett and Snyder., 1976). Chronic reserpine
treatment is reported (Sharma et al., 1979) not to influence \[^{3}H\] QNB binding sites in cortex and hippocampus.

The evidence against the central site of action of 'antihypertensive drug' has been provided by several authors. Thus after treatment with reserpine, no diminution in electrical discharge in the preganglionic sympathetic nerves was observed which is expected following depression of sympathetic centers (Kobinger & Pichler, 1976).

It is apparent from the available literature that even though a lot of work has been done on the physiological aspect of neurotransmitters but not much has been done to study the influence of repeated administration of antihypertensive drugs on central neurotransmitter receptors. It is important to determine the status of central receptors in animals treated chronically with antihypertensive drugs. The effect of antihypertensive drugs on these receptors will ultimately define the role of these receptors in the antihypertensive action of these drugs and in hypertension.
METHODOLOGY AND TECHNIQUE
Albino, male rats of the Charles Foster strain, bred in the animal house of the Central Drug Research Institute were used. Rats weighing 100-150 g were taken and kept in standard laboratory conditions of temperature (23 ± 1.5 °C), humidity (50 ± 10%) and dark light (5.00-18.00 h) cycle. The animals were allowed to acclimatize for four days before starting the experiment.

Antihypertensive drugs were administered orally to rats for two months. The doses of these drugs were selected on the basis of several reports, and preliminary work done in our laboratory, indicating the best dose which could be used chronically and effectively. Clonidine (0.1 mg/kg) dissolved in water, centhaquin (0.1 mg/kg) synthesized in the Central Drug Research Institute was dissolved in 10% ethanol, dihydralazine (Nepresol) (0.25 mg/kg) made by Ciba Geigy of India Ltd., was dissolved in water, reserpine (Serpasil) (1.25 mg/kg) made by Ciba Geigy of India Limited was also dissolved in water. These drugs were prepared fresh everyday just before the administration.

1. RECEPTOR BINDING STUDIES:

It is necessary, to differentiate between the two types of binding, one, which is specific to the type of receptor under investigation and the other, nonspecific binding to tissue biopolymers. The number of specific receptor sites are limited whereas the nonspecific sites are unlimited. The binding experiment was, therefore repeated in the presence of excess amount of non-radiolabelled ligand. Under these conditions it is likely that the radio labelled ligand would have minimal binding to the specific sites while
non-specific binding remained unaffected. The difference in the binding values for these two experiments gives the amount of specific binding. Repeating the two experiments for a range of labelled ligand concentrations helps in plotting down the results. This clearly shows that the amount of specifically bound ligand reaches a maximum despite the addition of increasing amounts of ligand. This situation would be expected if the number of specific receptors are limited. Such curves are capable of the mathematical analysis common to similar biochemical studies from which dissociation constants and the number of receptor sites are determined.

1.1 Preparation of membranes:

At the end of the two months of chronic drug treatment the animals were sacrificed and decapitated. Thereafter, specific areas of the brain like caudate, cortex, hypothalamus and medulla were dissected out in cold. Each brain area was weighed and then homogenized in 30 volumes of ice cold 50 mM Tris Hcl buffer (PH 7.4 at 20°C) containing 0.1% ascorbic acid, 120 nm pargyline, 5 nM potassium chloride and neuronal membranes were prepared.

All the experiments were repeated thrice and the data was analyzed using least square regression analysis and analysis of variance.

1.2 Binding of $^3$H-dihydroergocryptine to $\alpha$-adrenergic receptors

The freshly dissected brain regions were homogenized in 30 volumes of 50 mM Tris-HCl buffer (pH 7.7 at 25°C) using a motor driven teflon-glass tissue homogenizer. The tissue homogenate was centrifuged twice at 49,000 x g for 15 min in a refrigerated
Sorvall centrifuge after resuspending in fresh Tris buffer. The final pellet was suspended in 50 mM Tris buffer (pH 7.4 at 25 °C) consisting of 0.1% ascorbic acid, 120 mM NaCl, 5 mM KCl, 2 mM CaCl\textsubscript{2} and 10 μM pargyline to give a concentration of 10 mg wet tissue per ml of incubation buffer. The standard assay mixture contained 0.2 ml of the homogenate, containing approximately 200 μg of tissue, 0.1 ml of \textsuperscript{3}H-dihydroergocryptine, 0.1 ml of competing agent (phentolamine) and buffer to make up the total volume of 1 ml. Incubation was carried out in triplicate in a shaking water bath maintained at 37 °C for 10 min. At the end of the 10 min period, the contents of the incubation tubes were rapidly filtered under partial vacuum using Millipore manifold filtration unit and Whatman GF/C filters. It was followed by two 5 ml. washes of ice cold 50 mM Tris buffer (pH 7.4). The filters were transferred to liquid scintillation vials containing 10 ml of cocktail (composition: 2,5 diphenylonazole 4.9 M; 1,4-bis (5-phenylanzolyl) benzene 200 ng, naphthalein 60 g; ethylene glycol 20 ml; methanol 100 ml; dioxane up to 1 litre). After an overnight equilibration period, the radioactivity was determined in a Packard Tricarb liquid scintillation spectrometer. The specific binding of \textsuperscript{3}H-dihydroergocryptine was defined as the difference in binding observed in the absence and in the presence of 1 μM unlabeled phentolamine. The specific binding was expressed as pmoles of \textsuperscript{3}H-dihydroergocryptine bound per g tissue. The specific binding of the radioligand (8 to 10 concentrations) was determined. This was followed by the Scatchard analysis to determine the dissociation constant (K\text{d}) and the receptor density (B\text{max}) values. Four to five determinations were made to compute the means.
and their standard errors. The data were analyzed by the ANOVA.

1.3 Binding of $^3$H-dihydroalprenolol to $\beta$-adrenergic receptors

The freshly dissected brain regions were homogenized and membranes prepared as described earlier. The final pellet was suspended in 50 mM Tris buffer to give a concentration of 10 mg wet tissue per ml of incubation buffer. The standard assay mixture contained 0.2 ml of the homogenate, containing approximately 200 $\mu$g of tissue, 0.1 ml of $^3$H-dihydroalprenolol, 0.1 ml of competing agent (propranolol) and buffer to make up the total volume of 1 ml. Incubations were carried out in triplicate in a shaking water bath maintained at 37 °C for 10 min. The samples were filtered and the radioactivity was determined in a Packard Tricarb liquid scintillation spectrometer as described in section 1.2. The specific binding of $^3$H-dihydroalprenolol was defined as the difference in binding observed in the absence and in the presence of 1 $\mu$M unlabeled propranolol. The specific binding was expressed as pmoles of $^3$H-dihydroalprenolol bound per g tissue. The specific binding of the radioligand (8 to 10 concentrations) was determined. This was followed by the Scatchard analysis to determine the dissociation constant ($K_d$) and the receptor density ($B_{max}$) values. Four to five determinations were made to compute the means and their standard errors. The data were analyzed by the ANOVA.

1.4 Binding of $^3$H-quinuclydinyi benzylate to cholinergic (muscarinic) receptors

The membranes from freshly dissected brain regions were prepared as described in section 1.2. The standard assay mixture contained 0.2 ml of the homogenate,
containing approximately 200 µg of tissue, 0.1 ml of ³H-quinuclydinyd benzylate, 0.1 ml of competing agent (atropine) and buffer to make up the total volume of 1 ml. Incubation was carried out in triplicate in a shaking water bath maintained at 37 °C for 10 min. At the end of the 10 min period, the contents of the incubation tubes were rapidly filtered and the radioactivity was determined in a Packard Tricarb liquid scintillation spectrometer. The specific binding of ³H-quinuclydinyd benzylate was defined as the difference in binding observed in the absence and in the presence of 1 µM unlabeled atropine. The specific binding were expressed as pmoles of ³H-quinuclydinyd benzylate bound per g tissue. The specific binding of the radioligand (8 to 10 concentrations) was determined. This was followed by the Scatchard analysis to determine the dissociation constant (Kd) and the receptor density (Bmax) values. Four to five determinations were made to compute the means and their standard errors. The data were analyzed by the ANOVA.

1.5 Binding of ³H-spiroperidol to dopamine receptors

The membranes from freshly dissected brain regions were prepared as described earlier in section 1.2. The standard assay mixture contained 0.2 ml of the homogenate, containing approximately 200 µg of tissue, 0.1 ml of ³H-spiroperidol, 0.1 ml of competing agent (haloperidol) and buffer to make up the total volume of 1 ml. Incubation and filtration were carried out as described before. The filters were transferred to liquid scintillation vials and the radioactivity was determined in a Packard Tricarb liquid scintillation spectrometer. The specific binding of ³H-spiroperidol was
defined as the difference in binding observed in the absence and in the presence of 
1 µM unlabeled haloperidol. The specific binding was expressed as pmoles of \(^{3}\)H-
spiroperidol bound per g tissue. The specific binding of the radioligand (8 to 10 
concentrations) was determined. This was followed by the Scatchard analysis to
determine the dissociation constant (\(K_d\)) and the receptor density (\(B_{\text{max}}\)) values. Four
to five determinations were made to compute the means and their standard errors. The
data were analyzed by the ANOVA.

1.6 Binding of \(^{3}\)H-5-HT to 5HT\(_1\) receptors

The binding assays for \(^{3}\)H-5-HT was carried out as described before except that 
8-10 concentrations (0.25 to 20 nM) of the ligand were used. The tissue in each tube 
was about 300 µg. The incubation was carried out for 15 min in a shaking water bath 
maintained at 37 °C. At the end of the incubation period, the contents of the incubation 
tubes were filtered rapidly under partial vacuum using Whatman GF/C filter discs on a 
Millipore manifold filtration unit. The washing and counting of radioactivity was done 
as described before for other receptor studies. The specific binding was defined as 
the difference in binding of \(^{3}\)H-5-HT in the absence and presence of 10 µM 5-HT. The 
\(B_{\text{max}}\) and \(K_d\) values were determined by Scatchard analysis.

1.7 Binding of \(^{3}\)H-spiroperidol to 5-HT\(_2\) receptors

The freshly dissected brain regions were homogenized and neuronal membranes 
prepared as described in section 1.2. The standard assay mixture contained 0.2 ml of 
the homogenate, containing approximately 200 µg of tissue, 0.1 ml of \(^{3}\)H-spiroperidol,
0.1 ml of competing agent (ketanserin) and buffer to make up the total volume of 1 ml. Incubation and filtration was done and radioactivity determined as described earlier, followed by two 5 ml. washes of ice cold 50 mM Tris buffer (pH 7.4). The specific binding of $^3$H-spiroperidol was defined as the difference in binding observed in the absence and in the presence of 1 $\mu$M unlabeled ketanserin. The specific binding was expressed as pmoles of $^3$H-spiroperidol bound per g tissue. The specific binding of the radioligand (8 to 10 concentrations) was determined. This was followed by the

<table>
<thead>
<tr>
<th>Receptor</th>
<th>Hot ligand</th>
<th>Cold ligand</th>
</tr>
</thead>
<tbody>
<tr>
<td>a-adrenergic</td>
<td>$^3$H-Dihydroergocryptine 0.25-12 nM (Amersham)</td>
<td>Phentolamine (1 $\mu$M)</td>
</tr>
<tr>
<td>b-adrenergic</td>
<td>$^3$H-Dihydroalprenolol 0.12-4 nM (Amersham)</td>
<td>Propranolol (1 $\mu$M)</td>
</tr>
<tr>
<td>Muscarinic</td>
<td>$^3$H-Quinuclidinyl benzylate 0.1-2 nM (NEN)</td>
<td>Atropine (1 $\mu$M)</td>
</tr>
<tr>
<td>Dopamine</td>
<td>$^3$H-Spiperone 0.1-5 nM (NEN)</td>
<td>Haloperidol (1 $\mu$M)</td>
</tr>
<tr>
<td>5-hydroxytryptamine</td>
<td>$^3$H-5-hydroxytryptamine 0.25-15 nM (NEN)</td>
<td>5-hydroxytryptamine (5-HT) (1 $\mu$M)</td>
</tr>
<tr>
<td>5-hydroxytryptamine$_2$</td>
<td>$^3$H-Spiperone 0.15-10 nM (NEN)</td>
<td>Ketanserin (1 $\mu$M)</td>
</tr>
</tbody>
</table>

Table 1: Summary of the various ligands used for radioreceptor binding to different neurotransmitter receptors
Scatchard analysis to determine the dissociation constant ($K_d$) and the receptor density ($B_{max}$) values. Four to five determinations were made to compute the means and their standard errors. The data were analyzed by the ANOVA.

2. EFFECTS OF CENHAQUIN ON BLOOD PRESSURE AND HEART RATE IN NORMAL AND CERVICAL SECTIONED RATS

Albino rats were anaesthetized with urethane (1.2 g/kg, ip). The femoral artery was cannulated and connected to a Statham P23DC pressure transducer and a Grass low level 7P1DC preamplifier on a Grass Model 7 polygraph for recording the blood pressure. While the heart rate was recorded on another channel of the polygraph through a 7P44 tachograph preamplifier. The trachea was cannulated and the animal was maintained on artificial respiration. The skin was cut from the occipital protuberance to the lower cervical region. Then the atlanto-occipital membrane was exposed and the dorsal part of the cervical vertebra was nibbled, exposing the spinal cord. Spinal cord sectioning was performed and a part of the cervical (C2 to C4) region of spinal cord was removed so that there was absolutely no connection with the brain.

In some of the rats intrathecal cannulation was performed to determine the effect of intrathecal administration of centhaquin on blood pressure and heart rate. A PE10 tubing was guided into the spinal canal through a small opening made in the atlanto-occipital membrane. The cannula was pushed gently till 8 cm mark was reached. The location of cannula was confirmed after the experiment by injecting dye and dissecting open the spinal canal and locating the dye. Volume of injection was 2 μl for each drug.
and normal saline was without effect on blood pressure and heart rate.

3. MICROINJECTION STUDIES OF CENTHAQUIN IN CATS

Cats (2.5-4.5 kg) of either sex were used as experimental animal for the study. They were anaesthetized with alpha chloralose (80 mg/kg, iv). Blood pressure was recorded from the right femoral artery through a Statham P23DC pressure transducer and a Grass low level 7P1DC preamplifier on a Grass Model 7 polygraph. Heart rate was recorded on another channel of the polygraph through a 7P44 tachograph preamplifier. Trachea was cannulated and the animal maintained on artificial respiration. The head of the animal was fixed in David Kopf Stereotaxic frame, and the stereotaxic technique was used to hold the animal in the frame through out the experiment. Two burr holes were made on the skull to mark the position of the nucleus tractus solitarius (NTS) at parameters of p=10, L=2-3, V=-6 (Snider and Neimer, 1961). A cannula was placed in the NTS using the above parameters and drugs like centhaquin, clonidine, phentolamine, yohimbine and prazosin were injected with the help of a microsyringe. A maximum volume of 10 μl was injected in the NTS and saline (10 μl) did not produce any effect on blood pressure and heart rate.

3.1 Confirmation of Cannula Location: To confirm the location of the cannula, 10 μl of Evans blue or methylene blue was injected into the nucleus tractus solitarius (NTS) at the end of the experiment. The animals were sacrificed by an overdose of magnesium sulfate and the brain was removed from the skull and fixed in 10% formalin for 48 h. The brain was removed and its sections were examined. The location of dye
in the region of NTS confirmed the site of injection.

4. STUDIES ON THERMOREGULATION AND BLOOD PRESSURE

Groups of six pretreated rats were taken and allowed to acclimatize at the desired ambient temperature for two hours before starting the experiment. The experimental animals were administered drug into the lateral cerebral ventricle (icv) through a chronically implanted cannula.

4.1 Placement of the Cannula in Lateral Cerebral Ventricle: Rats were anaesthetized with pentobarbitone sodium (30 mg/kg, i.p). After shaving and cleaning the skull, the head was fixed in a rat stereotaxic frame (David Kopf). A 2 cm long midline incision was made to expose the skull, and a 1 mm hole was drilled with a dental burr, 2.5 mm lateral and 3.5 mm anterior to the interaural plane. A 14 mm long 20 gauge stainless steel tube with a small stainless steel plate fixed 2 mm from its lower end served as the cannula. The cannula was held in the electrode holder of the stereotaxic instrument and was lowered vertically into the hole after puncturing the dura with a cutting needle. The steel plate was fixed to the skull with dental cement, the wound was sutured in layers and sealed with cotton wool soaked in tincture benzoin. A stainless steel wire of appropriate length and thickness was used as stilette. Usually, the recovery was uneventful, and the wound healed within a week. After that, the animals exhibited normal behavior, without any neurological deficit indicating that they were fit for experimentation. The cannulated rats were used several times, with reproducible responses. All surgery was performed aseptically.
4.2 **Preparation of Drugs:** The drugs used were noradrenaline, dopamine, 5-HT and acetylcholine. The drug solutions were freshly prepared in pyrogen free normal saline. All the glassware was sterilized at 180 °C for 5 hours in a dry oven, syringes and needles were autoclaved and all solutions were filtered immediately before use, in a Maxiflow microfilter of 0.45 μm pore size to ensure that they were free from pyrogen producing bacteria.

4.3 **Drug Administration:** The amount of drug to be injected was dissolved in a constant volume of 10 μl, and injected into the lateral ventricle (icv) through the implanted cannula with the help of a Hamilton microsyringe of 50 μl, fitted with a number 27 hypodermic needle. A rubber disk fixed 12 mm from the tip of the needle served as a stopper during drug administration. The stilette was replaced immediately after injection of the drug.

4.4 **Confirmation of Cannula Location:** To confirm the location of the cannula, 10 μl of 1% Evans blue or methylene blue was injected into the lateral cerebral ventricle at the end of the experiment. The animals were sacrificed by an overdose of pentobarbital sodium, and the skull with the brain was fixed in 10% formalin for 48 h. The brain was then removed and its sections were examined. It showed that the dye was uniformly distributed throughout the cerebral ventricles.

4.5 **Temperature Measurements:** The core temperature of the albino rats was measured with a thermistor probe inserted 2 cm into the rectum and connected to a programmed Apple computer. The temperature was recorded before the drug
administration and immediately after it at regular intervals of 10 minutes for 5 hours.

The temperature duration from basal value for each animal and the mean duration for the group along with standard error (SE) were worked out. The time course of temperature change was plotted for each animal and the area under the curve measured with a planimeter.

4.6 Blood Pressure Measurement

The blood pressure of conscious albino rats was recorded by the IITC in 6 instrument. It serves to amplify photoelectrically detected tail pulses of mod B 60 type sensing cuffs. The available outputs also serve in conjunction with a dual channel recorded (IITC Model 45) to give permanent paper recordings to show the presence of tail pulses on one channel and the matching cuff pressure on the other channel. The animals were put into the rat holders which were connected to the manual scanner of the system. The tail pulses with the cuff pressure was recorded before the drug administration and after for 4 hours.

The IITC method measures the systolic as well as the mean blood pressure of rodents. The first onset of pulses is taken as the systolic point and the first maximum amplitude is taken as the Mean BP point. The diastolic BP is calculated by the formula Diastolic BP = 3(Systolic BP - Mean BP) + 2. The blood pressure before administration of the drug was compared to the change in blood pressure after the drug injection.

4.7 Statistics: The significance of the difference between the two groups was assessed by employing the Students 't' test.
RESULTS

1. STUDIES ON RADIORECEPTOR BINDING:

1.1 EFFECT OF CHRONIC ADMINISTRATION OF ANTIHYPERTENSIVE DRUGS ON α ADRENOCEPTORS:

The population ($B_{\text{max}}$) of the adrenoceptors in normal rat brain is fairly constant in all the four areas. In cortex the $B_{\text{max}}$ value was $30.21 \pm 1.38$ pmol/g tissue, while the $K_d$ value was $0.81 \pm 0.12$ nM. In caudate the density was ($B_{\text{max}}$) $41.49 \pm 2.19$ pmol/g tissue, while the affinity was ($K_d$) $1.24 \pm 0.23$ nM. Hypothalamus showed the $B_{\text{max}}$ value of $32.89 \pm 2.19$ pmol/g tissue, and the $K_d$ value was $0.68 \pm 0.11$ nM. In medulla the $B_{\text{max}}$ value was $31.09 \pm 1.30$ pmol/g tissue, while the $K_d$ value was $0.66 \pm 0.20$ nM.

In cortex the population ($B_{\text{max}}$) $29.01 \pm 1.79$ pmol/g tissue) and affinity ($K_d$) $0.77 \pm 0.16$ nM) of $^3$H-dihydroergocryptine binding, remained unchanged in the centhaquin treated rats. In the clonidine treated rats also the population ($B_{\text{max}}$) $30.53 \pm 1.54$ pmol/g tissue) and affinity ($K_d$) $0.83 \pm 0.14$ nM) remained unchanged. In hydralazine treated rats the population ($B_{\text{max}}$) $23.18 \pm 1.11$ pmol/g tissue) decreased ($p < 0.05$), but the affinity ($K_d$) $0.76 \pm 0.12$ nM) remained unchanged. In reserpine treated rats the density ($B_{\text{max}}$) $21.94 \pm 1.20$ pmol/g tissue) decreased ($p < 0.05$) but no change occurred in the affinity ($K_d$) $0.80 \pm 0.11$ nM) of $^3$H-dihydroergocryptine binding (Table 1; Figures 1 and 2).

In the caudate, treatment by centhaquin did not alter either the density ($B_{\text{max}}$) $44.80 \pm 2.31$ pmol/g tissue) or the affinity ($K_d$) $1.41 \pm 0.19$ nM) of $^3$H-dihydroergocryptine binding. Treatment by clonidine did not affect the density ($B_{\text{max}}$) $45.24 \pm 1.99$ pmol/g
Table 1. Effect of repeated administration of centhaquin, clonidine, hydralazine and reserpine on $^3$H-dihydroergocryptine binding to $\alpha$ adrenergic receptors in rat cerebral cortex.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>$^3$H-Dihydroergocryptine binding</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$B_{max}$ (pmol/g tissue)</td>
</tr>
<tr>
<td>Vehicle</td>
<td>30.21 ± 1.38</td>
</tr>
<tr>
<td>Centhaquin</td>
<td>29.00 ± 1.79</td>
</tr>
<tr>
<td>Clonidine</td>
<td>30.53 ± 1.54</td>
</tr>
<tr>
<td>Hydralazine</td>
<td>23.18 ± 1.11*</td>
</tr>
<tr>
<td>Reserpine</td>
<td>21.94 ± 1.20*</td>
</tr>
</tbody>
</table>

*P < 0.05 as compared to vehicle treated control

Table 2. Effect of repeated administration of centhaquin, clonidine, hydralazine and reserpine on $^3$H-dihydroergocryptine binding to $\alpha$ adrenergic receptors in rat corpus striatum.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>$^3$H-Dihydroergocryptine binding</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$B_{max}$ (pmol/g tissue)</td>
</tr>
<tr>
<td>Vehicle</td>
<td>41.49 ± 2.19</td>
</tr>
<tr>
<td>Centhaquin</td>
<td>44.80 ± 2.31</td>
</tr>
<tr>
<td>Clonidine</td>
<td>45.24 ± 1.99</td>
</tr>
<tr>
<td>Hydralazine</td>
<td>30.72 ± 1.24*</td>
</tr>
<tr>
<td>Reserpine</td>
<td>28.17 ± 1.16*</td>
</tr>
</tbody>
</table>

*P < 0.05 as compared to vehicle treated control
tissue) or affinity ($K_d = 1.43 \pm 0.14 \text{ nM}$) of these receptors. Treatment with reserpine decreased ($p < 0.05$) the population ($B_{\text{max}} = 28.17 \pm 1.16 \text{ pmol/g tissue}$) but the affinity ($K_d = 1.07 \pm 0.13 \text{ nM}$) of $^3\text{H}$-dihydroergocryptine binding did not change. Similarly, treatment with hydralazine decreased ($p < 0.05$) the population ($B_{\text{max}} = 30.72 \pm 1.24 \text{ pmol/g tissue}$) but the affinity ($K_d = 1.03 \pm 0.16 \text{ nM}$) of $^3\text{H}$-dihydroergocryptine binding did not change (Table 2; Figures 3 and 4).

In hypothalamus, the density ($B_{\text{max}}$) increased ($p < 0.05$) by centhaquin treatment ($50.12 \pm 2.32 \text{ pmol/g tissue}$) while $K_d$ ($0.94 \pm 0.20 \text{ nM}$) did not change. Clonidine treatment also increased the density ($B_{\text{max}} = 43.31 \pm 1.92 \text{ pmol/g tissue}$) while the affinity ($K_d = 0.87 \pm 0.16 \text{ nM}$) did not change. Hydralazine treatment decreased the density ($B_{\text{max}} = 23.67 \pm 1.1 \text{ pmol/g tissue}$), while the affinity ($K_d = 0.65 \pm 0.13 \text{ nM}$) was not altered. Reserpine treatment decreased ($p < 0.05$) the density ($B_{\text{max}} = 20.65 \pm 1.16 \text{ pmol/g tissue}$) while the $K_d$ ($0.57 \pm 0.09 \text{ nM}$) of $^3\text{H}$-dihydroergocryptine binding was unchanged (Table 3; Figures 5 and 6).

In the medulla of centhaquin treated rats the density ($B_{\text{max}} = 57.79 \pm 2.78 \text{ pmol/g tissue}$) increased ($p < 0.05$) and no change occurred in affinity ($K_d = 0.92 \pm 0.18 \text{ nM}$). In clonidine treated rats the density ($B_{\text{max}} = 49.41 \pm 2.29 \text{ pmol/g tissue}$) increased ($p < 0.05$) while the affinity ($K_d = 0.87 \pm 0.14 \text{ nM}$) did not alter. In hydralazine treated rats the density ($B_{\text{max}} = 23.49 \pm 1.12 \text{ pmol/g tissue}$) of these receptors decreased ($p < 0.05$), but no change occurred in the affinity ($K_d = 0.65 \pm 0.16 \text{ nM}$). In reserpine treated rats the density ($B_{\text{max}} = 19.65 \pm 1.11 \text{ pmol/g tissue}$) decreased ($p < 0.05$) but no change was
Table 3. Effect of repeated administration of centhaquin, clonidine, hydralazine and reserpine on $^3$H-dihydroergocryptine binding to $\alpha$ adrenergic receptors in rat hypothalamus.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>$^3$H-Dihydroergocryptine binding</th>
<th>Mean ± S.E.M., N=5</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$B_{\text{max}}$ (pmol/g tissue)</td>
<td>$K_d$ (nM)</td>
</tr>
<tr>
<td>Vehicle</td>
<td>32.89 ± 2.19</td>
<td>0.68 ± 0.11</td>
</tr>
<tr>
<td>Centhaquin</td>
<td>50.12 ± 2.32*</td>
<td>0.94 ± 0.20</td>
</tr>
<tr>
<td>Clonidine</td>
<td>43.31 ± 1.92*</td>
<td>0.87 ± 0.16</td>
</tr>
<tr>
<td>Hydralazine</td>
<td>23.67 ± 1.10*</td>
<td>0.65 ± 0.13</td>
</tr>
<tr>
<td>Reserpine</td>
<td>20.65 ± 1.16*</td>
<td>0.57 ± 0.09</td>
</tr>
</tbody>
</table>

*P < 0.05 as compared to vehicle treated control

Table 4. Effect of repeated administration of centhaquin, clonidine, hydralazine and reserpine on $^3$H-dihydroergocryptine binding to $\alpha$ adrenergic receptors in rat brain medulla.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>$^3$H-Dihydroergocryptine binding</th>
<th>Mean ± S.E.M., N=5</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$B_{\text{max}}$ (pmol/g tissue)</td>
<td>$K_d$ (nM)</td>
</tr>
<tr>
<td>Vehicle</td>
<td>31.09 ± 1.30</td>
<td>0.66 ± 0.20</td>
</tr>
<tr>
<td>Centhaquin</td>
<td>57.79 ± 2.78*</td>
<td>0.92 ± 0.18</td>
</tr>
<tr>
<td>Clonidine</td>
<td>49.41 ± 2.29*</td>
<td>0.87 ± 0.16</td>
</tr>
<tr>
<td>Hydralazine</td>
<td>23.49 ± 1.12*</td>
<td>0.65 ± 0.16</td>
</tr>
<tr>
<td>Reserpine</td>
<td>19.65 ± 1.11*</td>
<td>0.54 ± 0.11</td>
</tr>
</tbody>
</table>

*P < 0.05 as compared to vehicle treated control
Figure 1

CEREBRAL CORTEX

3H-Dihydropyroergocryptine binding to α-adrenergic receptors

Bmax (pmole/g tissue; Mean ± SEM)

- Reserpine
- Hydralazine
- Clonidine
- Centanquin
- Vehicle

p < 0.05
Figure 2

CEREBRAL binding to α adrenergic receptors

3H-Dihydroergocryptine binding to α adrenergic receptors

K_d (nM; Mean ± SEM)

0.0 0.2 0.4 0.6 0.8 1.0

Vehicle
Clonidine
Hydralazine
Reserpine
Figure 3
CORPUS STRIATUM

$\text{H}^{-}\text{Dihydroergocryptine binding to a adrenergic receptors}$

Bmax (pmole/g tissue; Mean ± SEM)

$\text{Reserpine}$

$\text{Hyderalazine}$

$\text{Clonidine}$

$\text{Centrithaquin}$

$\text{Vehicle}$

$\text{p} \geq 0.05$
Figure 4
CORPUS STRIATUM

$^{3}H$-Dihydroergocryptine binding to α-adrenergic receptors

Kd (nM; Mean ± SEM)
Figure 5

HYPOTHALAMUS

$^3$H-Dihydroergocryptine binding to α-adrenergic receptors

Bmax (pmole/g tissue; Mean ± SEM)
Figure 6

HYPOTHALAMUS

$3H$-Dihydroergocryptine binding to $\alpha$ adrenergic receptors

$K_d$ (nM; Mean ± SEM)

Reserpine
Hydergine
Clonidine
Centhaquin
Vehicle
observed in the affinity ($K_d 0.54 \pm 0.11 \text{ nM}$), of $^3$H-dihydroergocryptine binding (Table 4; Figures 7 and 8).

1.2 EFFECT OF CHRONIC ADMINISTRATION OF ANTIHYPERTENSIVE DRUGS ON $\beta$-ADRENOCEPTORS:

In normal rat brain the $\beta$-adrenoceptors have high affinity ($K_d$) binding sites. In cortex the $B_{\text{max}}$ value was $38.76 \pm 2.57 \text{ pmol/g tissue}$ and the $K_d$ value was $0.80 \pm 0.05 \text{ nM}$. In caudate the density was ($B_{\text{max}} 35.77 \pm 1.44 \text{ pmol/g tissue}$). While the affinity was ($K_d 1.76 \pm 0.07 \text{ nM}$). In hypothalamus the $B_{\text{max}}$ value was $24.88 \pm 2.11 \text{ pmol/g tissue}$, while the $K_d$ value was $0.58 \pm 0.04 \text{ nM}$. Medulla showed the $B_{\text{max}}$ value of $31.77 \pm 1.73 \text{ pmol/g tissue}$, and the $K_d$ value of $0.68 \pm 0.06 \text{ nM}$.

In the cortex, centhaquin treatment did not affect the density ($B_{\text{max}} 39.50 \pm 1.88 \text{ pmol/g tissue}$) and affinity ($K_d 0.74 \pm 0.04 \text{ nM}$) of $^3$H-dihydroalprenolol binding. Clonidine also did not affect either the density ($B_{\text{max}} 38.37 \pm 2.30 \text{ pmol/g tissue}$) or the affinity ($K_d 0.72 \pm 0.11 \text{ nM}$) of $\beta$-adrenoceptors. Hydralazine treatment increased ($p < 0.05$) both the density ($B_{\text{max}} 52.41 \pm 2.02 \text{ pmol/g tissue}$) and affinity ($K_d 0.96 \pm 0.04 \text{ nM}$) of $^3$H-dihydroalprenolol binding. While reserpine treatment increased only the density ($B_{\text{max}} 47.96 \pm 2.01 \text{ pmol/g tissue}$) but the affinity ($K_d 0.68 \pm 0.03 \text{ nM}$) of $\beta$-adrenoceptors did not change (Table 5; Figures 9 and 10).

In caudate, no change was observed in the density ($B_{\text{max}} 38.88 \pm 1.86 \text{ pmol/g tissue}$) and affinity ($K_d 1.54 \pm 0.06 \text{ nM}$) by centhaquin treatment. Clonidine treatment also produced no change in the density ($B_{\text{max}} 35.21 \pm 1.11 \text{ pmol/g tissue}$) and affinity
Figure 7

MEDULLA

3H-Dihydroergocryptine binding to α adrenergic receptors

Bmax (pmole/g tissue: Mean ± SEM)

- Reserpine
- Hydralazine
- Clonidine
- Centhagulin
- Vehicle

p < 0.05

*
Figure 8

MEDIUMLA

3H-Dihydroergocryptine binding to α adrenergic receptors

Kd (nM; Mean ± SEM)

Reserpine
Hydralazine
Clonidine
Centraquin
Vehicle
Table 5. Effect of repeated administration of centhaquin, clonidine, hydralazine and reserpine on $^3$H-dihydroalprenolol binding to β adrenergic receptors in rat cerebral cortex.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>$^3$H-Dihydroalprenolol binding</th>
<th>Mean ± S.E.M., N=5</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$B_{\text{max}}$ (pmol/g tissue)</td>
<td>$K_d$ (nM)</td>
</tr>
<tr>
<td>Vehicle</td>
<td>38.76 ± 2.57</td>
<td>0.80 ± 0.05</td>
</tr>
<tr>
<td>Centhaquin</td>
<td>39.50 ± 1.88</td>
<td>0.74 ± 0.04</td>
</tr>
<tr>
<td>Clonidine</td>
<td>38.37 ± 2.30</td>
<td>0.72 ± 0.11</td>
</tr>
<tr>
<td>Hydralazine</td>
<td>52.41 ± 2.02*</td>
<td>0.96 ± 0.04*</td>
</tr>
<tr>
<td>Reserpine</td>
<td>47.96 ± 2.01*</td>
<td>0.68 ± 0.03</td>
</tr>
</tbody>
</table>

*P < 0.05 as compared to vehicle treated control

Table 6. Effect of repeated administration of centhaquin, clonidine, hydralazine and reserpine on $^3$H-dihydroalprenolol binding to β adrenergic receptors in rat corpus striatum.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>$^3$H-Dihydroalprenolol binding</th>
<th>Mean ± S.E.M., N=5</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$B_{\text{max}}$ (pmol/g tissue)</td>
<td>$K_d$ (nM)</td>
</tr>
<tr>
<td>Vehicle</td>
<td>35.77 ± 1.44</td>
<td>1.76 ± 0.07</td>
</tr>
<tr>
<td>Centhaquin</td>
<td>38.88 ± 1.86</td>
<td>1.54 ± 0.06</td>
</tr>
<tr>
<td>Clonidine</td>
<td>35.21 ± 1.11</td>
<td>1.89 ± 0.05</td>
</tr>
<tr>
<td>Hydralazine</td>
<td>46.11 ± 1.86*</td>
<td>1.47 ± 0.06</td>
</tr>
<tr>
<td>Reserpine</td>
<td>41.40 ± 1.06*</td>
<td>1.58 ± 0.05</td>
</tr>
</tbody>
</table>

*P < 0.05 as compared to vehicle treated control
Table 7. Effect of repeated administration of centhaquin, clonidine, hydralazine and reserpine on $^3$H-dihydroalprenolol binding to β adrenergic receptors in rat hypothalamus.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>$^3$H-Dihydroalprenolol binding</th>
<th>Mean ± S.E.M., N=5</th>
<th>$K_d$ (nM)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$B_{max}$ (pmol/g tissue)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vehicle</td>
<td>24.8 ± 2.11</td>
<td>0.58 ± 0.04</td>
<td></td>
</tr>
<tr>
<td>Centhaquin</td>
<td>25.33 ± 1.90</td>
<td>0.66 ± 0.05</td>
<td></td>
</tr>
<tr>
<td>Clonidine</td>
<td>25.74 ± 1.41</td>
<td>0.52 ± 0.05</td>
<td></td>
</tr>
<tr>
<td>Hydralazine</td>
<td>32.00 ± 1.12*</td>
<td>0.46 ± 0.07</td>
<td></td>
</tr>
<tr>
<td>Reserpine</td>
<td>35.87 ± 1.11*</td>
<td>0.55 ± 0.06</td>
<td></td>
</tr>
</tbody>
</table>

*P < 0.05 as compared to vehicle treated control

Table 8. Effect of repeated administration of centhaquin, clonidine, hydralazine and reserpine on $^3$H-dihydroalprenolol binding to β adrenergic receptors in rat brain medulla.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>$^3$H-Dihydroalprenolol binding</th>
<th>Mean ± S.E.M., N=5</th>
<th>$K_d$ (nM)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$B_{max}$ (pmol/g tissue)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vehicle</td>
<td>31.77 ± 1.73</td>
<td>0.68 ± 0.06</td>
<td></td>
</tr>
<tr>
<td>Centhaquin</td>
<td>31.70 ± 2.04</td>
<td>0.68 ± 0.08</td>
<td></td>
</tr>
<tr>
<td>Clonidine</td>
<td>32.27 ± 2.29</td>
<td>0.66 ± 0.05</td>
<td></td>
</tr>
<tr>
<td>Hydralazine</td>
<td>24.44 ± 1.66*</td>
<td>0.68 ± 0.04</td>
<td></td>
</tr>
<tr>
<td>Reserpine</td>
<td>21.59 ± 1.08*</td>
<td>0.59 ± 0.08</td>
<td></td>
</tr>
</tbody>
</table>

*P < 0.05 as compared to vehicle treated control
(K_d 1.89 ± 0.05 nM). In hydralazine treated rats the receptor density (B_max 46.11 ± 1.86 pmol/g tissue) increased (p < 0.05) but the affinity (K_d 1.47 ± 0.06 nM) of ^3^H-dihydroalprenolol binding remained unchanged. In reserpine treated rats the density (B_max 41.40 ± 1.06 pmol/g tissue) increased (p < 0.05) and the affinity (K_d 1.58 ± 0.05 nM) remained unchanged (Table 6; Figures 11 and 12).

In hypothalamus the population (B_max 25.33 ± 1.90 pmol/g tissue) and affinity (K_d 0.66 ± 0.05 nM) did not change by centhaquin treatment. With clonidine treatment also the population (B_max 25.74 ± 1.41 pmol/g tissue) and affinity (K_d 0.52 ± 0.05 nM) remained unchanged. Hydralazine treatment increased (p < 0.05) the density (B_max 32.00 ± 1.12 pmol/g tissue) while the affinity was unchanged (K_d 0.46 ± 0.07 nM). Reserpine treatment increased (p < 0.05) the density (B_max 35.87 ± 1.11 pmol/g tissue) but no change was seen in affinity (K_d 0.55 ± 0.06 nM) of ^3^H-dihydroalprenolol binding (Table 7; Figures 13 and 14).

In medulla, centhaquin treatment did not change the density (B_max 31.70 ± 2.04 pmol/g tissue) or the affinity (K_d 0.68 ± 0.08 nM) of the ^3^H-dihydroalprenolol binding. Clonidine treatment also did not change either the density (B_max 32.27 ± 2.29 pmol/g tissue) or affinity (K_d 0.66 ± 0.05 nM). By hydralazine treatment the density (B_max 24.44 ± 1.66 pmol/g tissue) decreased (p < 0.05), no significant change was observed in the affinity (K_d 0.68 ± 0.04 nM). Reserpine treatment produced a decrease in the density (B_max 21.59 ± 1.08 pmol/g tissue) (p < 0.05) while no change occurred in the affinity (K_d 0.59 ± 0.08 nM) of β-adrenoceptors (Table 8; Figures 15 and 16).
Figure 9
CEREBRAL CORTEX
3H-Dihydroliprenol binding to G-adenergic receptors

Bmax (pmol/g tissue; Mean ± SEM)

Vehicle
Clonidine
Centhaquin
Hyderazine
Reserpine

p < 0.05

*
Figure 10

CEREBRAL CORTEX

$[^{3}H]-$Dihydroalprenolol binding to $\beta$ adrenergic receptors

<table>
<thead>
<tr>
<th>Group</th>
<th>Kd (nM; Mean ± SEM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reserpine</td>
<td>$p &gt; 0.05$</td>
</tr>
<tr>
<td>Hydrazine</td>
<td></td>
</tr>
<tr>
<td>Clonidine</td>
<td></td>
</tr>
<tr>
<td>Centiniquin</td>
<td></td>
</tr>
<tr>
<td>Vehicle</td>
<td></td>
</tr>
</tbody>
</table>
Figure 11
CORPUS STRIATUM

$^{3}H$-Dihydroalprenolol binding to $\alpha$ adrenergic receptors

$B_{\text{max}}$ (pmole/g tissue; Mean ± SEM)
CORPUS STRIATUM

$^{3}H$-dihydroalprenolol binding to $\beta$ adrenergic receptors

Figure 12

Kd (nM; Mean ± SEM)

3H-dihydroalprenolol binding to $\beta$ adrenergic receptors

Vehicle
Centhaquin
Clonidine
Hydralazine
Reserpine
Figure 13

HYPOTHALAMUS

$^{3}H$-Dihydroliprenol binding to $\beta$ adrenergic receptors

Bmax (pmole/g tissue; Mean ± SEM)

- Reserpine
- Hydralazine
- Clonidine
- Cenethaquin
- Vehicle

* $p < 0.05$
**Figure 14**

**HYPOTHALAMUS**

$^3$H-Dihydrolpranolol binding to $\beta$ adrenergic receptors

![Bar chart showing $K_d$ values for different compounds in the hypothalamus.](image-url)
Figure 15

MEDULLA

$^3$H-Dihydralpranolol binding to $g$ adrenergic receptor

Bmax (pmole/g tissue; Mean ± SEM)

- Reserve
- Hydrazine
- Clonidine
- Centraquin
- Vehicle

p < 0.05

*
1.3 EFFECT OF CHRONIC ADMINISTRATION OF ANTIHYPERTENSIVE DRUGS ON DOPAMINERGIC RECEPTORS:

The normal rat brain dopaminergic sites, labelled by $^3$H-spiroperidol in the caudate show high density ($B_{\text{max}}$ 58.68 ± 2.09 pmol/g tissue) and high affinity ($K_d$ 0.03 ± 0.01 nM) binding sites. In cortex the density was ($B_{\text{max}}$ 46.63 ± 1.70 pmol/g tissue) whereas the $K_d$ was 0.43 ± 0.09 nM. In caudate the $B_{\text{max}}$ value was 58.68 ± 1.33 pmol/g tissue, while the $K_d$ value was 0.03 ± 0.01 nM. The hypothalamus showed $B_{\text{max}}$ value of 25.23 ± 0.92 pmol/g tissue and $K_d$ value of 0.31 ± 0.04 nM. In medulla the $B_{\text{max}}$ was 44.49 ± 1.68 pmol/g tissue, while $K_d$ was 0.39 ± 0.05 nM.

In cortex, centhaquin treatment decreased ($p < 0.05$) the population ($B_{\text{max}}$ 34.86 ± 1.39 pmol/g tissue) and no change occurred in the affinity ($K_d$ 0.35 ± 0.15 nM). Clonidine treatment increased ($p < 0.05$) the density ($B_{\text{max}}$ 54.84 ± 1.96 pmol/g tissue), while no change occurred in the affinity ($K_d$ 0.48 ± 0.09 nM). Hydralazine treatment decreased ($p < 0.05$) the density ($B_{\text{max}}$ 31.49 ± 1.24 pmol/g tissue) without any change in the affinity ($K_d$ 0.44 ± 0.06 nM). Reserpine treatment also decreased ($p < 0.05$) the density ($B_{\text{max}}$ 37.37 ± 1.86 pmol/g tissue) with no change in the affinity ($K_d$ 0.43 ± 0.08 nM) (Table 9; Figures 17 and 18).

In caudate, no change occurred in the density ($B_{\text{max}}$ 58.03 ± 1.33 pmol/g tissue) or affinity ($K_d$ 0.03 ± 0.01 nM) of centhaquin treated rats. In the clonidine treated rats also there was no change in the density ($B_{\text{max}}$ 58.05 ± 1.26 pmol/g tissue) or affinity ($K_d$ 0.03 ± 0.01 nM). In hydralazine treated rats there was no change in the density.
Table 9. Effect of repeated administration of centhaquin, clonidine, hydralazine and reserpine on $^3$H-spiroperidol binding to dopaminergic receptors in rat cerebral cortex.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>$^3$H-Spiroperidol binding</th>
<th>Mean ± S.E.M., N=5</th>
<th>$K_d$ (nM)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$B_{max}$ (pmol/g tissue)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vehicle</td>
<td>46.63 ± 1.70</td>
<td>0.43 ± 0.09</td>
<td></td>
</tr>
<tr>
<td>Centhaquin</td>
<td>34.86 ± 1.39*</td>
<td>0.35 ± 0.15</td>
<td></td>
</tr>
<tr>
<td>Clonidine</td>
<td>54.84 ± 1.96*</td>
<td>0.48 ± 0.09</td>
<td></td>
</tr>
<tr>
<td>Hydralazine</td>
<td>31.49 ± 1.24*</td>
<td>0.44 ± 0.06</td>
<td></td>
</tr>
<tr>
<td>Reserpine</td>
<td>37.37 ± 1.86*</td>
<td>0.43 ± 0.08</td>
<td></td>
</tr>
</tbody>
</table>

*P < 0.05 as compared to vehicle treated control

Table 10. Effect of repeated administration of centhaquin, clonidine, hydralazine and reserpine on $^3$H-spiroperidol binding to dopaminergic receptors in rat corpus striatum.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>$^3$H-Spiroperidol binding</th>
<th>Mean ± S.E.M., N=5</th>
<th>$K_d$ (nM)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$B_{max}$ (pmol/g tissue)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vehicle</td>
<td>58.68 ± 2.09</td>
<td>0.03 ± 0.01</td>
<td></td>
</tr>
<tr>
<td>Centhaquin</td>
<td>58.03 ± 1.33</td>
<td>0.03 ± 0.01</td>
<td></td>
</tr>
<tr>
<td>Clonidine</td>
<td>58.05 ± 1.26</td>
<td>0.03 ± 0.01</td>
<td></td>
</tr>
<tr>
<td>Hydralazine</td>
<td>59.26 ± 1.94</td>
<td>0.03 ± 0.01</td>
<td></td>
</tr>
<tr>
<td>Reserpine</td>
<td>56.42 ± 1.84</td>
<td>0.04 ± 0.01</td>
<td></td>
</tr>
</tbody>
</table>

*P < 0.05 as compared to vehicle treated control
Table 11. Effect of repeated administration of centhaquin, clonidine, hydralazine and reserpine on $^3$H-spiroperidol binding to dopaminergic receptors in rat hypothalamus.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>$^3$H-Spiroperidol binding</th>
<th>$^3$H-Spiroperidol binding</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$B_{\text{max}}$ (pmol/g tissue)</td>
<td>$K_d$ (nM)</td>
</tr>
<tr>
<td>Vehicle</td>
<td>25.23 ± 0.92</td>
<td>0.31 ± 0.04</td>
</tr>
<tr>
<td>Centhaquin</td>
<td>24.74 ± 0.90</td>
<td>0.30 ± 0.05</td>
</tr>
<tr>
<td>Clonidine</td>
<td>26.66 ± 0.97</td>
<td>0.32 ± 0.07</td>
</tr>
<tr>
<td>Hydralazine</td>
<td>19.01 ± 1.26*</td>
<td>0.31 ± 0.05</td>
</tr>
<tr>
<td>Reserpine</td>
<td>31.39 ± 1.01*</td>
<td>0.29 ± 0.06</td>
</tr>
</tbody>
</table>

*P < 0.05 as compared to vehicle treated control

Table 12. Effect of repeated administration of centhaquin, clonidine, hydralazine and reserpine on $^3$H-spiroperidol binding to dopaminergic receptors in rat brain medulla.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>$^3$H-Spiroperidol binding</th>
<th>$^3$H-Spiroperidol binding</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$B_{\text{max}}$ (pmol/g tissue)</td>
<td>$K_d$ (nM)</td>
</tr>
<tr>
<td>Vehicle</td>
<td>44.49 ± 1.68</td>
<td>0.39 ± 0.05</td>
</tr>
<tr>
<td>Centhaquin</td>
<td>43.37 ± 1.63</td>
<td>0.43 ± 0.08</td>
</tr>
<tr>
<td>Clonidine</td>
<td>43.84 ± 1.49</td>
<td>0.43 ± 0.07</td>
</tr>
<tr>
<td>Hydralazine</td>
<td>37.71 ± 1.16*</td>
<td>0.32 ± 0.07</td>
</tr>
<tr>
<td>Reserpine</td>
<td>37.13 ± 1.11*</td>
<td>0.30 ± 0.05</td>
</tr>
</tbody>
</table>

*P < 0.05 as compared to vehicle treated control
(B_max 59.26 ± 1.94 pmol/g tissue) or affinity (K_d 0.03 ± 0.01 nM). And also in the
reserpine treated rats no change was seen in either density (B_max 56.42 ± 1.84 pmol/g
tissue) or affinity (K_d 0.04 ± 0.01 nM) of the dopamine receptors (Table 10; Figures 19
and 20).

In hypothalamus no change was observed in the population (B_max 24.74 ± 0.90
pmol/g tissue) and affinity (K_d 0.30 ± 0.05 nM) by centhaquin. Clonidine also produced
no change in the density (B_max 26.66 ± 0.97 pmol/g tissue) and affinity (K_d 0.32 ± 0.07
nM), of these receptors. Hydralazine treatment produced a decrease (p < 0.05) in the
density (B_max 19.01 ± 1.26 pmol/g tissue) while the affinity (K_d 0.31 ± 0.05 nM) of ³H-
spiperone binding remained unchanged. Reserpine treatment increased (p < 0.05) the
density (B_max 31.39 ± 1.01 pmol/g tissue) while the affinity (K_d 0.29 ± 0.06 nM) of ³H-
spiperone binding remained unchanged (Table 11; Figures 21 and 22).

In the medulla, the dopaminergic receptor population (B_max 43.37 ± 1.63 pmol/g
tissue) and affinity (K_d 0.43 ± 0.08 nM) remained unchanged by centhaquin treatment.
In the clonidine treated rats also the population (B_max 43.84 ± 1.49 pmol/g tissue) and
affinity (K_d 0.43 ± 0.07 nM) of ³H-spiperone binding remained unchanged. In the
hydralazine treated rats the density (B_max 37.71 ± 1.16 pmol/g tissue) decreased (p <
0.05) and the affinity (K_d 0.32 ± 0.07 nM) remained unchanged. In the reserpine treated
rats the density (B_max 37.13 ± 1.11 pmol/g tissue) decreased (p < 0.05) and the affinity
(K_d 0.30 ± 0.05 nM) of the ³H-spiperone binding remained unchanged (Table 12;
Figures 23 and 24).
CEREBRAL CORTEX

$[^3]$H-Spiropiperidol binding to dopaminergic receptors

$B_{max}$ (pmole/g tissue; Mean ± SEM)

- Reserpine
- Hydralazine
- Clonidine
- Centhachtin
- Vehicle

* $p < 0.05$
Figure 16
CEREBRAL CORTEX

3H-Spiropenidol binding to dopaminergic receptors

Kd (nM; Mean ± SEM)

Vehicle
Clonidine
Centhaquin
Hydralazine
Reserpine

0.0
0.2
0.4
0.6

CORPUS STRIATUM

$^{3}H$-Spiropenadol binding to dopaminergic receptors

![Graph showing $B_{\text{max}}$ (pmole/g tissue; Mean ± SEM) for different treatments.

- Reserpine
- Hydralazine
- Clonidine
- Centraquin
- Vehicle

Legend for treatments with corresponding bar patterns.
Figure 20
CORPUS STRIATUM
3H-Spiroperdol binding to dopaminergic receptors

Kd (nM; Mean ± SEM)

Reserpine
Hydralazine
Clonidine
Centraquin
Vehicle

0.0
2.0E-2
4.0E-2
6.0E-2
Figure 21

HYPOTHALAMUS

3H-Spiropiperidol binding to dopaminergic receptors

Bmax (pmole/g tissue: Mean ± SEM)

Reserpine  
Hydralazine  
Clonidine  
Centrathacin  
Vehicle

p < 0.05

*
Figure 22

HYPOTHALAMUS

3H-Dioperiodic binding to dopaminergic receptors

Kd (nM; Mean ± SEM)
Figure 24

3H-Spiroperidol binding to dopaminergic receptors

MEDULLA

K_d (nM; Mean ± SEM)

Vehicle
Clonidine
Hydralazine
Reserpine
1.4 EFFECT OF CHRONIC ADMINISTRATION OF ANTIHYPERTENSIVE DRUGS ON CHOLINERGIC (MUSCARINIC)-RECEPTORS:

In the normal rat brain the distribution of muscarinic receptor sites show that in cortex the $B_{\text{max}}$ value was $51.13 \pm 1.92 \text{ pmol/g tissue}$ and $K_d$ value was $0.78 \pm 0.09 \text{ nM}$. Caudate shows the $B_{\text{max}}$ of $63.10 \pm 2.20 \text{ pmol/g tissue}$ and $K_d$ of $0.77 \pm 0.11 \text{ nM}$. In hypothalamus the density was ($B_{\text{max}}$ 36.53 ± 1.63 pmol/g tissue) and affinity was $K_d$ 1.42 ± 0.09 nM). In medulla the density was ($B_{\text{max}}$ 39.93 ± 1.83 pmol/g tissue, and affinity was ($K_d$ 0.73 ± 0.06 nM). These receptors have high affinity binding sites in various brain regions.

In cortex, centhaquin treatment produced no change in the density ($B_{\text{max}}$ 50.53 ± 1.00 pmol/g tissue) and affinity ($K_d$ 0.77 ± 0.08 nM) of $^3\text{H}$-quinuclidinyl benzylate binding. Clonidine produced a decrease ($p < 0.05$) in the density ($B_{\text{max}}$ 38.13 ± 1.53 pmol/g tissue) and affinity ($K_d$ 0.96 ± 0.06 nM) of the muscarinic receptors was not affected. No change was observed by hydralazine treatment in the density ($B_{\text{max}}$ 52.93 ± 1.95 pmol/g tissue) and affinity ($K_d$ 0.92 ± 0.10 nM) of $^3\text{H}$-quinuclidinyl benzylate binding. Reserpine did not change the density ($B_{\text{max}}$ 56.19 ± 2.12 pmol/g tissue) but increased ($p < 0.05$) the affinity ($K_d$ 0.95 ± 0.10 nM) of $^3\text{H}$-QNB binding (Table 13; Figures 25 and 26).

In caudate, no change was observed in the density ($B_{\text{max}}$ 59.20 ± 2.42 pmol/g tissue) or affinity ($K_d$ 0.69 ± 0.08 nM) by centhaquin. In clonidine treated rats also there was no change in the density ($B_{\text{max}}$ 60.10 ± 2.95 pmol/g tissue) or affinity ($K_d$ 0.76 ±
Table 13. Effect of repeated administration of centhaquin, clonidine, hydralazine and reserpine on \(^{3}\text{H}\)-quinuclidinyl benzilate binding to cholinergic (muscarinic) receptors in rat cerebral cortex.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>(^{3}\text{H})-Quinuclidinyl benzilate binding</th>
<th>B&lt;sub&gt;max&lt;/sub&gt; (pmol/g tissue)</th>
<th>K&lt;sub&gt;d&lt;/sub&gt; (nM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle</td>
<td></td>
<td>51.13 ± 1.92</td>
<td>0.78 ± 0.09</td>
</tr>
<tr>
<td>Centhaquin</td>
<td></td>
<td>50.53 ± 1.00</td>
<td>0.77 ± 0.08</td>
</tr>
<tr>
<td>Clonidine</td>
<td></td>
<td>38.13 ± 1.53*</td>
<td>0.96 ± 0.06</td>
</tr>
<tr>
<td>Hydralazine</td>
<td></td>
<td>52.93 ± 1.95</td>
<td>0.92 ± 0.10</td>
</tr>
<tr>
<td>Reserpine</td>
<td></td>
<td>56.19 ± 2.12</td>
<td>0.95 ± 0.10</td>
</tr>
</tbody>
</table>

*P < 0.05 as compared to vehicle treated control

Table 14. Effect of repeated administration of centhaquin, clonidine, hydralazine and reserpine on \(^{3}\text{H}\)-quinuclidinyl benzilate binding to cholinergic (muscarinic) receptors in rat corpus striatum.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>(^{3}\text{H})-Quinuclidinyl benzilate binding</th>
<th>B&lt;sub&gt;max&lt;/sub&gt; (pmol/g tissue)</th>
<th>K&lt;sub&gt;d&lt;/sub&gt; (nM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle</td>
<td></td>
<td>63.10 ± 2.20</td>
<td>0.77 ± 0.11</td>
</tr>
<tr>
<td>Centhaquin</td>
<td></td>
<td>59.20 ± 2.42</td>
<td>0.69 ± 0.08</td>
</tr>
<tr>
<td>Clonidine</td>
<td></td>
<td>60.10 ± 2.95</td>
<td>0.76 ± 0.09</td>
</tr>
<tr>
<td>Hydralazine</td>
<td></td>
<td>57.52 ± 2.06</td>
<td>0.73 ± 0.08</td>
</tr>
<tr>
<td>Reserpine</td>
<td></td>
<td>38.68 ± 1.50*</td>
<td>0.68 ± 0.10</td>
</tr>
</tbody>
</table>

*P < 0.05 as compared to vehicle treated control
Table 15. Effect of repeated administration of centhaquin, clonidine, hydralazine and reserpine on \(^3\text{H}\)-quinuclidinyl benzilate binding to cholinergic (muscarinic) receptors in rat hypothalamus.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>(^3\text{H})-Quinuclidinyl benzilate binding</th>
<th>(B_{\text{max}}) (pmol/g tissue)</th>
<th>(K_d) (nM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle</td>
<td>Mean ± S.E.M., N=5</td>
<td>36.53 ± 1.63</td>
<td>1.42 ± 0.09</td>
</tr>
<tr>
<td>Centhaquin</td>
<td></td>
<td>35.72 ± 2.18</td>
<td>1.20 ± 0.12</td>
</tr>
<tr>
<td>Clonidine</td>
<td></td>
<td>55.00 ± 2.06*</td>
<td>1.13 ± 0.18</td>
</tr>
<tr>
<td>Hydralazine</td>
<td></td>
<td>35.89 ± 2.82</td>
<td>1.44 ± 0.10</td>
</tr>
<tr>
<td>Reserpine</td>
<td></td>
<td>33.13 ± 2.21</td>
<td>1.57 ± 0.11</td>
</tr>
</tbody>
</table>

*\(P < 0.05\) as compared to vehicle treated control

Table 16. Effect of repeated administration of centhaquin, clonidine, hydralazine and reserpine on \(^3\text{H}\)-quinuclidinyl benzilate binding to cholinergic (muscarinic) receptors in rat brain medulla.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>(^3\text{H})-Quinuclidinyl benzilate binding</th>
<th>(B_{\text{max}}) (pmol/g tissue)</th>
<th>(K_d) (nM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle</td>
<td>Mean ± S.E.M., N=5</td>
<td>39.93 ± 1.83</td>
<td>0.73 ± 0.06</td>
</tr>
<tr>
<td>Centhaquin</td>
<td></td>
<td>45.84 ± 2.00</td>
<td>0.74 ± 0.09</td>
</tr>
<tr>
<td>Clonidine</td>
<td></td>
<td>56.70 ± 2.12*</td>
<td>0.59 ± 0.09</td>
</tr>
<tr>
<td>Hydralazine</td>
<td></td>
<td>41.15 ± 2.31</td>
<td>0.75 ± 0.09</td>
</tr>
<tr>
<td>Reserpine</td>
<td></td>
<td>38.48 ± 2.55</td>
<td>0.71 ± 0.08</td>
</tr>
</tbody>
</table>

*\(P < 0.05\) as compared to vehicle treated control
$B_{\text{max}}$ (pmole/g tissue; Mean ± SEM)

CEREBRAL CORTEX

3H-Quinuclidinyl benzylate binding to cholinergic (muscarinic) receptors

Vehicle
Clonidine
Hydralazine
Reserpine

* $p < 0.05$
Figure 26
CEREBRAL CORTEX

3H-quinine/quinidine benzyliate binding to cholinergic (muscarinic) receptors

Kd (nM; Mean ± SEM)
Figure 27

**3H-Quinuclidinyl benzylate binding to cholinergic (muscarinic) receptors**

**CORPUS STRIATUM**

$B_{\text{max}}$ (pmole/g tissue; Mean ± SEM)

- Vehicle
- Clonidine
- Hydralazine
- Reserpine

* $p < 0.05$
Figure 28
CORPUS STRIATUM
3H-Quinucyclinyl benzyloate binding to cholinergic (muscarinic) receptors

Kd (nM; Mean ± SEM)

Reserpine
Hydralazine
Clonidine
Centhacolin
Vehicle
Figure 29

HYPOTHALAMUS

3H-quinuclidinyl benzilate binding to cholinergic (muscarinic) receptors

Bmax (pmole/g tissue; Mean ± SEM)

* p < 0.05

Reserpine
Hydralazine
Clonidine
Centraquin
Vehicle

Legend
Figure 30

Hypothalamus

$^3$H-Quinuclidinyl benzylate binding to cholinergic (muscarinic) receptors

$K_d$ (nM; Mean ± SEM)
**Figure 31**

**MEDULLA**

3H-Quinuclydine benzylate binding to cholinergic (muscarinic) receptors

![Bar chart showing binding levels with error bars for different conditions.](image-url)
Figure 32

MEDULLA

3H-Quinuclidinyl benzylate binding to cholinergic (muscarinic) receptors

Kd (nM; Mean ± SEM)
0.09 nM). Similarly in hydralazine treated rats no change occurred in the density ($B_{\text{max}}$ 57.52 ± 2.06 pmol/g tissue) or affinity ($K_d$ 0.73 ± 0.08 nM). In reserpine treated rats the density ($B_{\text{max}}$ 38.68 ± 1.50 pmol/g tissue) decreased ($p < 0.05$), but the affinity ($K_d$ 0.68 ± 0.10 nM) remained unchanged (Table 14; Figures 27 and 28).

In the hypothalamus, centhaquin did not change the density ($B_{\text{max}}$ 35.72 ± 2.18 pmol/g tissue) and affinity ($K_d$ 1.20 ± 0.12 nM). Clonidine increased ($p < 0.05$) the density ($B_{\text{max}}$ 55.00 ± 2.06 pmol/g tissue) and affinity ($K_d$ 1.13 ± 0.18 nM) of $^3$H-QNB binding was not affected. Hydralazine did not effect the density ($B_{\text{max}}$ 35.89 ± 2.82 pmol/g tissue) or affinity ($K_d$ 1.44 ± 0.10 nM). Reserpine also did not effect either the density ($B_{\text{max}}$ 33.13 ± 2.21 pmol/g tissue) or the affinity ($K_d$ 1.57 ± 0.11 nM) of these receptors (Table 15; Figures 29 and 30).

In medulla, centhaquin did not change the density ($B_{\text{max}}$ 45.84 ± 2.00 pmol/g tissue) or the affinity ($K_d$ 0.74 ± 0.09 nM). Clonidine treatment increased ($p < 0.05$) the density ($B_{\text{max}}$ 56.70 ± 2.12 pmol/g tissue) and the affinity ($K_d$ 0.59 ± 0.09 nM) of $^3$H-QNB binding was unaltered. Hydralazine produced no change in the density ($B_{\text{max}}$ 41.15 ± 2.31 pmol/g tissue) and affinity ($K_d$ 0.75 ± 0.09 nM). Reserpine also did not change the density ($B_{\text{max}}$ 38.48 ± 2.55 pmol/g tissue) and affinity ($K_d$ 0.71 ± 0.08 nM) of the muscarinic receptors binding (Table 16; Figures 31 and 32).

1.5 EFFECT OF CHRONIC ADMINISTRATION OF ANTIHYPERTENSIVE DRUGS ON 5-HT$_1$ RECEPTORS:
In the normal rat brain the $^3$H-5-HT binding is fairly constant. In cortex the $B_{\text{max}}$ was $31.46 \pm 1.35$ pmol/g tissue. While $K_d$ was $1.34 \pm 0.11$ nM. In caudate the density was ($B_{\text{max}}$ $23.74 \pm 2.11$ pmol/g tissue) and affinity was ($K_d$ $0.86 \pm 0.05$ nM). Hypothalamus showed the density of ($B_{\text{max}}$ $26.02 \pm 1.82$ pmol/g tissue, and affinity of ($K_d$ $1.54 \pm 0.07$ nM). In medulla, the $B_{\text{max}}$ was $24.99 \pm 1.66$ pmol/g tissue, while $K_d$ was $1.35 \pm 0.07$ nM.

The cortex of the centhaquin treated rats did not show any change in the density ($B_{\text{max}}$ $30.25 \pm 1.80$ pmol/g tissue) or affinity ($K_d$ $1.24 \pm 0.22$ nM) of $^3$H-5-HT binding. Clonidine treatment also did not show any change in the density ($B_{\text{max}}$ $30.82 \pm 1.80$ pmol/g tissue) or affinity ($K_d$ $1.28 \pm 0.22$ nM). Hydralazine treatment increased ($p < 0.05$) the density ($B_{\text{max}}$ $39.31 \pm 1.20$ pmol/g tissue) and affinity ($K_d$ $0.84 \pm 0.07$ nM) by $^3$H-5-HT binding. Reserpine treatment also increased ($p < 0.05$) the density ($B_{\text{max}}$ $38.32 \pm 1.20$ pmol/g tissue) and affinity ($K_d$ $0.77 \pm 0.07$ nM) of the 5HT$_1$ receptors (Table 17; Figures 33 and 34).

In caudate, centhaquin produced no change in the density ($B_{\text{max}}$ $23.96 \pm 1.11$ pmol/g tissue) and affinity ($K_d$ $0.87 \pm 0.05$ nM). Clonidine also did not produce any change in the density ($B_{\text{max}}$ $25.25 \pm 1.98$ pmol/g tissue) and affinity ($K_d$ $0.76 \pm 0.06$ nM) of $^3$H-5-HT binding. Hydralazine decreased both ($p < 0.05$) the density ($B_{\text{max}}$ $16.27 \pm 1.80$ pmol/g tissue) and the affinity ($K_d$ $1.10 \pm 0.11$ nM), while Reserpine treatment increased ($p < 0.05$) the density ($B_{\text{max}}$ $27.39 \pm 1.99$ pmol/g tissue) but decreased ($p < 0.05$) the affinity ($K_d$ $1.22 \pm 0.09$ nM) of the $^3$H-5HT binding (Table 18; Figures 35.
and 36).

In hypothalamus, no change occurred in the density ($B_{\text{max}} 25.96 \pm 1.91 \text{ pmol/g tissue}$) and affinity ($K_d 1.41 \pm 0.17 \text{ nM}$) of the centhaquin treated rats. No change occurred in the density ($B_{\text{max}} 25.74 \pm 1.88 \text{ pmol/g tissue}$) and affinity ($K_d 1.43 \pm 0.15 \text{ nM}$) of the clonidine treated. Hydralazine treatment decreased ($p < 0.05$) the density ($B_{\text{max}} 17.05 \pm 1.60 \text{ pmol/g tissue}$) but no change occurred in affinity ($K_d 1.59 \pm 0.14 \text{ nM}$) of the $5\text{HT}_1$ receptors. Reserpine treatment also decreased the density ($B_{\text{max}} 15.95 \pm 1.81 \text{ pmol/g tissue}$) but did not change the affinity ($K_d 1.35 \pm 0.14 \text{ nM}$) of the $^3\text{H}$-5HT binding sites (Table 19; Figures 37 and 38).

In medulla, centhaquin increased ($p < 0.05$) the density ($B_{\text{max}} 35.13 \pm 2.71 \text{ pmol/g tissue}$) and affinity ($K_d 1.12 \pm 0.11 \text{ nM}$) of $^3\text{H}$-5HT binding. Clonidine increased ($p < 0.05$) the density ($B_{\text{max}} 36.76 \pm 2.60 \text{ pmol/g tissue}$) and decreased ($p < 0.05$) the affinity ($K_d 1.16 \pm 0.10 \text{ nM}$) of the $5\text{HT}_1$ receptors. Hydralazine treatment did not change the density ($B_{\text{max}} 27.39 \pm 1.90 \text{ pmol/g tissue}$) or affinity $K_d 1.54 \pm 0.15 \text{ nM}$) of $5\text{HT}_1$ receptors. Reserpine also did not produce any change in the density ($B_{\text{max}} 25.76 \pm 1.60 \text{ pmol/g tissue}$) and affinity ($K_d 1.39 \pm 0.11 \text{ nM}$) of $^3\text{H}$-5-HT binding sites (Table 20; Figures 39 and 40).

### 1.6 EFFECT OF CHRONIC ADMINISTRATION OF ANTIHYPERTENSIVE DRUGS ON 5HT$_2$ RECEPTORS:

The normal rat brain showed that in cortex the $B_{\text{max}}$ was $34.86 \pm 2.61 \text{ pmol/g tissue}$, while $K_d$ was $0.86 \pm 0.04 \text{ nM}$. In caudate, $B_{\text{max}}$ was $33.80 \pm 2.25 \text{ pmol/g tissue}$,
Table 17. Effect of repeated administration of centhaquin, clonidine, hydralazine and reserpine on $^3$H-5-hydroxytryptamine binding to 5-HT$_1$ receptors in rat cerebral cortex.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>$^3$H-5-hydroxytryptamine binding</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean ± S.E.M., N=5</td>
<td>Bmax (pmol/g tissue)</td>
</tr>
<tr>
<td>Vehicle</td>
<td></td>
<td>31.46 ± 1.35</td>
</tr>
<tr>
<td>Centhaquin</td>
<td></td>
<td>30.25 ± 1.80</td>
</tr>
<tr>
<td>Clonidine</td>
<td></td>
<td>30.82 ± 1.80</td>
</tr>
<tr>
<td>Hydralazine</td>
<td></td>
<td>39.31 ± 1.20*</td>
</tr>
<tr>
<td>Reserpine</td>
<td></td>
<td>38.32 ± 1.20*</td>
</tr>
</tbody>
</table>

*P < 0.05 as compared to vehicle treated control

Table 18. Effect of repeated administration of centhaquin, clonidine, hydralazine and reserpine on $^3$H-5-hydroxytryptamine binding to 5-HT$_1$ receptors in rat corpus striatum.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>$^3$H-5-hydroxytryptamine binding</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean ± S.E.M., N=5</td>
<td>Bmax (pmol/g tissue)</td>
</tr>
<tr>
<td>Vehicle</td>
<td></td>
<td>23.74 ± 2.11</td>
</tr>
<tr>
<td>Centhaquin</td>
<td></td>
<td>23.96 ± 1.11</td>
</tr>
<tr>
<td>Clonidine</td>
<td></td>
<td>25.25 ± 1.98</td>
</tr>
<tr>
<td>Hydralazine</td>
<td></td>
<td>16.27 ± 1.80*</td>
</tr>
<tr>
<td>Reserpine</td>
<td></td>
<td>27.39 ± 1.99*</td>
</tr>
</tbody>
</table>

*P < 0.05 as compared to vehicle treated control
Table 19. Effect of repeated administration of centhaquin, clonidine, hydralazine and reserpine on $^3$H-5-hydroxytryptamine binding to 5-HT$_1$ receptors in rat hypothalamus.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>$^3$H-5-hydroxytryptamine binding</th>
<th>Mean ± S.E.M., N=5</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Bmax (pmol/g tissue)</td>
<td>$K_d$ (nM)</td>
</tr>
<tr>
<td>Vehicle</td>
<td>26.02 ± 1.82</td>
<td>1.54 ± 0.07</td>
</tr>
<tr>
<td>Centhaquin</td>
<td>25.96 ± 1.91</td>
<td>1.41 ± 0.17</td>
</tr>
<tr>
<td>Clonidine</td>
<td>25.74 ± 1.88</td>
<td>1.43 ± 0.15</td>
</tr>
<tr>
<td>Hydralazine</td>
<td>17.05 ± 1.60*</td>
<td>1.59 ± 0.14</td>
</tr>
<tr>
<td>Reserpine</td>
<td>15.95 ± 1.81*</td>
<td>1.35 ± 0.14</td>
</tr>
</tbody>
</table>

*P < 0.05 as compared to vehicle treated control

Table 20. Effect of repeated administration of centhaquin, clonidine, hydralazine and reserpine on $^3$H-5-hydroxytryptamine binding to 5-HT$_1$ receptors in rat brain medulla.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>$^3$H-5-hydroxytryptamine binding</th>
<th>Mean ± S.E.M., N=5</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Bmax (pmol/g tissue)</td>
<td>$K_d$ (nM)</td>
</tr>
<tr>
<td>Vehicle</td>
<td>24.99 ± 1.66</td>
<td>1.35 ± 0.07</td>
</tr>
<tr>
<td>Centhaquin</td>
<td>35.13 ± 2.71*</td>
<td>1.12 ± 0.11*</td>
</tr>
<tr>
<td>Clonidine</td>
<td>36.76 ± 2.60*</td>
<td>1.16 ± 0.10*</td>
</tr>
<tr>
<td>Hydralazine</td>
<td>27.39 ± 1.90</td>
<td>1.54 ± 0.15</td>
</tr>
<tr>
<td>Reserpine</td>
<td>25.76 ± 1.60</td>
<td>1.39 ± 0.11</td>
</tr>
</tbody>
</table>

*P < 0.05 as compared to vehicle treated control
Figure 33

CEREBRAL CORTEX

$^3$H-5-HYDROXYTRYPTAMINE BINDING TO 5-HT$_1$ RECEPTORS

$B_{max}$ (pmole/g tissue; Mean ± SEM)

Reserpine
Hydralazine
Clonidine
Centhaquin
Vehicle
CEREBRAL CORTEX

3H-5-Hydroxytryptamine binding to 5-HT1 receptors

Kd (nM; Mean ± SEM)
$B_{\text{max}}$ (pmole/g tissue; Mean ± SEM)

3H-5-Hydroxytryptamine binding to 5-HT$_1$ receptors

CORPUS STRIATUM

Figure 35

Vehicle
Clonidine
Hydralazine
Reserpine

* $p < 0.05$
CORPUS STRIATUM

$^{3}$H-5-Hydroxytryptamine binding to 5-HT$ _4$ receptors

Figure 36

Kd (nM; Mean ± SEM)

Vehicle
Centhaquin
Chloridzone
Hydralazine
Reserpine

$* p < 0.05$
Figure 37

HYPOTHALAMUS

$^{3}H$-5-Hydroxytryptamine binding to 5-HT$_1$ receptors

Bmax (pmole/g tissue; Mean ± SEM)

- Reserpine
- Hydralazine
- Chloride
- Centraquin
- Vehicle

* p < 0.05
Figure 38

HYPOTHALAMUS

3H-5-Hydroxytryptamine binding to 5-HT1 receptors

Kd (nM; Mean ± SEM)
Figure 39

MEDULLA

$[^3]H-5$-Hydroxytryptamine binding to $5-HT_1$ receptors

Bmax (pmole/g tissue; Mean ± SEM)

Reserpine
Hydralazine
Clonidine
Centraquin
Vehicle

* $p < 0.05$
Figure 40

MEDULLA

$3^H$-5-Hydroxytryptamine binding to 5-HT$_1$ receptors

$K_d$ (nM; Mean ± SEM)

Vehicle
Clonidine
Hydralazine
Reserpine
and $K_d$ was $0.78 \pm 0.02$ nM. In hypothalamus the density was $(B_{\text{max}} 23.36 \pm 1.25$ pmol/g tissue. Where as affinity was $(K_d 0.86 \pm 0.06$ nM). In medulla the $B_{\text{max}}$ was $27.49 \pm 1.40$ pmol/g tissue and $K_d$ was $1.38 \pm 0.07$ nM.

In cortex, centhaquin did not show any change in the density $(B_{\text{max}} 34.91 \pm 2.63$ pmol/g tissue) and affinity $(K_d 0.86 \pm 0.04$ nM) of the $^3$H-spiperone binding. Clonidine also did not show any change in the density $(B_{\text{max}} 35.21 \pm 1.90$ pmol/g tissue) and affinity $(K_d 0.87 \pm 0.02$ nM) of the 5HT$_2$ receptors. Hydralazine increased $(p < 0.05)$ the density $(B_{\text{max}} 45.58 \pm 2.44$ pmol/g tissue) and produced no change in affinity $(K_d 0.71 \pm 0.02$ nM) of $^3$H-spiperone binding. Reserpine also increased $(p < 0.05)$ the density $(B_{\text{max}} 44.70 \pm 2.56$ pmol/g tissue) and affinity $(K_d 0.84 \pm 0.03$ nM) was not changed after $^3$H-spiperone binding (Table 21; Figures 41 and 42).

In caudate, centhaquin did not produce any change in the density $(B_{\text{max}} 34.45 \pm 1.91$ pmol/g tissue) and affinity $(K_d 0.82 \pm 0.02$ nM) of $^3$H-spiperone binding. Clonidine also did not change the density $(B_{\text{max}} 34.43 \pm 1.92$ pmol/g tissue) and affinity $(K_d 0.81 \pm 0.01$ nM) of 5HT$_2$ receptors. Hydralazine increased $(p < 0.05)$ the density $(B_{\text{max}} 47.24 \pm 2.55$ pmol/g tissue) but had no effect on the affinity $(K_d 0.64 \pm 0.04$ nM) of the $^3$H-spiperone binding. Reserpine also increased $(p < 0.05)$ the density $(B_{\text{max}} 48.03 \pm 2.46$ pmol/g tissue) but did not effect the affinity $(K_d 0.63 \pm 0.04$ nM) of the 5HT$_2$ receptors (Table 22; Figures 43 and 44).

In hypothalamus, centhaquin had no effect on either the density $(B_{\text{max}} 21.71 \pm 1.22$ pmol/g tissue) or affinity $(K_d 0.86 \pm 0.05$ nM) of the $^3$H-spiperone binding.
Table 21. Effect of repeated administration of centhaquin, clonidine, hydralazine and reserpine on $^3$H-spiroperidol binding to 5-HT$_2$ receptors in rat cerebral cortex.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>$^3$H-spiroperidol binding</th>
<th>$K_d$ (nM)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Bmax (pmol/g tissue)</td>
<td></td>
</tr>
<tr>
<td>Vehicle</td>
<td>34.86 ± 2.61</td>
<td>0.86 ± 0.04</td>
</tr>
<tr>
<td>Centhaquin</td>
<td>34.91 ± 2.63</td>
<td>0.86 ± 0.04</td>
</tr>
<tr>
<td>Clonidine</td>
<td>35.21 ± 1.90</td>
<td>0.87 ± 0.02</td>
</tr>
<tr>
<td>Hydralazine</td>
<td>45.58 ± 2.44$^*$</td>
<td>0.71 ± 0.02</td>
</tr>
<tr>
<td>Reserpine</td>
<td>44.70 ± 2.56$^*$</td>
<td>0.84 ± 0.03</td>
</tr>
</tbody>
</table>

*P < 0.05 as compared to vehicle treated control

Table 22. Effect of repeated administration of centhaquin, clonidine, hydralazine and reserpine on $^3$H-spiroperidol binding to 5-HT$_2$ receptors in rat corpus striatum.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>$^3$H-spiroperidol binding</th>
<th>$K_d$ (nM)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Bmax (pmol/g tissue)</td>
<td></td>
</tr>
<tr>
<td>Vehicle</td>
<td>33.80 ± 2.25</td>
<td>0.78 ± 0.02</td>
</tr>
<tr>
<td>Centhaquin</td>
<td>34.45 ± 1.91</td>
<td>0.82 ± 0.02</td>
</tr>
<tr>
<td>Clonidine</td>
<td>34.43 ± 1.92</td>
<td>0.81 ± 0.01</td>
</tr>
<tr>
<td>Hydralazine</td>
<td>47.24 ± 2.55$^*$</td>
<td>0.64 ± 0.04</td>
</tr>
<tr>
<td>Reserpine</td>
<td>48.03 ± 2.46$^*$</td>
<td>0.63 ± 0.04</td>
</tr>
</tbody>
</table>

*P < 0.05 as compared to vehicle treated control
Table 23. Effect of repeated administration of centhaquin, clonidine, hydralazine and reserpine on \(^3\text{H}\)-spiroperidol binding to 5-HT\(_2\) receptors in rat hypothalamus.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>(^3\text{H})-spiroperidol binding</th>
<th>Mean ± S.E.M., N=5</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Bmax (pmol/g tissue)</td>
<td>(K_D) (nM)</td>
</tr>
<tr>
<td>Vehicle</td>
<td>23.36 ± 1.25</td>
<td>0.86 ± 0.06</td>
</tr>
<tr>
<td>Centhaquin</td>
<td>21.71 ± 1.22</td>
<td>0.86 ± 0.05</td>
</tr>
<tr>
<td>Clonidine</td>
<td>24.35 ± 2.30</td>
<td>0.96 ± 0.08</td>
</tr>
<tr>
<td>Hydralazine</td>
<td>23.18 ± 1.11</td>
<td>0.87 ± 0.03</td>
</tr>
<tr>
<td>Reserpine</td>
<td>24.33 ± 2.11</td>
<td>0.83 ± 0.05</td>
</tr>
</tbody>
</table>

*P < 0.05 as compared to vehicle treated control

Table 24. Effect of repeated administration of centhaquin, clonidine, hydralazine and reserpine on \(^3\text{H}\)-spiroperidol binding to 5-HT\(_2\) receptors in rat brain medulla.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>(^3\text{H})-spiroperidol binding</th>
<th>Mean ± S.E.M., N=5</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Bmax (pmol/g tissue)</td>
<td>(K_D) (nM)</td>
</tr>
<tr>
<td>Vehicle</td>
<td>27.49 ± 1.40</td>
<td>1.38 ± 0.07</td>
</tr>
<tr>
<td>Centhaquin</td>
<td>26.16 ± 1.22</td>
<td>1.18 ± 0.17</td>
</tr>
<tr>
<td>Clonidine</td>
<td>27.06 ± 1.48</td>
<td>1.30 ± 0.11</td>
</tr>
<tr>
<td>Hydralazine</td>
<td>17.36 ± 1.21*</td>
<td>1.37 ± 0.14</td>
</tr>
<tr>
<td>Reserpine</td>
<td>17.44 ± 1.08*</td>
<td>1.54 ± 0.05</td>
</tr>
</tbody>
</table>

*P < 0.05 as compared to vehicle treated control
Clonidine also did not have any effect on the density ($B_{max}$ 24.35 ± 2.30 pmol/g tissue) and affinity ($K_d$ 0.96 ± 0.08 nM) of 5HT$_2$ receptors. Hydralazine had no effect on the density ($B_{max}$ 23.18 ± 1.11 pmol/g tissue) and affinity ($K_d$ 0.87 ± 0.03 nM) of $^3$H-spiperone binding. Reserpine had no effect on the density ($B_{max}$ 24.33 ± 2.11 pmol/g tissue) and affinity ($K_d$ 0.83 ± 0.05 nM) of the $^3$H-spiperone binding (Table 23; Figures 45 and 46).

In medulla, centhaquin did not change the density ($B_{max}$ 26.16 ± 1.22 pmol/g tissue) or affinity ($K_d$ 1.18 ± 0.17 nM) of the $^3$H-spiperone binding. Clonidine treatment also did not change the density ($B_{max}$ 27.06 ± 1.48 pmol/g tissue) or the affinity ($K_d$ 1.30 ± 0.11 nM) of the 5HT$_2$ receptors. Hydralazine treatment decreased ($p < 0.05$) the density ($B_{max}$ 17.36 ± 1.21 pmol/g tissue) but did not affect the affinity ($K_d$ 1.37 ± 0.14 nM). In reserpine treated rats, the density ($B_{max}$ 17.44 ± 1.08 pmol/g tissue) decreased ($p < 0.05$) but the affinity ($K_d$ 1.54 ± 0.05 nM) was not affected (Table 24; Figures 47 and 48).

2. EFFECT OF CENTHAQUIN ON BLOOD PRESSURE AND HEART RATE IN NORMAL AND CERVICAL SECTIONED RATS:

Centhaquin in a dose of 0.1 mg/kg, iv produced significant hypotension and bradycardia in rats. However in the cervical sectioned rats centhaquin in the same dose (0.1 mg/kg, iv) did not produce any change in either blood pressure or heart rate. Centhaquin (2 $\mu$g) when given intrathecally produced no change in blood pressure and heart rate (Figure 49).
$B_{\text{max}} \text{ (pmole/g tissue; Mean ± SEM)}$

CEREBRAL CORTEX

$3^\text{H}$-Spiroperidol binding to 5-HT$\text{2}$ receptors

Vehicle
Clonidine
Hydralazine
Reserpine

* $p < 0.05$
Figure 42

CEREBRAL CORTEX

$^{3}H$-Spiroperial binding to 5-HT$_2$ receptors

$K_d$ (nM; Mean ± SEM)
Figure 43

CORPUS STRIATUM

$^3$H-Spiropiperidol binding to 5-HT$_2$ receptors

B$_{max}$ (pmole/g tissue; Mean ± SEM)

- Reserpine
- Hydrazine
- Chlorzepate
- Centrahogin
- Vehicle

$p < 0.05$
Figure 44

CORPUS STRIATUM

\[ \text{H}-\text{Spiroperidol binding to 5-HT}_2 \text{ receptors} \]

\[ \text{Kd (nM; Mean ± SEM)} \]

* \( p < 0.05 \)

Reserpine

Hydrazine

Clonidine

Centraquin

Vehicle
$B_{max}$ (pmole/g tissue; Mean ± SEM)

HYPOTHALAMUS

$[^3]H$-Spiroperidol binding to 5-HT$_2$ receptors

Figure 45

Vehicle
Clonidine
Hydralazine
Reserpine
Figure 46

HYPOTHALAMUS

$^{3}H$-Spiropiperidol binding to 5-HT$_2$ receptors

Kd (nM; Mean ± SEM)
Figure 47

MEDULLA

$^3$H-Spiropiperidol binding to 5-HT$_2$ receptors

Bmax (pmole/g tissue; Mean ± SEM)

Reserpine
Hydralazine
Clonidine
Centhagulin
Vehicle

* $p > 0.05$
Figure 48

**MEDULLA**

$^3$H-Spiroperidol binding to 5-HT$_2$ receptors

![Bar chart showing Kd (nM; Mean ± SEM) for different compounds in the medulla.](image)
3. EFFECT OF CENTHAQUIN MICROINJECTIONS IN NTS OF CAT:

Centhaquin given in cats at doses of 0.05, 0.1 and 0.2 mg/kg, iv, produced a dose-dependent fall in blood pressure and heart rate (Figure 50). Microinjection of centhaquin in the NTS (100 ng) produced hypertension and bradycardia, which could be significantly blocked by phentolamine (50 μg) (Figure 51). The decrease in heart rate produced by centhaquin (100 ng) microinjection in the NTS could also be significantly blocked by yohimbine (50 ng) (Figure 52). Prazosin (10 ng) also significantly blocked the bradycardiac effect of centhaquin (100 ng) microinjected in the NTS (Figure 53).

4.1 EFFECT OF CHRONIC ADMINISTRATION OF ANTIHYPERTENSIVE DRUGS ON BLOOD PRESSURE RESPONSE TO INTRACEREBROVENTRICULAR INJECTION OF NORADRENALINE IN CONSCIOUS RATS:

Clonidine, hydralazine and reserpine produced a significant decrease in basal blood pressure as compared to control. In normal rats intracerebroventricular injection (icv) of noradrenaline (50 μg) caused hypotension. In centhaquin and clonidine treated rats hypertension was produced while in hydralazine and reserpine treated rats hypotension was produced after icv injection of noradrenaline as compared to normal rat. Compared to the control blood pressure, treatment with centhaquin and reserpine caused hypotension, while treatment with clonidine and hydralazine caused hypertension after intracerebroventricular injection of noradrenaline (Table 25; Figure 54).

4.2 EFFECT OF CHRONIC ADMINISTRATION OF ANTIHYPERTENSIVE DRUGS ON BLOOD PRESSURE RESPONSE TO INTRACEREBROVENTRICULAR INJECTION
Effect of centhaquin on blood pressure and heart rate in anesthetized rat. Centhaquin produced a significant fall in blood pressure and heart rate at the dose of 0.1 mg/kg iv. However, after cervical sectioning, centhaquin did not produce any effect either on blood pressure or heart rate. Intrathecal injection of centhaquin in normal rats did not produce any change in blood pressure or heart rate, indicating that centhaquin produces decrease in blood pressure and heart rate by acting at a site higher than spinal cord.
Figure 50

- 0.05 Centhaquin (mg/kg iv)
- 0.1 Centhaquin (mg/kg iv)
- 0.2 Centhaquin (mg/kg iv)

Graphs showing changes in blood pressure and heart rate in response to Centhaquin administration (mg/kg iv).
Effect of bilateral microinjection of centhaquin (100 ng) in NTS. Note there was not much effect on blood pressure but a significant bradycardia was produced. Phentolamine (50 ng) which blocks both $\alpha_1$ as well as $\alpha_2$ adrenergic receptors completely blocked the decrease in heart rate produced by centhaquin microinjection in NTS.
Effect of bilateral microinjection of centhaquin (100 ng) in NTS. Yohimbine (50 ng) which blocks \( \alpha_2 \) adrenergic receptors completely blocked the decrease in heart rate produced by centhaquin microinjection in NTS.
Effect of bilateral microinjection of centhaquin (100 ng) in NTS. Prazosin (10 ng) which blocks $\alpha_1$ adrenergic receptors completely blocked the decrease in heart rate produced by centhaquin microinjection in NTS.
OF DOPAMINE IN CONSCIOUS RATS:

Treatment with centhaquin, clonidine, hydralazine and reserpine produced a significant hypotension. Compared to basal blood pressure no change in mean blood pressure occurred by treatment with centhaquin and clonidine, decreased by hydralazine treatment but increased by treatment with reserpine after icv injections of dopamine (50 μg). As compared to normal rat, hypotension was produced by treatment with centhaquin, clonidine and hydralazine, while treatment with reserpine produced first decrease and then increase in blood pressure after intracerebroventricular injections of dopamine (Table 26; Figure 55).

4.3 EFFECT OF CHRONIC ADMINISTRATION OF ANTIHYPERTENSIVE DRUGS ON BLOOD PRESSURE RESPONSE TO INTRACEREBROVENTRICULAR INJECTION OF ACETYLCHOLINE IN CONSCIOUS RATS:

Treatment with centhaquin, clonidine, hydralazine and reserpine produced hypotension. In normal rat, hypotension was produced after icv injection of acetylcholine (10 μg). Treatment with centhaquin and reserpine did not change the mean blood pressure while treatment with clonidine and hydralazine produced hypotension after intracerebroventricular injection of acetylcholine as compared to the basal blood pressure. Centhaquin treatment did not affect the mean blood pressure, while clonidine, hydralazine and reserpine treatment decreased the blood pressure after intracerebroventricular injection of acetylcholine injection (Table 27; Figure 56).
MEAN BLOOD PRESSURE (mmHg)
Mean ± SEM (N=6)

EFFECT OF NORADRENALINE (50 µg, iv)

TIME (min)
0 30 60 120 180 240 300

CONTROL
HYDRAZINE
CLONIDINE
RESERPINE
MEAN BLOOD PRESSURE (mmHg)
Mean ± SEM (N=6)

EFFECT OF ACETYLCHOLINE (10 μg, icv)

TIME (min)
0 25 50 75 100 125 150

0 30 60 120 180 240 300

CONTROL HYDRAZINE CLONIDINE RESERPINE
Table 25: Effect of intraventricular administration of noradrenaline (50 μg) on blood pressure of conscious rats

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>BP</th>
<th>Control (mmHg; Mean ± SEM; N = 6)</th>
<th>Centhaquin</th>
<th>Clonidine</th>
<th>Hydralazine</th>
<th>Reserpine</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Systolic</td>
<td>Diastolic</td>
<td>Mean</td>
<td>Systolic</td>
<td>Diastolic</td>
<td>Mean</td>
</tr>
<tr>
<td>0</td>
<td>148.5 ± 0.67</td>
<td>114.6 ± 1.31</td>
<td>125.9 ± 0.61</td>
<td>134.2 ± 0.62</td>
<td>117.3 ± 0.41</td>
<td>122.9 ± 0.33</td>
</tr>
<tr>
<td>30</td>
<td>134.2a ± 1.13</td>
<td>117.3 ± 0.41</td>
<td>122.9a ± 0.33</td>
<td>131.38 ± 1.51</td>
<td>110.48 ± 1.16</td>
<td>117.48 ± 1.18</td>
</tr>
<tr>
<td>60</td>
<td>130.4a ± 1.36</td>
<td>113.4 ± 0.63</td>
<td>119.2a ± 0.81</td>
<td>131.38 ± 1.51</td>
<td>110.48 ± 1.16</td>
<td>117.48 ± 1.18</td>
</tr>
<tr>
<td>120</td>
<td>125.7a ± 1.60</td>
<td>94.8a ± 1.75</td>
<td>105.1a ± 1.32</td>
<td>131.38 ± 1.51</td>
<td>110.48 ± 1.16</td>
<td>117.48 ± 1.18</td>
</tr>
<tr>
<td>180</td>
<td>116.2a ± 0.76</td>
<td>78.0a ± 0.42</td>
<td>90.8a ± 0.43</td>
<td>131.38 ± 1.51</td>
<td>110.48 ± 1.16</td>
<td>117.48 ± 1.18</td>
</tr>
<tr>
<td>240</td>
<td>127.4a ± 0.76</td>
<td>91.9a ± 2.33</td>
<td>103.7a ± 1.72</td>
<td>131.38 ± 1.51</td>
<td>110.48 ± 1.16</td>
<td>117.48 ± 1.18</td>
</tr>
<tr>
<td>300</td>
<td>131.2a ± 0.76</td>
<td>103.2a ± 2.29</td>
<td>112.4a ± 1.99</td>
<td>131.38 ± 1.51</td>
<td>110.48 ± 1.16</td>
<td>117.48 ± 1.18</td>
</tr>
</tbody>
</table>

a = p < 0.05 compared to the basal blood pressure (0 min); B = p < 0.05 compared to the control group
Table 26: Effect of intraventricular administration of dopamine (50 μg) on blood pressure of conscious rats

<table>
<thead>
<tr>
<th>Time</th>
<th>BP</th>
<th>Control (mmHg; Mean ± SEM; N =6)</th>
<th>Centhaquin</th>
<th>Clonidine</th>
<th>Hydralazine</th>
<th>Reserpine</th>
</tr>
</thead>
</table>
|       | Systolic | Diastolic | Mean | Systolic | Diastolic | Mean | Systolic | Diastolic | Mean | Systolic | Diastolic | Mean | Systolic | Diastolic | Mean | Systolic | Diastolic | Mean | Systolic | Diastolic | Mean | Systolic | Diastolic | Mean | Systolic | Diastolic | Mean | Systolic | Diastolic | Mean | Systolic | Diastolic | Mean | Systolic | Diastolic | Mean | Systolic | Diastolic | Mean | Systolic | Diastolic | Mean | Systolic | Diastolic | Mean | Systolic | Diastolic | Mean | Systolic | Diastolic | Mean | Systolic | Diastolic | Mean | Systolic | Diastolic | Mean | Systolic | Diastolic | Mean | Systolic | Diastolic | Mean | Systolic | Diastolic | Mean | Systolic | Diastolic | Mean | Systolic | Diastolic | Mean | Systolic | Diastolic | Mean | Systolic | Diastolic | Mean | Systolic | Diastolic | Mean | Systolic | Diastolic | Mean | Systolic | Diastolic | Mean | Systolic | Diastolic | Mean | Systolic | Diastolic | Mean | Systolic | Diastolic | Mean | Systolic | Diastolic | Mean | Systolic | Diastolic | Mean | Systolic | Diastolic | Mean | Systolic | Diastolic | Mean | Systolic | Diastolic | Mean | Systolic | Diastolic | Mean | Systolic | Diastolic | Mean | Systolic | Diastolic | Mean | Systolic | Diastolic | Mean | Systolic | Diastolic | Mean | Systolic | Diastolic | Mean | Systolic | Diastolic | Mean | Systolic | Diastolic | Mean | Systolic | Diastolic | Mean | Systolic | Diastolic | Mean | Systolic | Diastolic | Mean | Systolic | Diastolic | Mean | Systolic | Diastolic | Mean | Systolic | Diastolic | Mean | Systolic | Diastolic | Mean | Systolic | Diastolic | Mean | Systolic | Diastolic | Mean | Systolic | Diastolic | Mean | Systolic | Diastolic | Mean | Systolic | Diastolic | Mean | Systolic | Diastolic | Mean | Systolic | Diastolic | Mean | Systolic | Diastolic | Mean | Systolic | Diastolic | Mean | Systolic | Diastolic | Mean | Systolic | Diastolic | Mean | Systolic | Diastolic | Mean | Systolic | Diastolic | Mean | Systolic | Diastolic | Mean | Systolic | Diastolic | Mean | Systolic | Diastolic | Mean | Systolic | Diastolic | Mean | Systolic | Diastolic | Mean | Systolic | Diastolic | Mean | Systolic | Diastolic | Mean | Systolic | Diastolic | Mean | Systolic | Diastolic | Mean | Systolic | Diastolic | Mean | Systolic | Diastolic | Mean | Systolic | Diastolic | Mean | Systolic | Diastolic | Mean | Systolic | Diastolic | Mean | Systolic | Diastolic | Mean | Systolic | Diastolic | Mean | Systolic | Diastolic | Mean | Systolic | Diastolic | Mean | Systolic | Diastolic | Mean | Systolic | Diastolic | Mean | Systolic | Diastolic | Mean | Systolic | Diastolic | Mean | Systolic | Diastolic | Mean | Systolic | Diastolic | Mean | Systolic | Diastolic | Mean | Systolic | Diastolic | Mean | Systolic | Diastolic | Mean | Systolic | Diastolic | Mean | Systolic | Diastolic | Mean | Systolic | Diastolic | Mean | Systolic | Diastolic | Mean | Systolic | Diastolic | Mean | Systolic | Diastolic | Mean | Systolic | Diastolic | Mean | Systolic | Diastolic | Mean | Systolic | Diastolic | Mean | Systolic | Diastolic | Mean | Systolic | Diastolic | Mean | Systolic | Diastolic | Mean | Systolic | Diastolic | Mean | Systolic | Diastolic | Mean | Systolic | Diastolic | Mean | Systolic | Diastolic | Mean | Systolic | Diastolic | Mean | Systolic | Diastolic | Mean | Systolic | Diastolic | Mean | Systolic | Diastolic | Mean | Systolic | Diastolic | Mean | Systolic | Diastolic | Mean | Systolic | Diastolic | Mean | Systolic | Diastolic | Mean | Systolic | Diastolic | Mean | Systolic | Diastolic | Mean | Systolic | Diastolic | Mean | Systolic | Diastolic | Mean | Systolic | Diastolic | Mean | Systolic | Diastolic | Mean | Systolic | Diastolic | Mean | Systolic | Diastolic | Mean | Systolic | Diastolic | Mean | Systolic | Diastolic | Mean | Systolic | Diastolic | Mean | Systolic | Diastolic | Mean | Systolic | Diastolic | Mean | Systolic | Diastolic | Mean | Systolic | Diastolic | Mean | Systolic | Diastolic | Mean | Systolic | Diastolic | Mean | Systolic | Diastolic | Mean | Systolic | Diastolic | Mean | Systolic | Diastolic | Mean | Systolic | Diastolic | Mean | Systolic | Diastolic | Mean | Systolic | Diastolic | Mean | Systolic | Diastolic | Mean | Systolic | Diastolic | Mean | Systolic | Diastolic | Mean | Systolic | Diastolic | Mean | Systolic | Diastolic | Mean | Systolic | Diastolic | Mean | Systolic | Diastolic | Mean | Systolic | Diastolic | Mean | Systolic | Diastolic | Mean | Systolic | Diastolic | Mean | Systolic | Diastolic | Mean | Systolic | Diastolic | Mean | Systolic | Diastolic | Mean | Systolic | Diastolic | Mean | Systolic | Diastolic | Mean | Systolic | Diastolic | Mean | Systolic | Diastolic | Mean | Systolic | Diastolic | Mean | Systolic | Diastolic | Mean | Systolic | Diastolic | Mean | Systolic | Diastolic | Mean | Systolic | Diastolic | Mean | Systolic | Diastolic | Mean | Systolic | Diastolic | Mean | Systolic | Diastolic | Mean | Systolic | Diastolic | Mean | Systolic | Diastolic | Mean | Systolic | Diastolic | Mean | Systolic | Diastolic | Mean | Systolic | Diastolic | Mean | Systolic | Diastolic | Mean | Systolic | Diastolic | Mean | Systolic | Diastolic | Mean | Systolic | Diastolic | Mean | Systolic | Diastolic | Mean | Systolic | Diastolic | Mean | Systolic | Diastolic | Mean | Systolic | Diastolic | Mean | Systolic | Diastolic | Mean | Systolic | Diastolic | Mean | Systolic | Diastolic | Mean | Systolic | Diastolic | Mean | Systolic | Diastolic | Mean | Systolic | Diastolic | Mean | Systolic | Diastolic | Mean | Systolic | Diastolic | Mean | Systolic | Diastolic | Mean | Systolic | Diastolic | Mean | Systolic | Diastolic | Mean | Systolic | Diastolic | Mean | Systolic | Diastolic | Mean | Systolic | Diastolic | Mean | Systolic | Diastolic | Mean | Systolic | Diastolic | Mean | Systolic | Diastolic | Mean | Systolic | Diastolic | Mean | Systolic | Diastolic | Mean | Systolic | Diastolic | Mean | Systolic | Diastolic | Mean | Systolic | Diastolic | Mean | Systolic | Diastolic | Mean | Systolic | Diastolic | Mean | Systolic | Diastolic | Mean | Systolic | Diastolic | Mean | Systolic | Diastolic | Mean | Systolic | Diastolic | Mean | Systolic | Diastolic | Mean | Systolic | Diastolic | Mean | Systolic | Diastolic | Mean | Systolic | Diastolic | Mean | Systolic | Diastolic | Mean | Systolic | Diastolic | Mean | Systolic | Diastolic | Mean | Systolic | Diastolic | Mean | Systolic | Diastolic | Mean | Systolic | Diastolic | Mean | Systolic | Diastolic | Mean | Systolic | Diastolic | Mean | Systolic | Diastolic | Mean | Systolic | Diastolic | Mean | Systolic | Diastolic | Mean | Systolic | Diastolic | Mean | Systolic | Diastolic | Mean | Systolic | Diastolic | Mean | Systolic | Diastolic | Mean | Systolic | Diastolic | Mean | Systolic | Diastolic | Mean | Systolic | Diastolic | Mean | Systolic | Diastolic | Mean | Systolic | Diastolic | Mean | Systolic | Diastolic | Mean | Systolic | Diastolic | Mean | Systolic | Diastolic | Mean | Systolic | Diastolic | Mean | Systolic | Diastolic | Mean | Systolic | Diastolic | Mean | Systolic | Diastolic | Mean | Systolic | Diastolic | Mean | Systolic | Diastolic | Mean | Systolic | Diastolic | Mean | Systolic | Diastolic | Mean | Systolic | Diastolic | Mean | Systolic | Diastolic | Mean | Systolic | Diastolic | Mean | Systolic | Diastolic | Mean | Systolic | Diastolic | Mean | Systolic | Diastolic | Mean | Systolic | Diastolic | Mean | Systolic | Diastolic | Mean | Systolic | Diastolic | Mean | Systolic | Diastolic | Mean | Systolic | Diastolic | Mean | Systolic | Diastolic | Mean | Systolic | Diastolic | Mean | Systolic | Diastolic | Mean | Systolic | Diastico
Table 27: Effect of intraventricular administration of acetylcholine (10 µg) on blood pressure of conscious rats

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>BP</th>
<th>Control (mmHg; Mean ± SEM; N =6)</th>
<th>Centhaquin</th>
<th>Clonidine</th>
<th>Hydralazine</th>
<th>Reserpine</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>Systolic</td>
<td>139.9 ± 3.15</td>
<td>131.68 ± 2.21</td>
<td>135.08 ± 0.71</td>
<td>140.68 ± 1.01</td>
<td>138.78 ± 1.87</td>
</tr>
<tr>
<td>Diastolic</td>
<td>119.3 ± 1.42</td>
<td>107.08 ± 0.93</td>
<td>106.88 ± 0.95</td>
<td>106.98 ± 1.60</td>
<td>104.98 ± 1.40</td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>126.2 ± 1.90</td>
<td>115.29 ± 0.89</td>
<td>118.28 ± 1.52</td>
<td>116.18 ± 1.12</td>
<td>116.28 ± 0.76</td>
<td></td>
</tr>
<tr>
<td>30</td>
<td>Systolic</td>
<td>130.0a ± 1.44</td>
<td>130.4 ± 1.60</td>
<td>127.5a ± 1.21</td>
<td>138.78 ± 1.87</td>
<td>130.0a ± 1.44</td>
</tr>
<tr>
<td>Diastolic</td>
<td>107.4a ± 5.56</td>
<td>104.9 ± 1.43</td>
<td>105.0 ± 1.17</td>
<td>104.9 ± 1.40</td>
<td>107.4 ± 5.56</td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>114.9a ± 4.02</td>
<td>112.2a ± 1.21</td>
<td>112.5a ± 5.20</td>
<td>116.2 ± 0.76</td>
<td>114.9 ± 4.02</td>
<td></td>
</tr>
<tr>
<td>60</td>
<td>Systolic</td>
<td>127.5a ± 2.50</td>
<td>131.7 ± 1.47</td>
<td>123.7a ± 1.11</td>
<td>136.28 ± 2.11</td>
<td>132.6a ± 1.11</td>
</tr>
<tr>
<td>Diastolic</td>
<td>106.7a ± 4.49</td>
<td>103.1 ± 1.46</td>
<td>95.6a ± 1.52</td>
<td>109.2 ± 3.81</td>
<td>111.0 ± 0.81</td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>113.7a ± 3.81</td>
<td>112.2 ± 1.24</td>
<td>112.5a ± 1.29</td>
<td>118.2 ± 1.21</td>
<td>116.2a ± 1.02</td>
<td></td>
</tr>
<tr>
<td>120</td>
<td>Systolic</td>
<td>132.4a ± 0.73</td>
<td>131.0 ± 1.23</td>
<td>135.0 ± 0.71</td>
<td>135.0 ± 1.54</td>
<td>133.7a ± 0.37</td>
</tr>
<tr>
<td>Diastolic</td>
<td>113.7 ± 2.19</td>
<td>106.2 ± 0.84</td>
<td>94.8a ± 0.55</td>
<td>106.88 ± 1.42</td>
<td>111.1a ± 0.52</td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>119.9a ± 1.25</td>
<td>114.6 ± 1.73</td>
<td>108.2a ± 1.19</td>
<td>116.2 ± 2.19</td>
<td>118.7 ± 1.31</td>
<td></td>
</tr>
<tr>
<td>180</td>
<td>Systolic</td>
<td>133.7 ± 0.73</td>
<td>131.6 ± 1.98</td>
<td>128.0a ± 2.35</td>
<td>134.0a ± 2.33</td>
<td>134.9 ± 1.52</td>
</tr>
<tr>
<td>Diastolic</td>
<td>111.1a ± 2.52</td>
<td>105.0 ± 3.81</td>
<td>89.4a ± 2.05</td>
<td>105.3 ± 2.61</td>
<td>110.5a ± 0.27</td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>118.7a ± 1.44</td>
<td>115.2 ± 1.93</td>
<td>102.3a ± 0.93</td>
<td>115.9 ± 2.01</td>
<td>118.7 ± 0.91</td>
<td></td>
</tr>
<tr>
<td>240</td>
<td>Systolic</td>
<td>132.6a ± 1.34</td>
<td>131.8 ± 4.11</td>
<td>125.7a ± 1.60</td>
<td>134.0a ± 2.50</td>
<td>132.6a ± 1.11</td>
</tr>
<tr>
<td>Diastolic</td>
<td>111.0a ± 2.33</td>
<td>107.0 ± 1.13</td>
<td>94.8a ± 1.75</td>
<td>105.8 ± 1.18</td>
<td>111.0 ± 0.81</td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>116.2a ± 2.15</td>
<td>115.2 ± 1.89</td>
<td>105.1a ± 1.32</td>
<td>115.9 ± 1.46</td>
<td>116.2 ± 1.02</td>
<td></td>
</tr>
<tr>
<td>300</td>
<td>Systolic</td>
<td>132.6a ± 1.34</td>
<td>132.9 ± 2.70</td>
<td>135.1 ± 3.15</td>
<td>133.0a ± 1.01</td>
<td>130.0a ± 0.48</td>
</tr>
<tr>
<td>Diastolic</td>
<td>111.0a ± 2.33</td>
<td>108.0 ± 1.71</td>
<td>101.8a ± 1.14</td>
<td>104.0 ± 0.99</td>
<td>107.4 ± 1.66</td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>116.2a ± 2.15</td>
<td>116.9 ± 2.00</td>
<td>113.0a ± 1.40</td>
<td>114.0 ± 1.10</td>
<td>114.9 ± 1.02</td>
<td></td>
</tr>
</tbody>
</table>

α = p < 0.05 compared to the basal blood pressure (0 min)
β = p < 0.05 compared to the control group
Table 28: Effect of intraventricular administration of 5-hydroxytryptamine (100 μg) on the blood pressure of conscious rats

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>BP</th>
<th>Control (mmHg; Mean ± SEM; N = 6)</th>
<th>Centhaquin</th>
<th>Clonidine</th>
<th>Hydralazine</th>
<th>Reserpine</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>Systolic</td>
<td>144.3 ± 2.23</td>
<td>136.18 ± 1.44</td>
<td>140.6 ± 1.01</td>
<td>126.78 ± 1.53</td>
<td>138.7 ± 1.87</td>
</tr>
<tr>
<td></td>
<td>Diastolic</td>
<td>119.1 ± 2.75</td>
<td>102.08 ± 4.03</td>
<td>106.98 ± 1.60</td>
<td>112.9 ± 1.00</td>
<td>104.98 ± 1.40</td>
</tr>
<tr>
<td></td>
<td>Mean</td>
<td>127.4 ± 2.53</td>
<td>113.48 ± 3.44</td>
<td>118.18 ± 1.12</td>
<td>117.78 ± 1.00</td>
<td>116.28 ± 0.76</td>
</tr>
<tr>
<td>30</td>
<td>Systolic</td>
<td>146.9 ± 1.00</td>
<td>130.88 ± 2.59</td>
<td>134.9 ± 0.76</td>
<td>137.2 ± 1.13</td>
<td>144.3 ± 1.63</td>
</tr>
<tr>
<td></td>
<td>Diastolic</td>
<td>122.7 ± 2.89</td>
<td>93.68 ± 3.37</td>
<td>98.5 ± 1.13</td>
<td>119.1 ± 0.97</td>
<td>106.3 ± 2.11</td>
</tr>
<tr>
<td></td>
<td>Mean</td>
<td>124.1 ± 1.93</td>
<td>106.08 ± 3.06</td>
<td>110.6 ± 0.93</td>
<td>125.2 ± 0.85</td>
<td>119.0 ± 0.89</td>
</tr>
<tr>
<td>60</td>
<td>Systolic</td>
<td>133.1 ± 0.54</td>
<td>130.3 ± 0.89</td>
<td>127.4 ± 0.76</td>
<td>143.9 ± 1.36</td>
<td>146.9 ± 0.81</td>
</tr>
<tr>
<td></td>
<td>Diastolic</td>
<td>104.9 ± 1.27</td>
<td>89.4 ± 3.36</td>
<td>91.9 ± 2.33</td>
<td>116.8 ± 1.22</td>
<td>120.4 ± 0.41</td>
</tr>
<tr>
<td></td>
<td>Mean</td>
<td>113.4 ± 2.71</td>
<td>103.1 ± 3.06</td>
<td>103.7 ± 1.72</td>
<td>125.9 ± 0.67</td>
<td>128.9 ± 0.40</td>
</tr>
<tr>
<td>120</td>
<td>Systolic</td>
<td>133.1 ± 0.58</td>
<td>130.3 ± 0.89</td>
<td>122.8 ± 1.40</td>
<td>148.5 ± 0.67</td>
<td>146.9 ± 0.81</td>
</tr>
<tr>
<td></td>
<td>Diastolic</td>
<td>103.5 ± 1.27</td>
<td>89.4 ± 3.36</td>
<td>89.1 ± 2.33</td>
<td>114.6 ± 1.31</td>
<td>120.4 ± 0.41</td>
</tr>
<tr>
<td></td>
<td>Mean</td>
<td>113.4 ± 2.71</td>
<td>103.0 ± 3.06</td>
<td>100.3 ± 1.72</td>
<td>125.9 ± 0.67</td>
<td>128.9 ± 0.40</td>
</tr>
<tr>
<td>180</td>
<td>Systolic</td>
<td>130.9 ± 0.46</td>
<td>126.5 ± 2.71</td>
<td>119.0 ± 1.21</td>
<td>146.8 ± 0.62</td>
<td>150.0 ± 0.40</td>
</tr>
<tr>
<td></td>
<td>Diastolic</td>
<td>104.5 ± 1.58</td>
<td>88.9 ± 3.82</td>
<td>85.4 ± 1.98</td>
<td>120.4 ± 0.53</td>
<td>121.8 ± 0.40</td>
</tr>
<tr>
<td></td>
<td>Mean</td>
<td>113.5 ± 1.43</td>
<td>101.5 ± 3.17</td>
<td>96.6 ± 1.44</td>
<td>128.9 ± 0.53</td>
<td>131.0 ± 0.21</td>
</tr>
<tr>
<td>240</td>
<td>Systolic</td>
<td>131.4 ± 1.43</td>
<td>132.1 ± 1.24</td>
<td>124.6 ± 0.47</td>
<td>150.0 ± 0.98</td>
<td>150.0 ± 0.66</td>
</tr>
<tr>
<td></td>
<td>Diastolic</td>
<td>106.6 ± 1.24</td>
<td>94.18 ± 0.47</td>
<td>95.1 ± 2.08</td>
<td>121.8 ± 0.51</td>
<td>127.5 ± 0.71</td>
</tr>
<tr>
<td></td>
<td>Mean</td>
<td>114.9 ± 1.50</td>
<td>106.88 ± 1.63</td>
<td>105.0 ± 3.14</td>
<td>131.0 ± 1.22</td>
<td>135.0 ± 2.11</td>
</tr>
<tr>
<td>300</td>
<td>Systolic</td>
<td>141.5 ± 2.26</td>
<td>127.4 ± 2.46</td>
<td>131.2 ± 2.28</td>
<td>138.0 ± 0.99</td>
<td>138.0 ± 1.11</td>
</tr>
<tr>
<td></td>
<td>Diastolic</td>
<td>110.3 ± 2.31</td>
<td>118.3 ± 3.00</td>
<td>103.2 ± 2.29</td>
<td>117.6 ± 0.95</td>
<td>116.8 ± 0.88</td>
</tr>
<tr>
<td></td>
<td>Mean</td>
<td>120.7 ± 2.26</td>
<td>115.3 ± 3.00</td>
<td>112.5 ± 2.29</td>
<td>124.4 ± 0.95</td>
<td>125.9 ± 0.88</td>
</tr>
</tbody>
</table>

α = p < 0.05 compared to the basal blood pressure (0 min)
β = p < 0.05 compared to the control group
EFFECT OF 5-HYDROXYTRYPTAMINE (100 µg, iv)

MEAN BLOOD PRESSURE (mmHg)

Mean ± SEM (N=6)
Figure S8

EFFECT OF NORADRENALINE (10 nM, ICy)

TIME (min)

TEMPERATURE (°C)

Mean ± SEM (N=6)

RESERPINE

HYDRAZLINE

CLONIDINE

CENTHAQUIN

CONTROL

0 100 200 300

40 43 46
Figure 59

Effect of Dopamine (50 μg, iv)

Time (min)

TEMPERATURE (°C)

Mean ± SEM (N=6)

Reserpine □——□
Hydralazine ▼——▼
Clonidine ▼——▼
Centhaquin ●——●
Control ○——○
Figure 60

EFFECT OF ACETECHOLINE (10 μg, iv)

TIME (min)

TEMPERATURE (°C)

Mean ± SEM (N=6)

- Reserpine
- Hydralazine
- Clonidine
- Centraurin
- Control
Figure 61

Effect of 5-Hydroxytryptamine (100 µg, iv)

TIME (min)

TEMPERATURE (°C)

Mean ± SEM (N=6)

RESERPINE □ — □
HYDRAZINE ▼ — ▼
CLONIDINE ▼ — ▼
CENTHAQUIN □ — □
CONTROL □ — □
4.4 EFFECT OF CHRONIC ADMINISTRATION OF ANTIHYPERTENSIVE DRUGS ON BLOOD PRESSURE RESPONSE TO INTRACEREBROVENTRICULAR INJECTION OF 5HT:

In the centhaquin, clonidine, hydralazine and reserpine treated rats, lower basal blood pressure was observed as compared to normal rat. Intracerebroventricular injection of 5HT produced hypotension in normal rat and centhaquin and clonidine treated rats, while hypertension was produced in hydralazine and reserpine treated rats. Treatment with centhaquin and clonidine produced hypotension, but treatment with hydralazine and reserpine produced hypertension after intracerebroventricular 5HT injections (Table 28; Figure 57).

5.1 EFFECT OF CHRONIC ADMINISTRATION OF ANTIHYPERTENSIVE DRUGS ON COLONIC TEMPERATURE (CT) RESPONSE TO INTRACEREBROVENTRICULAR INJECTION OF NORADRENALINE IN CONSCIOUS RATS:

In the control rats the basal CT was 37.6 ± 0.18°C. In the centhaquin treated rats the basal CT was significantly increased (p < 0.05) as compared to control; in clonidine treated rats the CT was 39.1 ± 0.03°C; in the hydralazine treated rats the basal CT was 38.4 ± 0.02°C and in reserpine treated rats the basal CT was 38.8 ± 0.05°C. An insignificant decrease of 0.33 ± 0.06°C in CT was observed at 140 mins of noradrenaline administration in the lateral cerebral ventricles. Centhaquin treated rats produced a significant decrease of 1.06°C in CT at 240 mins of noradrenaline administration. Similarly clonidine produced a significant decrease of 1.52°C in CT, at
260 mins after icv administration of noradrenaline. Treatment with hydralazine and reserpine increased the CT by 1.17°C and 1.06°C respectively at 320 mins after noradrenaline administration (Table 29; Figure 58).

5.2 EFFECT OF CHRONIC ADMINISTRATION OF ANTIHYPERTENSIVE DRUGS ON COLONIC TEMPERATURE (CT) RESPONSE TO INTRACEREBROVENTRICULAR INJECTION OF DOPAMINE IN CONSCIOUS RATS:

In the control rats the basal rectal temperature was 37.4 ± 0.11°C. Chronic treatment with antihypertensive drugs produced a significant (p < 0.05) increase in CT. Intracerebroventricular administration of dopamine in control rats produced a maximum decrease of 0.3°C at 320 mins. In rats treated with centhaquin a maximum increase (1.26°C) (p < 0.05) in colonic temperature was observed at 60 mins after icv administration of dopamine. In the hydralazine treated rats the maximum increase (1.11°C)(p < 0.05) occurred at 280 mins after the intracerebroventricular administration of dopamine. In reserpine treated group the maximum decrease was 0.52°C at 140 mins after the intracerebroventricular administration of dopamine (Table 30; Figure 59).

5.3 EFFECT OF CHRONIC ADMINISTRATION OF ANTIHYPERTENSIVE DRUGS ON COLONIC TEMPERATURE (CT) RESPONSE TO INTRACEREBROVENTRICULAR INJECTION OF ACETYLCHOLINE IN CONSCIOUS RATS:

In the control rats the basal CT was 37.6 ± 0.08°C. Treatment with antihypertensive drugs (centhaquin, clonidine, hydralazine and reserpine) produced a significant (p < 0.05) increase in basal CT. Intracerebroventricular injection of
Table 29: Effect of intraventricular administration of noradrenaline (10 µg) on colonic temperature of conscious rats

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Control (°C; Mean ± SEM; N = 6)</th>
<th>Centhaquin</th>
<th>Clonidine</th>
<th>Hydralazine</th>
<th>Reserpine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Basal(0)</td>
<td>37.6±0.18</td>
<td>38.3±0.128</td>
<td>38.6±0.038</td>
<td>38.4±0.028</td>
<td>38.8±0.058</td>
</tr>
<tr>
<td>20</td>
<td>37.9±0.22</td>
<td>38.3±0.10</td>
<td>38.6±0.08</td>
<td>38.5±0.09</td>
<td>39.2±0.15*</td>
</tr>
<tr>
<td>40</td>
<td>37.7±0.22</td>
<td>38.2±0.22</td>
<td>38.5±0.06</td>
<td>38.6±0.20</td>
<td>39.2±0.11*</td>
</tr>
<tr>
<td>60</td>
<td>37.8±0.24</td>
<td>38.0±0.12</td>
<td>38.4±0.06*</td>
<td>38.4±0.12</td>
<td>39.2±0.13*</td>
</tr>
<tr>
<td>80</td>
<td>37.6±0.09</td>
<td>38.0±0.15</td>
<td>38.3±0.06*</td>
<td>38.5±0.12</td>
<td>39.2±0.13*</td>
</tr>
<tr>
<td>100</td>
<td>37.8±0.22</td>
<td>37.9±0.15</td>
<td>38.3±0.06*</td>
<td>38.6±0.12</td>
<td>39.3±0.15*</td>
</tr>
<tr>
<td>120</td>
<td>37.7±0.22</td>
<td>37.9±0.15</td>
<td>38.2±0.06*</td>
<td>38.7±0.12</td>
<td>39.4±0.15*</td>
</tr>
<tr>
<td>140</td>
<td>37.6±0.24</td>
<td>37.6±0.14*</td>
<td>38.2±0.06*</td>
<td>38.8±0.10*</td>
<td>39.4±0.20*</td>
</tr>
<tr>
<td>160</td>
<td>37.6±0.28</td>
<td>37.6±0.14*</td>
<td>38.0±0.08*</td>
<td>38.8±0.11*</td>
<td>39.4±0.22*</td>
</tr>
<tr>
<td>180</td>
<td>37.7±0.24</td>
<td>37.6±0.18*</td>
<td>37.9±0.06*</td>
<td>38.8±0.14*</td>
<td>39.4±0.25*</td>
</tr>
<tr>
<td>200</td>
<td>37.6±0.28</td>
<td>37.7±0.18*</td>
<td>37.8±0.06*</td>
<td>38.9±0.25</td>
<td>39.4±0.20*</td>
</tr>
<tr>
<td>220</td>
<td>37.7±0.33</td>
<td>37.5±0.22*</td>
<td>36.6±0.08*</td>
<td>38.8±0.28</td>
<td>39.5±0.20*</td>
</tr>
<tr>
<td>240</td>
<td>37.6±0.30</td>
<td>37.3±0.24*</td>
<td>36.3±0.18*</td>
<td>38.6±0.20</td>
<td>39.5±0.26*</td>
</tr>
<tr>
<td>260</td>
<td>37.6±0.18</td>
<td>37.5±0.19*</td>
<td>36.1±0.22*</td>
<td>38.8±0.25</td>
<td>39.5±0.26*</td>
</tr>
<tr>
<td>280</td>
<td>37.9±0.18</td>
<td>37.5±0.19*</td>
<td>36.0±0.23*</td>
<td>38.9±0.20*</td>
<td>39.4±0.18*</td>
</tr>
<tr>
<td>300</td>
<td>37.9±0.18</td>
<td>37.6±0.22*</td>
<td>36.3±0.22*</td>
<td>39.1±0.23*</td>
<td>39.4±0.11*</td>
</tr>
<tr>
<td>320</td>
<td>38.0±0.18</td>
<td>37.6±0.22*</td>
<td>36.4±0.28*</td>
<td>39.1±0.23*</td>
<td>39.4±0.10*</td>
</tr>
</tbody>
</table>

* = p < 0.05 compared to the basal temperature
β = p < 0.05 compared to the control group
Table 30: Effect of intraventricular administration of dopamine (50 µg) on colonic temperature of conscious rats

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Control</th>
<th>Centhaquin (°C; Mean ± SEM; N =6)</th>
<th>Clonidine</th>
<th>Hydralazine</th>
<th>Reserpine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Basal(0)</td>
<td>37.6±0.06</td>
<td>38.2±0.08</td>
<td>38.4±0.09</td>
<td>38.5±0.06</td>
<td>38.8±0.06</td>
</tr>
<tr>
<td>20</td>
<td>37.6±0.06</td>
<td>38.5±0.12</td>
<td>38.6±0.12</td>
<td>38.7±0.03*</td>
<td>38.8±0.20</td>
</tr>
<tr>
<td>40</td>
<td>37.6±0.06</td>
<td>38.9±0.12*</td>
<td>38.6±0.09</td>
<td>39.0±0.16*</td>
<td>38.8±0.20</td>
</tr>
<tr>
<td>60</td>
<td>37.4±0.06</td>
<td>39.0±0.10*</td>
<td>38.6±0.09</td>
<td>38.9±0.14*</td>
<td>38.7±0.20</td>
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<tr>
<td>80</td>
<td>37.4±0.06</td>
<td>39.0±0.08*</td>
<td>38.5±0.03</td>
<td>38.9±0.10*</td>
<td>38.5±0.25</td>
</tr>
<tr>
<td>100</td>
<td>37.5±0.12</td>
<td>39.1±0.08*</td>
<td>38.4±0.03</td>
<td>38.9±0.10*</td>
<td>38.6±0.22</td>
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<tr>
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<td>37.4±0.12</td>
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<td>38.4±0.03</td>
<td>38.9±0.10*</td>
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<tr>
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<td>38.9±0.11*</td>
<td>38.4±0.09</td>
</tr>
<tr>
<td>160</td>
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<td>38.4±0.09</td>
<td>39.2±0.10*</td>
<td>38.5±0.07</td>
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<td>39.3±0.17*</td>
<td>38.4±0.09</td>
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<td>39.5±0.08*</td>
<td>38.3±0.17</td>
</tr>
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<td>38.3±0.10</td>
<td>39.4±0.08*</td>
<td>38.4±0.25</td>
</tr>
<tr>
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<td>38.2±0.09</td>
<td>39.3±0.10*</td>
<td>38.4±0.25</td>
</tr>
<tr>
<td>260</td>
<td>37.1±0.24</td>
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<td>38.1±0.18</td>
<td>39.3±0.14*</td>
<td>38.4±0.33</td>
</tr>
<tr>
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<td>38.0±0.15</td>
<td>39.2±0.22*</td>
<td>38.3±0.37</td>
</tr>
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<td>37.9±0.20</td>
<td>39.1±0.10*</td>
<td>38.3±0.44</td>
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<td>39.1±0.10*</td>
<td>38.3±0.44</td>
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</table>

* = p < 0.05 compared to the basal temperature
β = p < 0.05 compared to the control group
Table 31: Effect of intraventricular administration of acetylcholine (10 µg) on colonic temperature of conscious rats

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Control</th>
<th>Centhaquin</th>
<th>Clonidine</th>
<th>Hydralazine</th>
<th>Reserpine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Basal(O)</td>
<td>37.6±0.08</td>
<td>38.6±0.01</td>
<td>39.0±0.08</td>
<td>38.1±0.06</td>
<td>38.0±0.06</td>
</tr>
<tr>
<td>20</td>
<td>37.6±0.07</td>
<td>38.6±0.01</td>
<td>39.3±0.05*</td>
<td>38.1±0.03</td>
<td>38.0±0.04</td>
</tr>
<tr>
<td>40</td>
<td>37.4±0.07</td>
<td>38.5±0.02*</td>
<td>39.4±0.05*</td>
<td>38.1±0.04</td>
<td>38.0±0.03</td>
</tr>
<tr>
<td>60</td>
<td>37.2±0.08*</td>
<td>38.4±0.05*</td>
<td>39.4±0.05*</td>
<td>38.1±0.03</td>
<td>37.9±0.03</td>
</tr>
<tr>
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<td>37.2±0.07*</td>
<td>38.3±0.05*</td>
<td>39.5±0.06*</td>
<td>38.1±0.03</td>
<td>37.9±0.05</td>
</tr>
<tr>
<td>100</td>
<td>37.1±0.13*</td>
<td>38.3±0.04*</td>
<td>39.3±0.06*</td>
<td>38.1±0.03</td>
<td>37.9±0.07</td>
</tr>
<tr>
<td>120</td>
<td>36.9±0.12*</td>
<td>38.2±0.05*</td>
<td>39.3±0.04*</td>
<td>38.1±0.07</td>
<td>37.9±0.02</td>
</tr>
<tr>
<td>140</td>
<td>36.8±0.12*</td>
<td>38.1±0.05*</td>
<td>39.2±0.05</td>
<td>38.2±0.06</td>
<td>37.8±0.03</td>
</tr>
<tr>
<td>160</td>
<td>36.8±0.13*</td>
<td>38.1±0.06*</td>
<td>39.1±0.04</td>
<td>38.2±0.08</td>
<td>37.8±0.02*</td>
</tr>
<tr>
<td>180</td>
<td>36.8±0.17*</td>
<td>38.0±0.06*</td>
<td>39.0±0.04</td>
<td>38.2±0.03</td>
<td>37.9±0.08</td>
</tr>
<tr>
<td>200</td>
<td>36.8±0.17*</td>
<td>38.0±0.06*</td>
<td>39.1±0.06</td>
<td>38.2±0.03</td>
<td>37.8±0.02*</td>
</tr>
<tr>
<td>220</td>
<td>36.8±0.20*</td>
<td>37.9±0.03*</td>
<td>38.9±0.06</td>
<td>38.2±0.02</td>
<td>37.9±0.02</td>
</tr>
<tr>
<td>240</td>
<td>36.6±0.18*</td>
<td>37.8±0.03*</td>
<td>39.0±0.06</td>
<td>38.2±0.02</td>
<td>37.9±0.03</td>
</tr>
<tr>
<td>260</td>
<td>36.9±0.17</td>
<td>37.5±0.04</td>
<td>39.0±0.08</td>
<td>38.2±0.03</td>
<td>37.9±0.03</td>
</tr>
<tr>
<td>280</td>
<td>36.9±0.16*</td>
<td>37.5±0.06</td>
<td>39.1±0.05</td>
<td>38.1±0.03</td>
<td>37.9±0.03</td>
</tr>
<tr>
<td>300</td>
<td>37.0±0.15*</td>
<td>37.5±0.06</td>
<td>38.9±0.03</td>
<td>38.1±0.02</td>
<td>37.9±0.02</td>
</tr>
<tr>
<td>320</td>
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<td>37.5±0.05</td>
<td>38.9±0.03</td>
<td>38.1±0.02</td>
<td>38.0±0.02</td>
</tr>
</tbody>
</table>

* = p < 0.05 compared to the basal temperature
β = p < 0.05 compared to the control group
Table 32: Effect of intraventricular administration of 5-HT (100 µg) on colonic temperature of conscious rats

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Control</th>
<th>Centhac</th>
<th>Clonidine</th>
<th>Hydralazine</th>
<th>Reserpine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Basal(0)</td>
<td>37.6±0.08</td>
<td>38.0±0.12</td>
<td>38.4±0.10</td>
<td>38.1±0.06</td>
<td>38.2±0.03</td>
</tr>
<tr>
<td>20</td>
<td>37.6±0.10</td>
<td>38.5±0.07*</td>
<td>38.6±0.12</td>
<td>38.1±0.06</td>
<td>38.2±0.03*</td>
</tr>
<tr>
<td>40</td>
<td>37.6±0.12</td>
<td>38.9±0.07*</td>
<td>38.7±0.12</td>
<td>38.1±0.08</td>
<td>38.0±0.03*</td>
</tr>
<tr>
<td>60</td>
<td>37.4±0.10</td>
<td>39.2±0.11*</td>
<td>38.8±0.11*</td>
<td>38.4±0.08</td>
<td>38.0±0.03*</td>
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<tr>
<td>80</td>
<td>37.2±0.09*</td>
<td>39.3±0.11*</td>
<td>38.6±0.12</td>
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</tr>
<tr>
<td>100</td>
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<td>39.3±0.11*</td>
<td>38.6±0.11</td>
<td>38.5±0.09</td>
<td>37.8±0.04*</td>
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</tr>
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</tr>
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<tr>
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<tr>
<td>280</td>
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<td>38.8±0.08*</td>
<td>38.6±0.06</td>
<td>38.3±0.04*</td>
</tr>
</tbody>
</table>

* = p < 0.05 compared to the basal temperature
β = p < 0.05 compared to the control group
acetylcholine in the control rats caused a maximum decrease in CT of 1.10°C (p < 0.05) at 320 mins after intracerebroventricular administration of acetylcholine. In the clonidine treated rats the maximum increase in CT was 0.50°C (p < 0.05) at 80 mins after icv administration of acetylcholine. In the hydralazine treated rats, a maximum increase (0.91°C) in CT was observed at 260 mins. In rats treated with reserpine, the CT increased by 0.93°C (p < 0.05) 260 mins after acetylcholine administration (Table 31; Figure 60).

5.4 EFFECT OF CHRONIC ADMINISTRATION OF ANTIHYPERTENSIVE DRUGS ON COLONIC TEMPERATURE RESPONSE TO INTRACEREBROVENTRICULAR INJECTION OF 5-HT IN CONSCIOUS RATS:

In the control rats the basal CT was 37.6 ± 0.08°C. In antihypertensive drug (centhaquin, clonidine hydralazine and reserpine) treated rats, the CT temperature was 38.0 ± 0.12°C which was significantly (p < 0.05) higher than control. In the control rats the maximum decrease in CT (0.80 ± 0.07°C) following 5-HT administration occurred at 200 mins. In centhaquin treated rats, the rectal temperature increased (2.51°C) at 200 mins after intracerebroventricular injection of 5-HT as compared to control. Treatment with clonidine increased rectal temperature (1.3°C) at 200 mins after icv injections of 5-HT as compared to control. By treatment with hydralazine the rectal temperature increased (1.26°C) at 200 mins after 5-HT administration as compared to control. Treatment with reserpine showed a maximum of (0.6°C) decrease in CT following 5-HT administration in the lateral ventricle (Table 32; Figure 61).
The role of the central nervous system (CNS) in regulating arterial pressure has been known since long time. The areas in the CNS, first described by Wang and Ranson (1939), were divided into vasopressor and vasodepressor areas by Alexander (1946) and have been conventionally described as medullary vasomotor or cardiovascular center. The information on the neuroanatomical organization of central autonomic pathways is very scanty and this has limited our understanding of how the CNS controls the sympathetic activity and the circulation. Much of our knowledge stems from the effects of electrical stimulation and ablation studies of different parts of the brain on blood pressure and heart rate. Neuroanatomical studies performed earlier, relied on the production of lesions with resultant neuronal degeneration. However, with the newer techniques the axonal transport, visualization of some neurotransmitters and their enzymes has enabled the tracing of pathways utilizing a definite transmitter. Till now, not much attention has been given to the status of central neurotransmitter receptors in hypertension, and the effect of antihypertensive drugs on central neurotransmitter receptors.

It was thought that if, antihypertensive drugs, like centhaquin, clonidine (which are centrally acting), hydralazine (which acts through a peripheral mechanism) and reserpine (which has a complex mechanism of action, but mainly peripheral) are administered chronically, they are likely to produce different actions on various central neurotransmitter receptors. Broadly centrally acting antihypertensives will have direct effect on the CNS receptors, while peripherally acting antihypertensives are not likely
to produce any change in central receptors. If peripherally acting antihypertensives do produce alteration in central receptors, it could be due to a secondary (feedback) effect i.e. due to a decreased blood pressure the central receptors are altered. Since medulla and hypothalamus are the main areas of brain involved in blood pressure regulation, alteration of receptor characteristics in these areas will be of direct relevance to regulation of blood pressure. Other brain regions like striatum and cortex are less involved in cardiovascular regulation.

In the present study the effect of chronic administration of centhaquin, clonidine, hydralazine and reserpine on medullary, hypothalamic, striatal and cortical neurotransmitter receptors has been studied.

Radioligand binding technique has been used to determine the differences in the receptor density and affinity between normal rats and those treated with antihypertensive drugs. Since receptor population alters in an inverse manner to changes in neural impulse flow (Morris et al., 1981), it is clear that changes in the radio receptor binding will indirectly reflect the neuronal activity. The drugs investigated in the present study are anti-hypertensive drugs centhaquin, clonidine, hydralazine and reserpine. The effect has been studied on the adrenergic, dopaminergic, cholinergic (muscarinic) and serotonergic receptors. The selection of the drugs has been guided by an effort to ensure that they act by different mechanisms.
Effect of chronic administration of antihypertensive drugs on adrenergic receptors

The density ($B_{\text{max}}$) of the $\alpha$-adrenoceptors in the normal rat brain is fairly constant in all the four areas and the $K_d$ values show high affinity binding sites. In the cortex, the density ($B_{\text{max}}$) and affinity ($K_d$) of $^3$H-dihydroergocryptine binding remained unchanged in the centhaquin treated rats. In clonidine treated rats also the density and affinity remained unchanged in the cerebral cortex. In hydralazine treated rats the density decreased but the affinity remained unchanged. In reserpine treated rats the density decreased but no change occurred in the affinity of the $^3$H-dihydroergocryptine binding. In the caudate of centhaquin and clonidine treated rats, the density and affinity of $^3$H-dihydroergocryptine binding did not change. Treatment with hydralazine and reserpine decreased the density but the affinity of $^3$H-dihydroergocryptine binding did not change. In hypothalamus, the density and affinity of $^3$H-dihydroergocryptine, increased in centhaquin treated rats. Clonidine also increased both the density and affinity. Hydralazine and reserpine treatment decreased the density while the affinity of $^3$H-dihydroergocryptine binding did not change significantly. In medulla of centhaquin and clonidine treated rats, the density increased while the affinity decreased. In hydralazine and reserpine treated rats the density of the $\alpha$-adrenoceptors decreased without changing the affinity of the $^3$H-dihydroergocryptine binding.

Central catecholaminergic neurons and $\alpha$-adrenoceptors are implicated in blood pressure regulation (Axelrod, 1976; Chalmers, 1975; Huchet et al., 1982). There are several reports on the differences between spontaneously hypertensive (SHR) rats and
Wistar-Kyoto (WKY) rats in terms of uptake, content, metabolism and release of catecholamines (Yamori et al., 1970; Nagatsu et al., 1976; Fuxe et al., 1979; Howes et al., 1984). No consistent differences between SHR and WKY rats have been reported in the catecholamine content of the brain nuclei. Noradrenaline content in the hypothalamic nuclei has been found to be lower in age matched SHR as compared to WKY rats (Saavedra et al., 1979). In the brain stem nuclei SHR rats have been found to have greater, lesser or even similar noradrenaline levels as compared to WKY rats (Versteeg et al., 1976; Fuxe et al., 1979; Saavedra et al., 1979; Wijnen et al., 1980). With this background it becomes very difficult to predict the status of α-adrenoceptors in SHR in comparison to WKY rats.

Several reports are available, with conflicting results on the characteristics of α-adrenoceptors in SHR as compared to WKY rats. Yamada et al. (1985) determined the α₁, α₂ and β-adrenoceptors using ³H-WB4101, ³H-yohimbine and ³H-dihydroalprenolol in brain regions of SHR, SHRSP and renal hypertensive rats, and they found a specific increase in α₁-adrenoceptors in hypothalamus of SHR and SHRSP as compared to WKY rats. Parini et al. (1986) found that five weeks of high sodium intake induced a decrease in α₂-adrenoceptor density in cortex and an increase in hypothalamus only in salt-resistant rats as compared to salt-sensitive rats. Further studies by Yamada et al. (1989) show that there is a specific loss of α₂-adrenoceptors in the medulla oblongata of SHR and SHRSP as compared to WKY rats. The increase in hypothalamic α₂ adrenoceptors has been reported in SHR as compared to WKY rats (Morris et al., 1981). Cantor et al.
(1981) reported that the enhanced number of $\alpha_1$-adrenoceptors in hypothalamus of SHR rats is neither a consequence of the increased blood pressure, nor a phenomenon common to all models of hypertension.

No study has yet been performed to determine and compare the effect of repeated administration of centrally and peripherally acting antihypertensive drugs, clonidine and centhaquin increase both the affinity and density of $\alpha$-adrenoceptors in hypothalamus and medulla, while peripherally acting antihypertensive drugs, hydralazine and reserpine decreased the density of $\alpha$-adrenoceptors in all four brain regions. The affinity of clonidine for presynaptic $\alpha$-adrenoceptors is at least ten times higher than its affinity for postsynaptic $\alpha$-adrenoceptors (Starke et al., 1974). It could be possible that repeated administration of clonidine decreases the release of norepinephrine in hypothalamus and medulla by acting on presynaptic receptors. The decreased concentration of norepinephrine in the synapse will result in an increase in the density of post synaptic $\alpha$-adrenoceptors, as observed in the present study. The decrease in affinity of $\alpha$-adrenoceptors in hypothalamus and medulla indicates a decrease in the neural transmission.

The normal rat brain showed a fairly constant density, and high affinity binding of beta receptors in cortex, caudate, hypothalamus and medulla. Centhaquin or clonidine treatment did not affect the density and affinity of $^3$H-dihydroalprenolol binding to $\beta$-adrenoceptors in any of the four brain regions studied. Hydralazine treatment increased the density of these receptors in cortex, striatum and hypothalamus. No change in the affinity was
observed in cortex, striatum and hypothalamus. In medulla, hydralazine treatment decreased the density but no change in affinity of β adrenoceptors was observed. Reserpine treatment increased the density of the ³H-dihydroalprenolol binding in cortex, striatum, hypothalamus and medulla. The affinity was not affected in these brain regions.

Noradrenaline (10 μg, ICV) produced no significant effect on colonic temperature in control saline treated rats. However in clonidine and centhaquin treated rats a significant decrease in colonic temperature was produced. It may be noted that both clonidine and centhaquin are centrally acting drugs. Hydralazine and reserpine treated rats produced no change in colonic temperature upon noradrenaline administration. Similar effects were observed on blood pressure. Noradrenaline (10 μg, ICV) lowered the mean blood pressure in control, hydralazine and reserpine treated rats, while no change was observed in clonidine as well as centhaquin treated rats. A clear cut differentiation between responses of noradrenaline in centrally acting and peripherally acting antihypertensive drugs were obtained. This further strengthens our contention that besides clonidine, centhaquin a newly developed antihypertensive drug acts on central adrenergic receptors. In all probability these receptors are α adrenergic in nature.

It is clear from the results that β adrenoceptors are not involved in the action of centrally acting antihypertensive drugs (centhaquin and clonidine). Only drugs (hydralazine and reserpine) known to produce hypotension through a peripheral mechanism caused alterations in either Bₘₐₓ or Kᵅ values of ³H-dihydroalprenolol.
It is known that adrenergic receptor concentration in tissues is modulated by sympathetic nervous activity (Williams and Lefkowitz, 1978). Increased sympathetic stimulation caused a reduction in rat pineal β adrenoceptors (Kebabian et al., 1975), while central sympathectomy resulted in increase of β adrenoceptors in rat salivary glands (Pointon and Banerjee, 1979). β adrenoceptors reduced significantly in the hearts of rats made hypertensive by renal artery occlusion or DOCA-salt treatment (Woodcock et al., 1979; Woodcock and Johnston, 1980). In hypertensive baboon model a down regulation of myocardial β adrenoceptors was observed (Hurwitz and Rosendorff, 1985). Yamada et al. (1985) did not observe any change in ³H-dihydroalprenolol binding in the brain regions of SHR, SHRS and renal hypertensive rats as compared to age matched WKY rats. Similarly, Morris et al. (1981) could not find any change in ¹²⁵I-iodohydroxybenzilpinodolol binding in hypothalamus, brain stem or cortex of young SHR in comparison to age matched WKY rats. These studies support our finding that central β adrenoceptors are not involved in hypotensive action of centrally acting antihypertensive drugs. In the present study both biochemical and pharmacological evidences have been provided indicating an alteration in central adrenergic receptors. Centrally acting antihypertensive drugs, centhaquin and clonidine, increase the B_max values of α adrenergic receptors. This correlates with the response of noradrenaline administered intracerebroventricularly on blood pressure and temperature.
Effect of chronic administration antihypertensive drugs on dopaminergic receptors

The spontaneously hypertensive rat (SHR) and the related, normotensive, Wistar Kyoto rat (WKY) have been employed extensively in the study of genetically predetermined hypertensive disease (Yamabe et al., 1973). The application of the matched strains as a model of human essential hypertension has been critically debated (McGiff and Quilley, 1981; Trippodo and Frohlich, 1981). It has been reported that SHR exhibit differences in dopaminergic receptor systems in various brain regions as compared to WKY (Bhargava, 1984; Chiu et al., 1982; Le Fur et al., 1981, 1983). In these studies $^3$H-spiperone was used to label $D_2$ receptors. Hypothalamus was the main region where changes were observed. It has been recognized that monoaminergic nerve activity in the hypothalamus is related to regulation of cardiovascular function (Juskevich et al., 1978) and may contribute to the genesis and/or maintenance of experimental genetic hypertension (Trippodo and Frohlich, 1981).

The role of central dopamine receptors in the regulation of blood pressure was shown by Jaszlits et al. (1985). They found that GYKI-32 887 reveals an antihypertensive action, similar to that of the known ergoline derivatives, in conscious SHR, anesthetized normotensive rats and in cats. It exerts its action first of all by stimulation of the central DA receptors and by this it reduces the sympathetic activity. The hypotensive effect cannot be detected after ICV administration, but both the hypotension and bradycardia can be antagonized by sulpiride administered either ICV or IV (Jaszlits et al., 1985).
Studies performed till date do not define cause and effect in the relationship between dopaminergic receptor function and hypertension, they do support a concept that changes in dopaminergic neuronal activity are related to hypertensive disease.

The normal rat brain dopaminergic sites labeled by $^3$H-spiperone in the striatum show high density and affinity. In the other three areas the density and affinity did not vary to a great extent. In the cortex, centhaquin treatment decreased the density while no change occurred in the affinity. Clonidine treatment increased the density but did not change the affinity of the dopaminergic receptors. Hydralazine treatment decreased the density without any change in the affinity of these receptors. Reserpine treatment also decreased the density with no change in the affinity of $^3$H-spiperone binding. In the striatum, no change occurred in the density or the affinity of the centhaquin treated rats. In clonidine treated rats also there was no change in the density or affinity of these receptors. In hydralazine treated rats, the density and affinity of $^3$H-spiperone binding remained unchanged. In reserpine treated rats also the dopaminergic receptors density and affinity remained unchanged. In hypothalamus, no change occurred in the density and affinity of $^3$H-spiperone binding by centhaquin treatment. By clonidine also there was no change in the density or affinity of these receptors. Hydralazine treatment decreased the density while the affinity remained unchanged. By reserpine treatment the density increased, while the affinity remained unchanged to $^3$H-spiperone binding. In medulla centrally acting antihypertensive drugs clonidine and centhaquin did not affect either the density or the affinity of $^3$H-spiperone binding. In hydralazine treated
rats the density decreased and affinity remained unchanged. In reserpine treated rats the density decreased and affinity remained unchanged to \(^3\)H-spiperone.

Dopamine (50 \(\mu\)g, ICV) produced no significant change in either colonic temperature or mean blood pressure in control, clonidine and reserpine treated rats. In hydralazine treated rats a significant decrease in blood pressure and increase in colonic temperature were observed. In centhaquin treated no change in blood pressure occurred, however a significant increase in colonic temperature was observed.

It is clear from the above results which provide both biochemical and pharmacological evidence that central dopaminergic receptors are not involved in blood pressure regulation or in the action of centrally acting antihypertensive drugs.

**Effect of chronic administration of antihypertensive drugs on cholinergic (muscarinic) receptors**

In the normal rat brain the distribution of muscarinic receptor sites in the medulla show low density, whereas, the other three brain areas have higher density. These receptors have high affinity binding sites in all the four areas of brain. In cortex, centhaquin treatment produced no change in the density and affinity of \(^3\)H-quinuclidinyl benzylate binding. Clonidine treatment produced a decrease in the density and affinity of the muscarinic receptors. No change was observed in the density and affinity of the muscarinic receptors by hydralazine treatment. Reserpine treatment did not change density but increased the affinity of \(^3\)H-quinuclidinyl benzylate binding. In caudate, no change was deserved in the density or affinity by centhaquin treatment. By clonidine
treatment also no change occurred in the density and affinity of muscarinic receptors. Hydralazine treatment also did not affect the density or affinity of $^3$H-quinuclidinyl binding. By reserpine treatment the density of the muscarinic receptors decreased but the affinity remained unchanged. In hypothalamus, centhaquin treatment did not change the density and affinity of the $^3$H-quinuclidinyl binding. Clonidine treatment increased the density and affinity of the muscarinic receptors. Hydralazine treatment did not effect the density or affinity of $^3$H-quinuclidinyl benzylate binding. Reserpine also did not change the density or affinity of the muscarinic receptors. In the medulla, centhaquin treatment did not change the density or affinity of $^3$H-quinuclidinyl benzylate binding. Clonidine treatment increased the density and affinity of muscarinic receptors. Hydralazine treatment produced no change in the density and affinity of these receptors. Reserpine treatment also did not change the density or affinity of muscarinic receptors.

Acetylcholine (10 $\mu$g, ICV) produced a decrease in colonic temperature in control rats. Peripherally acting antihypertensive drugs, hydralazine and reserpine treated rats showed no change in colonic temperature. Centrally acting antihypertensive drug, centhaquin treated rats showed a decrease in colonic temperature. However, clonidine treated rats showed a significant increase in colonic temperature in response to acetylcholine. Blood pressure was decreased by acetylcholine (10 $\mu$g, ICV) in control rats as well as in centhaquin, hydralazine and reserpine treated rats. However clonidine treated rats showed a significantly greater decrease in blood pressure in
comparison to other groups of rats. These results indicate that clonidine does act on cholinergic receptors and might be producing some antihypertensive effect through these receptors.

Although it has been reported that SHR exhibit differences in muscarinic (Hershkowitz et al., 1983) and nicotinic (Yamada et al., 1987) receptor systems in brain as compared to WKY. However, the cause and effect relationship between receptor changes and blood pressure have not been defined.

Our results indicate that cholinergic (muscarinic) receptors are involved in the action of centrally acting antihypertensive drug, clonidine. Repeated administration of clonidine produced an increase in the $B_{max}$ value of cholinergic (muscarinic) receptors as well as pharmacological evidences of change in blood pressure and temperature responses to centrally administered acetylcholine. Srimal et al. (1977) suggested that intact cholinergic link in the brain stem is essential for the hypotensive action of clonidine. Criscione et al. (1983) have proposed that cholinergic mechanism in the NTS tends tonically to lower arterial pressure after clonidine administration and may modulate the baroreflex without being an integral part of the reflex arc.

Effect of chronic administration of antihypertensive drugs on 5-HT receptors

The evidences for the involvement of serotonergic system in the regulation of blood pressure are very strong. Despite considerable investigations, the modulatory contributions of the different serotonergic neural pathways and receptor systems are not completely understood. Different studies have shown that intraventricularly or
intravenously administered 5-HT or 5-HT precursors increase or decrease sympathetic nervous activity, blood pressure and heart rate (McCall and Humphery, 1982). Recent studies using selective agonists and antagonists have provided additional information. 5-HT1A agonists decrease blood pressure and heart rate (Martin and Lis, 1985; Gardin et al., 1985; Fozard et al., 1987) whereas 5-HT2 agonists increase blood pressure (McCall et al., 1987; Alper and Snider, 1987; McCall and Harris, 1988). The mechanisms of action of these compounds are very complex. 5-HT1A agonists require intact adrenergic pathways to affect blood pressure and heart rate (Fozard et al., 1987) whereas 5-HT2 agonists can increase renin secretion independently of sympathetic activation (van de Kar and Richardson-Morton, 1986; Lorens and van de Kar, 1987).

In the normal rat brain constant density and high affinity 5HT1 binding sites have been detected. Cortex, of the centhaquin treated rats, did not show any change in the density or affinity of 3H-5-HT binding. Clonidine treatment also did not show any change in the density or affinity of 3H-5-HT binding. Hydralazine treatment increased the density and affinity of 5-HT1 receptors. Reserpine treatment also increased the density and affinity of 3H-5-HT binding. In the caudate, centhaquin treatment produced no change in the density and affinity of the 5-HT1 receptors. Clonidine treatment also did not produce any change in the density and affinity of 3H-5-HT binding. Hydralazine treatment decreased the density and affinity of 5-HT1 receptors. Reserpine treatment increased the density but decreased the affinity of 3H-5-HT binding. In hypothalamus, no change occurred in the density and affinity of the centhaquin treated rats by 3H-5-
HT. No change was deserved in the density and affinity of $^{3}$H-5-HT binding of the clonidine treated rats. Hydralazine treatment decreased the density but did not affect the affinity of the 5-HT$_1$ receptors. Reserpine treatment also decreased the density, but did not affect the affinity of $^{3}$H-5-HT binding. In medulla, centhaquin increased the density and affinity of $^{3}$H-5-HT binding. Clonidine treatment increased the density and decreased the affinity of the 5-HT$_1$ receptors. Hydralazine treatment did not alter either the density or affinity of 5-HT$_1$ receptors. Reserpine treatment also did not produce any change in the density or affinity of $^{3}$H 5-HT binding.

The normal rat brain have a fairly constant density and high affinity 5-HT$_2$ binding sites. In cortex, centhaquin treatment did not show any change in the density and affinity of $^{3}$H-spiperone binding. Clonidine treatment also did not change the density or affinity of 5-HT$_2$ receptors. Hydralazine treatment increased the density but did not affect the affinity of $^{3}$H-spiperone binding. Reserpine treatment also increased the density but affinity of 5-HT$_2$ receptors did not change by $^{3}$H-spiperone binding. In caudate, centhaquin treatment did not change either the density or affinity of $^{3}$H-spiperone binding. Clonidine treatment also did not produce any change in the density or affinity of 5-HT$_2$ receptors. Hydralazine treatment increased the density but had no effect on the affinity of $^{3}$H-spiperone binding. Reserpine treatment also increased the density but did not change the affinity of 5-HT$_2$ receptors. In hypothalamus, centhaquin had no effect on either the density or affinity of $^{3}$H-spiperone binding. Clonidine treatment also did not produce any change in density and affinity of 5-HT$_2$ receptors. Hydralazine
treatment had no effect on the density and affinity of $^3$H-spiperone binding. Reserpine treatment also did not effect either the density or affinity of $5$-$HT_2$ receptors. In medulla, no change was seen in the density and affinity of $^3$H-spiperone binding by centhaquin treatment. In clonidine treated rats the density and affinity of $5$-$HT_2$ receptors did not change. In hydralazine treated rats, the density decreased, while affinity did not alter by $^3$H-spiperone binding. Reserpine treatment also decreased the density but did not effect the affinity of $^3$H-spiperone binding.

$5$-$HT$ (100 $\mu$g, ICV) produced a slight decrease in colonic temperature in control group of rats. Reserpine treated group also showed a fall in colonic temperature. Clonidine and hydralazine treated group showed no effect of $5$-$HT$ on colonic temperature. Centhaquin treated group showed a marked increase in colonic temperature. The effect of $5$-$HT$ (100 $\mu$g, ICV) on mean blood pressure was not significant in control rats while in reserpine and hydralazine (peripherally acting antihypertensive drugs) treated rats, $5$-$HT$ produced a significant increase in blood pressure. However, in clonidine and centhaquin (centrally acting antihypertensive drugs) treated rats a significant decrease in blood pressure was observed.

These results indicate both biochemically and pharmacologically $5$-$HT$ receptors of the brain might be involved in the action of centrally acting antihypertensive drugs.
Effect of Centhaquin on Blood Pressure and heart Rate in Normal and Cervical sectioned rats

Centhaquin when administered intravenously produced hypotension and bradycardia in normal rats. In cervical sectioned rats it did not produce any effect on blood pressure or heart rate. This suggests that centhaquin produces hypotension and bradycardia by acting directly in the central nervous system. In order to find out the spinal action of centhaquin on blood pressure and heart rate, centhaquin was given intrathecally, which did not produce any effect on blood pressure and heart rate. This showed that the site of action of centhaquin was above the spinal cord.

Effect of centhaquin microinjections in the NTS of cat

Microinjection of centhaquin in the NTS produced mainly bradycardia with little effect on blood pressure. In order to determine the nature of receptors involved several antagonists were administered prior to the administration of centhaquin. It was found that phentolamine, which blocks both $\alpha_1$ and $\alpha_2$ adrenergic receptors could block the effect of centhaquin. In order to determine which subtype of receptor is involved $\alpha_1$ adrenergic receptor antagonist prazosin was given prior to the administration of centhaquin. The effect of centhaquin was blocked by prazosin. Similarly $\alpha_2$ adrenergic receptor antagonist yohimbine blocked the effect of centhaquin. It became difficult to comment which subtype of receptor is involved. It could be possible that in NTS the $\alpha$ adrenergic receptors are of undifferentiated type and are responsive to both prazosin and yohimbine.
CONCLUSIONS

1. Clonidine and centhaquin, centrally acting antihypertensive drugs, increase the density of $\alpha$ adrenoceptors in hypothalamus and medulla, while hydralazine and reserpine, peripherally acting antihypertensive drugs, decreased the density of $\alpha$ adrenergic receptors in all the four regions of the brain.

2. A clear cut differentiation in the blood pressure and temperature responses of noradrenaline in centrally acting hypertensive drugs and peripherally acting antihypertensive drugs was obtained.

3. Centrally acting antihypertensive drugs clonidine and centhaquin do not involve $\beta$ adrenergic receptors in producing hypotensive effect.

4. Clonidine treatment produced an increase in the density of cholinergic (muscarinic) receptors and the rats treated chronically with clonidine showed a significant increase in colonic temperature and decrease in blood pressure in response to centrally administered acetylcholine. These results indicate that clonidine might be producing a decrease in blood pressure by involving cholinergic (muscarinic) receptors.

5. Centhaquin, a newly discovered antihypertensive drug, has been found to produce hypotension by acting at the $\alpha$ adrenergic receptors. The nature of these receptors is not known, since the hypotensive effect could be blocked by phentolamine, yohimbine and prazosin.
SUMMARY
Summary

Hypertension is a frequently occurring disease of the developed as well as the developing countries. Although a persistent elevation of blood pressure beyond the normal range may be devoid of clinical symptoms but the complications resulting from this disorder are severe. Peripheral vascular resistance, vascular reactivity, cardiac output, the autonomic nervous control, blood viscosity etc. may all be involved in a very complex and interdependent manner.

The life span of hypertensive patients can be increased by many years if appropriate therapeutic measures bring about a reduction in blood pressure to near normal level. The main thrust of antihypertensive therapy consists of treatment with drugs. Usually the treatment is symptomatic regardless of the type of hypertension. However, the cause should be treated as soon as possible, for which, reasonable knowledge of the cause of hypertensive disease and the mechanism of action of antihypertensive drugs is important. Many potential sites and mechanisms exist through which a drug may exert its antihypertensive effect.

The first attempt at modern pharmacotherapy of hypertension began around 1955 with the availability of drugs like reserpine, hydralazine, veratrum alkaloids, thiazide diuretics and guanethidine. Although, these drugs made the first real impact on the morbidity and mortality associated with the complications of hypertension, the side
effects of these agents remained unacceptable to many patients. As a result some of
these compounds were discarded or had to be used in combination with other drugs.
Attention was subsequently drawn towards the influence of the central nervous system
in the development of hypertensive disease. Thus clonidine was developed, which
decreased blood pressure by acting mainly on the central nervous system. The brain
stem has been shown to be an important site of action.

The etiology of primary hypertension is obscure and hence the choice of drug
treatment cannot be wholly adequate. Moreover, the pharmacotherapy remains the most
useful tool in the management of primary hypertension. The treatment has to be often
life long and a high standard of efficacy and safety is required of the drugs used. The
antihypertensive drugs can be mainly classified as (1) centrally acting antihypertensives,
mainly adrenergic agonists (2) peripherally acting antihypertensives, which can be
antiadrenergic, mineralocorticoids, diuretics and direct acting drugs. We have selected
clonidine a centrally acting drug, hydralazine a peripherally acting antihypertensive,
reserpine which acts by releasing catecholamines and centhaquin a new centrally
acting antihypertensive drug.

1. Radioreceptor binding studies: Two types of binding sites exist, one which is specific
to the type of receptor under investigation and the other is nonspecific binding to the
biopolymers of the tissue. Since the number of specific receptors sites are limited, and
the nonspecific sites are unlimited, the binding experiment was repeated in the
presence of excess amount of non-radiolabelled ligand. The various ligands used for
Radioreceptor binding to different neurotransmitter receptors are as follows:

<table>
<thead>
<tr>
<th>Receptor</th>
<th>Hot ligand</th>
<th>Cold ligand</th>
</tr>
</thead>
<tbody>
<tr>
<td>α-adrenergic</td>
<td>$^3$H-Dihydroergocryptine 0.25-12 nM (Amersham)</td>
<td>Phentolamine (1 μM)</td>
</tr>
<tr>
<td>β-adrenergic</td>
<td>$^3$H-Dihydroalprenolol 0.12-4 nM (Amersham)</td>
<td>Propranolol (1 μM)</td>
</tr>
<tr>
<td>Muscarinic</td>
<td>$^3$H-Quinuclidinyl benzylate 0.1-2 nM (NEN)</td>
<td>Atropine (1 μM)</td>
</tr>
<tr>
<td>Dopamine</td>
<td>$^3$H-Spiperone 0.1-5 nM (NEN)</td>
<td>Haloperidol (1 μM)</td>
</tr>
<tr>
<td>5-hydroxytryptamine ($5HT_1$)</td>
<td>$^3$H-5-hydroxytryptamine 0.25-15 nM (NEN)</td>
<td>5-hydroxytryptamine ($5HT$) (1 μM)</td>
</tr>
<tr>
<td>5-hydroxytryptamine$_2$ ($5HT_2$)</td>
<td>$^3$H-Spiperone 0.15-10 nM (NEN)</td>
<td>Ketanserin (1 μM)</td>
</tr>
</tbody>
</table>

Radioreceptor binding studies were carried out with these ligands for the specific receptors in corpus striatum, cortex, hypothalamus and medullary regions of the rat brain. The changes in receptor affinity and density was studied.

2. Effects of centhaquin on blood pressure and heart rate in normal and cervical sectioned rats was recorded on a Grass Model 7 Polygraph.

3. Microinjection studies were performed in cats by placing a cannula in the nucleus tractus solitarius (NTS). Drugs like centhaquin, clonidine, phentolamine, yohimbine and prazosin were injected by the help of a microsyringe.
4. Studies on thermoregulation and blood pressure. Rats were cannulated at the lateral cerebral ventricle (lcv) and allowed to recover. Then they were chronically treated with the oral administration of four antihypertensive drugs selected in this study. The rats were then tested for their responses to noradrenaline, dopamine, acetylcholine and 5-HT. Temperature measurements were done by measuring the core temperature of the albino rats with a thermistor probe inserted 2 cm into the rectum and connected to a programmed apple computer. The temperature was recorded before the drug administration and immediately after it at regular intervals of 10 minutes for 5 hours. Blood pressure was recorded by the IITC in 6 instrument which measures the systolic as well as the mean blood pressure. The blood pressure before administration of the drug was compared to the change in blood pressure after the drug injection.

Till now, not much attention has been given to the status of central neurotransmitter receptors in hypertension, and the effect of antihypertensive drugs on central neurotransmitter receptors. It was thought that if, antihypertensive drugs, like centhaquin, clonidine (which are centrally acting), hydralazine (which acts through a peripheral mechanism) and reserpine (which has a complex mechanism of action, but mainly peripheral) are administered chronically, they are likely to produce different actions on various central neurotransmitter receptors. Broadly centrally acting antihypertensives will have direct effect on the CNS receptors, while peripherally acting antihypertensives are not likely to produce any change in central receptors. If peripherally acting antihypertensives do produce alteration in central receptors, it could
be due to a secondary (feedback) effect i.e. due to decreased blood pressure the central receptors are altered. Since medulla and hypothalamus are the main areas of brain involved in blood pressure regulation, alteration of receptor characteristics in these areas will be of direct relevance to regulation of blood pressure. Other brain regions like striatum and cortex are less involved in cardiovascular regulation.

Receptor binding studies show that clonidine and centhaquin increase both the affinity and density of α-adrenoceptors in hypothalamus and medulla of rat brain, while peripherally acting antihypertensive drugs, hydralazine and reserpine decrease the density of α-adrenoceptors in all four brain regions. Since the affinity of clonidine for presynaptic α-adrenoceptors is at least ten times higher than its affinity for postsynaptic α-adrenoceptors, it could be possible that repeated administration of clonidine decreases the release of norepinephrine in hypothalamus and medulla by acting on presynaptic receptors. Thus the decreased concentration of norepinephrine in the synapse will result in increase in the density of post synaptic α-adrenoceptors, as observed in the present study. The mean blood pressure response to noradrenaline in hydralazine and reserpine treated rats was similar to the control rats, while it was opposite in clonidine and centhaquin treated rats. This shows a clear differentiation between responses of noradrenaline in centrally and peripherally acting antihypertensive drugs and also strengthens our contention that besides clonidine, centhaquin a newly developed antihypertensive drug acts on central adrenergic receptors, which in all probability could be α adrenergic receptors. Centhaquin and
clonidine did not affect the density or affinity of β adrenergic receptors. Further supporting the fact that β adrenergic receptors are not involved in the action of centrally acting antihypertensive drugs. In the present study both biochemical and pharmacological evidences have been provided indicating an alteration in central adrenergic receptors.

In hypothalamus and medulla clonidine and centhaquin did not affect either the density or the affinity of [H]-spiperone binding. Some non specific changes was observed in hydralazine and reserpine treated rats. Dopamine (50 µg, ICV) produced no significant change in either colonic temperature or mean blood pressure in control, clonidine and reserpine treated rats. In hydralazine treated rats a significant decrease in blood pressure and increase in colonic temperature were observed. In centhaquin treated rat no change in blood pressure occurred, however a significant increase in colonic temperature was observed. It is clear from the above results which provide both biochemical and pharmacological evidence that central dopaminergic receptors are not involved in blood pressure regulation or in the action of centrally acting antihypertensive drugs.

Centhaquin treatment produced no change in the density and affinity of [H]-quinuclidinyl benzylate binding. Clonidine treatment produced a decrease in the density and affinity of the muscarinic receptors. No change was observed in the density and affinity of the muscarinic receptors by hydralazine and reserpine treatment. Acetylcholine (10 µg, ICV) produced a decrease in colonic temperature in control rats. Peripherally
acting antihypertensive drugs, hydralazine and reserpine treated rats showed no change in colonic temperature. Centrally acting antihypertensive drug, centhaquin treated rats showed a decrease in colonic temperature, as control rats. However, clonidine treated rats showed a significant increase in colonic temperature in response to acetylcholine. Blood pressure was decreased by acetylcholine (10 μg, ICV) in control rats as well as in centhaquin, hydralazine and reserpine treated rats. However clonidine treated rats showed a significantly greater decrease in blood pressure in comparison to other groups of rats. These results indicate that clonidine does act on cholinergic receptors and might be producing some of antihypertensive effect through these receptors.

The evidences for the involvement of serotonergic system in the regulation of blood pressure are very strong. These results of the present study indicate both biochemically and pharmacologically that 5-HT receptors of the brain might be involved in the action of centrally acting antihypertensive drugs.

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