ANTIHYPERTENSIVE ACTIVITY OF PARA-SUBSTITUTED CLONIDINE ANALOGUES AND THEIR ACYLATED DERIVATIVES.

BY

ALAN MARTIN LEWIS

THESIS SUBMITTED IN FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF

MASTER OF SCIENCE

UNIVERSITI SAINS MALAYSIA
FEBRUARY 1994

For my wife Daisy,
Adrian, Bryan and Mark

ACKNOWLEDGEMENT

I wish to express sincere gratitude and thanks to Professor Gan Ee Kiang for his invaluable guidance, support and encouragement to whom in large measure may be attributed the success of this study.

I also wish to sincerely thank Professor Sam Teng Wah and his staff for the synthesis and characterization of the clonidine analogues.

Thanks also to Dr. Aishah Latiff for making available the facilities to conduct the research.

Staff of the Department of Pharmacology, Physiology and Pharmaceutical Chemistry for their invaluable assistance in particular Mr. R. Tangisuran, Mr. Arumugam, Mr. Cheah Boon Huat, Mr. Tan Siow Peng, En. Roseli Hassan, En. Adnan and En. Hassan.

The Ministry of Health Malaysia for the opportunity to pursue these studies in Pharmacology.

Table of Contents

Lists of Figures

LIST OF TABLES

AKTIVITI ANTIHIPERTENSIF ANALOG-ANALOG KLONIDIN PENUKARGANTIAN PARA DAN TERBITAN-TERBITAN ASILNYA.

Kegunaan klonidin sebagai ubat dalam regimen-regimen antihipertensif adalah terhad disebabkan keujudannya hipertensi pantulan semasa pemberian ubat dihentikan secara mengejut. Adalah tidak diketahui kemungkinan pengasingan efek hipertensi pantulan daripada aktiviti hipotensif dapat dilakukan melalui perubahan dalam struktur induk. Kajian ini telah menumpu perhatian kepada efek penukargantian para dan pengasilan-*N* ke atas potensi hipotensif, hipertensi pantulan dan selektiviti untuk adrenoseptor-α. Aktiviti antihipertensif dan kemungkinannya kejadian hipertensi pantulan sebelas analog-analog dibandingkan dengan klonidin dalam haiwan-haiwan SHR. Pemberian 56 nmol./kg secara intraperitoneal selama 20 hari kepada SHR yang diikuti dengan pemberhentian mengejut tidak menunjukkan aktiviti hipertensif yang berkesan diantara analog-analog kecuali sebatian AL-1.2. Semasa fasa pemberhentian tekanan darah sistolik tikus-tikus yang dirawati dengan AL-1.2 tidak menunjukkan kesan peningkatan pantulan atau "overshoot". Ketiadaan hipertensi pantulan dipercayai disebabkan oleh penukargantian para kumpulan isobutiril. Peranan

pengasilan-*N* tidak begitu jelas. Pemberian intravena dalam tikus normotensif terbius dengan pentobarbiton menunjukkan kekurangan dalam efikasi hipotensi analog-analog berbanding dengan klonidin. Penukargantian para dengan kumpulan nitril hampir sama sekali menghilangkan aktiviti hipotensif tetapi gantian dengan kumpulan hidroksilmetil dan karboetoksil membenarkan pembendungan sedikit aktiviti hipotensif. Dalam eksperimen yang sama di mana denyutan jantung diuji, pengasilan-*N* kelihatan hampir sama-sekali menghilangkan kesan bradikardia jika dibandingkan dengan penukargantian para yang kurang berkesan. Dalam persediaan anococcygeus yang telah digunakan untuk menilai aktiviti rangsangan adrenoseptor-α_1, adalah dikelihatan bahawa sebatian-sebatian CL-1.1, CL-1.2, AL-1.0, AL-1.1 dan AL-1.2 mempamerkan perangsangan adrenoseptor-α_1 yang lemah. AL-1.2 telah menunjukkan afiniti untuk adrenoseptor-α_1 yang terendah dan autoinhibisi pada dos-dos yang lebih tinggi. Dalam persediaan vas deferens tikus terasingan yang telah digunakan untuk menilai aktiviti adrenoseptor-α_2 presinaptik, adalah diperhatikan bahawa untuk aktiviti agonisme adrenoseptor-α_2 yang tinggi, tapak para perlu dikosongkan. Tambahan pula, untuk afiniti-α_2 yang tinggi sekurang-kurangnya satu nitrogen imidazolin diperlukan bebas.

ABSTRACT

The use of clonidine in antihypertensive drug regimens has been severely limited by the appearance of withdrawal hypertension upon abrupt discontinuation of treatment. It is not known if a separation of rebound hypertension from hypotensive activity is possible through structural alteration. The present investigation involved a study of the effects of *p*-substitution and *N*-acylation of clonidine on the hypotensive potency, rebound hypertension and α-adrenoceptor selectivity. The antihypertensive activity and potential for exhibiting withdrawal hypertension of eleven analogues were compared to clonidine in Spontaneously Hypertensive Rats(SHRs). Intraperitoneal administration at 56 nmol./kg over a period of 20 days in the SHRs followed by abrupt withdrawal did not reveal significant antihypertensive activity in any of the analogues except for AL-1.2. During the withdrawal phase, the systolic blood pressure of AL-1.2-treated rats did not show a rebound increase or overshoot. The absence of rebound hypertension was believed to be due to the isobutyryl functional group in the para position. The role of *N*-acylation was unclear. The intravenous administration in pentobarbitone-anesthetized

normotensive rats revealed a lower hypotensive efficacy in comparison to clonidine. *p*-Substitution with nitrile functional groups resulted in compounds with almost no hypotensive activity while an appreciable amount of activity was retained in compounds with *p*-substitutions involving the hydroxylmethyl and carboethoxyl functional groups. In the same experiments in which heart rate was monitored, *N*-acylation was more effective in reducing bradycardia when compared to *p*-substitution. In the rat anococcygeus preparation, it was observed that the compounds CL-1.1, CL-1.2, AL-1.0, AL-1.1 and AL-1.2 displayed weak α_1-adrenoceptor stimulatory activity. AL-1.2 had the lowest affinity for α_1-adrenoceptors and displayed autoinhibition at higher doses. In the isolated rat vas deferens which was used to assess presynaptic α_2-adrenoceptor activity, it was observed that for high α_2-adrenoceptor agonist activity, the para position should be left unsubstituted. Further, for high α_2-affinity at least one imidazolidine nitrogen should be left unsubstituted.

INTRODUCTION

1.0. Introduction

Hypertension may be defined as a condition of raised blood pressure which is persistently elevated beyond normal limits. If the cause cannot be clearly defined, it is termed as primary or essential hypertension. Secondary hypertension is the condition where the cause is known and this may be in the form of an imbalance in endocrine function, kidney disease, hypertension in pregnancy and that resulting from the use of contraceptive pills.

Although it is impossible to clearly define normal or abnormal blood pressure, the World Health Organisation has provided the following guideline for consideration in the diagnosis of the disease(WHO, 1978). Blood pressure can be considered normal for an adult with a systolic pressure equal to or below 140 mmHg and a diastolic pressure equal to or below 90 mmHg. An individual is considered hypertensive if systolic pressure equals to or exceeds 160 mmHg and the diastolic pressure is equal to or greater than 95 mmHg.

Whatever the difficulties there may be in identifying hypertensive individuals, hypertension if left untreated will increase the risks of development and

death from cardiovascular disease. Persistently elevated blood pressure will lead to irreversible changes in the walls of blood vessels. There is an increase in peripheral resistance and the left ventricle of the heart gradually becomes hypertrophied. The blood vessels of the heart undergo adaptive changes with the lumen becoming progressively smaller. Eventually lesions develop in the heart due to ischaemia and the result is heart failure. Cerebral stroke and damage to blood vessels in the kidney are other complications of raised arterial pressure(Sleight, 1977). Reducing elevated blood pressure is thus the primary objective of antihypertensive therapy and will drastically reduce the risks of many cardiovascular complications and reverse the structural changes seen in the vasculature.

Hypertension may be treated in part by a modification in lifestyle such as the avoidance of stress, a low salt diet, maintenance of normal bodyweight, regular exercise and immediate cessation of smoking. Drug therapy remains the main approach in relieving hypertension and a large number of drugs(Van Zwieten 1984) are presently available for this purpose. They are classified in table 1 shown below.

Table 1.

Classification of antihypertensive drugs

Class	Drugs
1. Beta-adrenoceptor blocking agents	Atenolol Metoprolol Propranolol
2. Alpha-adrenoceptor blocking agents	Prazosin Phentolamine Phenoxybenzamine
3. Adrenergic and Beta adrenoceptor blocking drugs	Labetolol
4. Angiotensin-converting enzyme inhibitors	Captopril Enalapril
5. Calcium-channel bocking agents	Nifedipine
6. Direct-acting vasodilators	Diazoxide Hydralazine
7. Centrally acting agents	Clonidine Guanabenz Guanfacine Methyldopa
8. Diuretics	Hydrochlorothiazide
9. Ganglion-blocking agents	Trimetaphan
10. Adrenergic neurone blocking agents	Reserpine Bethanidine Guanethidine
11. Angiotensin-II receptor blocking agents	Saralasin
12. Inhibitors of renin	pepstatin
13. Serotonin blocking agent	Ketanserin

While the primary pharmacological action of many of these agents are known, the exact mechanisms by which they act to reducing blood pressure in the long term are not clearly understood. Since antihypertensive therapy involves prolonged administration of one or more of these drugs in combination, there is the possibility that many of the patients will experience side-effects. Patients unable to tolerate the side-effects may require a change in the drug or treatment combination used and this would be facilitated by having a broad range to choose from. It is worthwhile therefore, to experiment with new compounds with a clearly understood mode of action which could provide good control of blood pressure and be relatively free or have a reduced number of side-effects . They may not necessarily be more active compounds.

1.1. The regulation of blood pressure

A number of mechanisms involved in the control of blood pressure have been identified(Struyker Boudier, 1984). Among them are neural, endocrine, renal and vascular mechanisms.

1.1.1. Neural mechanisms

Neural mechanisms are most important for the rapid stabilisation of blood pressure. They are further subdivided into the sinoaortic baroreceptor, the cardiopulmonary baroreceptor, the chemoreceptor, the central nervous system ischaemic, somatic afferent and the renal afferent reflexes.

1.1.1(a). The sinoaortic baroreceptor reflex

The sinoaortic baroreceptor reflex acts by detecting changes in arterial pressure through its stretch sensitive receptors located in the aortic arch and the carotid bifurcation. Information is then transmitted along afferent nerves to the central nervous system. The response that follows involves both the sympathetic and parasympathetic nervous system and has direct consequences on the heart and vasculature. It is the most important of the neural control mechanisms. Baroreceptors function within a certain minimum or maximum pressure limit. They are activated above a certain minimum level and their activity does not increase above a certain maximum pressure level. They also show reflex adaptation in that they may reset to

higher pressure thresholds at which they are activated, for instance in hypertensive individuals.

1.1.1(b). The cardiopulmonary baroreceptor reflex

This reflex detects changes in blood volume of the circulation through stretch-sensitive receptors located in the atrium and pulmonary arteries. Renal function is influenced by the activity of these reflexes and it has been shown that increased receptor activity may cause inhibition of renin release and also affect the water balance of the body.

1.1.1(c). Other reflexes

The chemoreceptor, central nervous system ischemic, somatic afferent and renal afferent reflexes are less important in the acute control of blood pressure. The central nervous system ischaemic reflex only functions when there is a drastic drop in mean arterial pressure below 40-50 mmHg. The result is a very large increase in sympathetic nervous activity aimed at restoring normal blood pressure.

1.1.2. Hormonal mechanisms

They are divided into those involving the cathecolamines, renin-angiotensin system, aldosterone, antidiuretic hormone, prostaglandins, vasoactive principles and natriuretic hormone. The role of hormonal mechanisms in the long term control of blood pressure are relatively minor.

1.1.2(a). The cathecolamines

The cathecolamines like adrenaline and noradrenaline are partially responsible for the maintenance of vascular tone by their action on α_1 and α_2-adrenergic receptor subtypes present in the blood vessels. Their role in the pathogenesis of hypertension remains controversial. Plasma noradrenaline has been found to be elevated in only some studies of patients with essential hypertension(Goldstein, 1981).

1.1.2(b). The renin-angiotensin system

Renin, secreted by the juxtaglomerular apparatus of the kidney is an inactive molecule. In the plasma, renin acts on an angiotensinogen to form angiotensin-I.

Angiotensin converting enzyme facilitates the conversion of Angiotensin-I into angiotensin-II which is the pharmacologically active molecule. Angiotensin-II has direct vascular effects and causes constriction of veins and arteries. Vascular permeability is increased. Renal sodium and water excretion is altered. The synthesis and release of aldosterone is stimulated by angiotensin-II and by increases in sympathetic nerve activity via both a central nervous system mechanism and a peripheral presynaptic effect. The role of aldosterone in the regulation of blood pressure is briefly described below.

1.1.2(c). Aldosterone

Aldosterone is a mineralocorticoid hormone secreted by the zona glomerulosa cells of the adrenal cortex. The hormone has its primary role in enhancing the renal tubular reabsorption of sodium in exchange for potassium and hydrogen ions. This results in an expansion of blood volume. Alterations in the renin-angiotensin system regulates the the secretion of aldosterone. Angiotensisn-II is the main stimulus for the release of aldosterone as described previously.

1.1.2(d). Antidiuretic hormone

The antidiuretic hormone(ADH) or vasopressin is secreted by the neurohypophysis. Secretion of this hormone is promoted by a fall in plasma volume as well as an increase in plasma osmotic pressure. This causes an increase in the reabsorption of water in the distal tubules and collecting ducts of the kidney. Other factors also modulate the release of this hormone(Bayliss, 1977). ADH has also been implicated as a contributory factor toward raised arterial pressure in hypertension because of its vasoconstrictor effects on vascular smooth muscle(Monos *et al*, 1978).

1.1.2(e). Vasoactive substances

These are composed of mainly the prostaglandins, the kalleikrin-kinin system, the antihypertensive principle of the renal medulla and the natriuretic hormone. The prostaglandins are thought to function as local tissue hormones adjusting the local blood flow to the changing metabolic requirements of the tissue. The kalleikrin-kinin system has components in the plasma, the exocrine glands and the kidney. Kinins are formed from plasma substrates called kininogens by the action of kalleikrin. Kinins are potent vasodilators and affect

sodium and water reabsorption in the distal tubules. The antihypertensive principle and the natriuretic hormone have not yet been clearly identified. Their role, much like the role of the prostaglandins and kallekrin-kinin have not been clearly established.

1.1.3. Renal mechanisms

Some of the changes in arterial pressure brought about by neural and endocrine mechanisms involve an alteration in renal function. On its own, however, the kidney has a dominant function in the long-term maintenance of arterial pressure(Borst & Borst-De Geus, 1963 . This has been attributed to the intimate relationship between renal perfusion pressure and renal output which under normal circumstances are equal. Slight increases in perfusion pressure cause a very large increase in glomerular filtration rate. After sometime, extracellular volume and blood volume decrease and return to pre-existing levels. The important role of the kidney in long-term maintenance of blood pressure does not presuppose that the causative factor of hypertension lies in this organ.

1.1.4. Vascular mechanisms

Like renal function, vascular mechanisms are controlled in part by neural and endocrine mechanisms. A number of organs such as the brain, the kidneys and heart regulate their own blood flow in response to changing stimuli such as stretch(pressure) and chemical factors such as oxygen and other blood-borne substances. In the long term control of blood pressure, changes may occur in the walls of these vessels. There may be an increase in vascularity or a thickening of the walls of blood vessels as seen in high blood pressure conditions.

1.2. The role of α-adrenoceptors in the regulation of blood pressure.

1.2.1. Classification

α-Adrenoceptors that are located within the cardiovascular system play an important role in the regulation of blood pressure in the body. They are also the targets for a wide range of therapeutically useful antihypertensive drugs. α-Adrenoceptors belong to two subgroups namely α and ß as proposed by

Ahlquist(1948). A further division was later proposed in which α-adrenoceptors were divided into α_1 and α_2 subtypes and ß-adrenoceptors into $ß_1$ and $ß_2$ subtypes(Langer, 1974). The anatomical location of α_1-adrenoceptors was post-junctional and that of α_2-adrenoceptors, pre-junctional. Receptors of the α_2 subtype were later shown to be present postjunctionally making the anatomical classification of receptor subtypes by location inappropriate(Timmermans & Van Zwieten, 1982). A pharmacological classification based on receptor demand(Ruffolo *et al*, 1991) is presently used in the classification of α-adrenoceptor subtypes. In this classification, an α_1-adrenoceptor responds toward stimulation by methoxamine, cirazoline or phenylephrine and the responses are competitively antagonized by WB-4101 or corynanthine. An α_2-adrenoceptor is stimulated by UK-14,301, B-HT 920, B-HT 933 or α-methylnorepinephrine and the responses blocked competitively by low concentrations of yohimbine, rauwolscine or idazoxan.

1.2.2. Distribution of α-adrenoceptors participating in blood pressure regulation.

The presence of α-adrenoceptors in various tissues to some extent explains the probable mechanisms by which

some antihypertensive drugs act. In the peripheral circulation, both α_1 and α_2 receptor subtypes are present on the smooth muscle cell membranes of the arteries(Timmermans *et al*, 1990). Stimulation of α_1 and α_2-adrenoceptors evokes vasoconstriction.

In the veins, both receptor subtypes are present and are thought to be responsible for the maintenance of venous tone. Blockade of α_1-adrenoceptors, for example with prazosin causes vasodilatation.

α-Adrenoceptors are also present in the heart. They are mainly of the α_1 subtype(Timmermans *et al*, 1990) and mediate increases in heart rate and contractility. The presence of α_2-adrenoceptors have not been demonstrated. Clonidine depresses heart rate and it is believed that this effect is mediated by its action on α_1-adrenoceptors.

It has already been mentioned previously that renal function is of considerable importance to the long-term control of blood pressure. It is richly innervated by noradrenergic neurones which extend to the afferent and efferent arterioles, the juxtaglomerular apparatus, the nephrons and the collecting ducts. Adrenergic receptors that are present are mainly of the α_2 subtype

with α_1-adrenoceptors being less abundant. Renal excretory functions are controlled by α-adrenoceptors. Stimulation of α_1-adrenoceptors is thought to promote the tubular reabsorption of sodium and water while stimulation of extrasynaptically located α_2-adrenoceptors by catecholamines results in opposing effects(Olson, 1976).

The central nervous system controls many important physiological functions. In particular, the pontomedullary region contains the vital centres regulating respiratory, cardiac and vasomotor function which are contained in the grey matter. Motor and sensory fibres that are present interconnect these centres with higher brain centres and with various receptors such as those involved in baroreceptor and carotid sinus occlusion reflex(Chalmers, 1975). The presence of a dense adrenergic innervation has made this region a natural target for centrally acting hypotensive drugs which are thought to act by interacting with α-adrenoceptors. Both α_1 and α_2 subtypes are present in the brain. Research so far has centred mainly on the interaction of hypotensive drugs with α_2-adrenoceptors. The role played by α_1-adrenoceptors in mediating the response of blood pressure toward antihypertensive drugs remain unclear. The spinal cord has been shown to contain

α-adrenoceptors of the α_2 subtype(Kubo *et al*, 1987) that may play a role in the regulation of blood pressure.

1.3. Interaction of antihypertensive drugs and blood pressure control mechanisms.

The process by which an antihypertensive drug lowers blood pressure is complex and depends on the pharmacological action of the drug at the cellular level and responsiveness of other regulatory mechanisms. This interaction occurs at different levels and often more than one mechanism may be involved. When hemodynamic parameters are altered, other mechanisms are activated either through reflex pathways, for example, the baroreceptor reflex, which may in turn stimulate the release of ADH. Renal excretory function may be altered to adapt to changes in blood pressure in order to maintain normal fluid balance. As a result of this, there is a gradual shift in blood pressure from the previous level to a new set-point. Prolonged therapy also allows the chronic effects of antihypertensive therapy to be known. The reversal of structural changes commonly seen in hypertensive disease(Struyker Boudier, 1984) may also be observed in the vasculature during this time.

1.4. α-Adrenergic agonists

They may be divided into two major groups. These are the phenethylamines and the imidazoli(di)nes. The first group includes the compounds phenylephrine and methoxamine which are specific for α_1-adrenergic receptors. In the second group are clonidine, naphazoline and xylometazoline. Clonidine is specific for α_2-adrenoceptors with the latter two being relatively non-specific(Timmermans *et al*, 1990). Clonidine has a potent central hypotensive action(Constantine & McShane, 1968; Bolme & Fuxe, 1971). The structure and pharmacological actions of clonidine are discussed in more detail below.

1.4.1. Clonidine

Clonidine or 2-(2,6-dichlorophenylimino)-2-imidazolidine was first synthesized in 1962. The structure is depicted in figure 1 below.

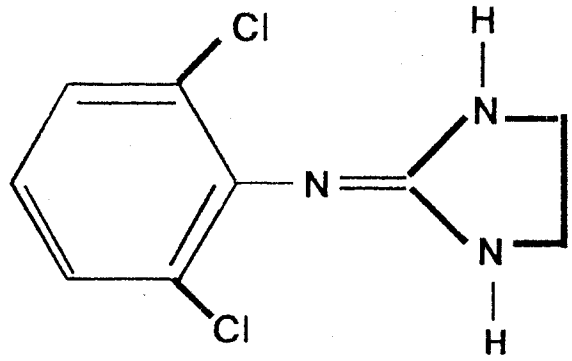

Figure 1. The structure of Clonidine.

1.4.1(a) Mechanism of action

The centrally mediated hypotension seen after systemic administration of clonidine is believed to be due to post-synaptic activation of α_2-adrenoceptors(Kobinger & Pichler, 1976; Timmermans *et al*, 1981) of the cardiovascular regulatory centres of the brain. Clonidine in its unionized, lipophilic form, crosses the blood-brain barrier into the medulla where the protonated imidazolidine drug molecules bind to α_2-adrenoceptors(Ruffolo *et al*, 1982). Stimulation of these receptors results in the inhibition of sympathetic outflow together with an increase in vagal output. The resultant vasodilation and bradycardia lead to a fall in mean arterial pressure. Interaction of clonidine with cardiac prejunctional α_2-adrenoceptors(Huchet *et al*, 1981) has been found to contribute to the bradycardia seen.

It has also been suggested, that clonidine may act by stimulating presynaptic instead of postsnyaptic α_2-adrenoceptors. These α_2-adrenoceptors are believed to be connected to a facilitatory neurone, the stimulation of which results in a reduction of peripheral sympathetic tone and a fall in arterial

pressure(Van Zwieten *et al*, 1973). This mechanism is less likely as the hypotensive effects of clonidine(Kobinger & Pichler, 1976) are not abolished even if central noradrenaline stores are depleted by reserpine or 6-hydroxydopamine.

While these are the predominant effects, interactions with other blood pressure control mechanisms are known to occur. Baroreceptor and chemoreceptor activity in the region of the carotid arteries are amplified. Endocrine effects such as a decrease in renin secretion and angiotensin-II levels are seen. Inhibition of secretion of ACTH and aldosterone leading to a lower blood volume contribute to the hypotension in chronic therapy(Walland, 1977).

The medullary site at which clonidine acts and the associated nerve pathways involved in the mediation of the hypotensive and bradycardiac responses have been investigated intensively in rats and cats. They correspond closely with the neural pathways involved in both the reflex and tonic control of arterial pressure. An integral component of the neuronal circuitry involved is the nucleus tractus solitarii(NTS). It has been shown that destruction of the NTS reduces but does not abolish the hypotensive and bradycardiac effects of

clonidine, indicating that clonidine has its primary site of action elsewhere (Lipski *et al*, 1976, Zandberg *et al*, 1979).

The NTS also provides major input to the nucleus ambiguus and dorsal motor nucleus of the vagus, which is the site of origin of preganglionic cardiac vagal neurones. The NTS is known to provide neurones which innervate the region of the medulla known as the rostral ventrolateral medulla(RVLM) which is believed to be responsible for vasomotor tone(Spyer, 1994). The RVLM receives major innervation from the A1 group of noradrenergic containing neurones found in the caudal ventrolateral medulla which has been shown to further contain a subgroup of adrenergic neurones, the C1 group(Spyer, 1994). These neurones are believed to relay baroreceptor inputs from the NTS to the RVLM to regulate its activity(Granata *et al*, 1985). The C1 neurons also project into the spinal cord providing major innervation for the sympathetic preganglionic neurones of the intermediolateral cell column(Zagon & Smith, 1993). The activity of these neurones are largely dependent on baroreceptor activity and have been observed to fire with a cardiac rythym(Spyer, 1994). They are believed to be responsible for the maintenance of resting levels of arterial pressure(Granata *et al*, 1985).

The RVLM is believed to be the main site of action of clonidine in the medulla(Ernsberger *et al*, 1987; Reis *et al*, 1988). In the framework of the neuronal circuits described above, clonidine may be envisaged as acting on α_2-adrenoceptors in the RVLM(Ernsberger *et al*, 1987). This causes a reduced sympathetic output which is transmitted to the sympathetic preganglionic neurones of the intermediolateral cell column. The activity of postganglionic sympathetic neurones are inhibited by the release of acetylcholine. At the level of the blood vessels and the heart, there is a modulation in the release of noradrenaline which is accompanied by vasodilatation and bradycardia. The increase in vagal output may be explained as a stimulation of the dorsal vagal nuclei in the pressor region of the reticular system of the medulla by clonidine. This results in an increase in nerve activity of the preganglionic cardiac neurones. Subsequently, there is an increased discharge in postganglionic cardiac neurones and the release of acetylcholine at the synapses in the heart. This is accompanied by bradycardia.

1.4.1(b) Common side-effects of clonidine

The administration of clonidine in therapy of hypertension is accompanied by a number of side-effects. Among the most commonly seen during the initial stages are drowsiness, dry-mouth, dizziness, headache and constipation. Less common side-effects are depression, anxiety, fatigue, nausea, slight orthostatic hypotension and impotence. In animals, chronic administration of clonidine has been shown to produce acidosis(Gan & Abdul Satar, 1982). A temporary loss in hypotensive efficacy has been observed sometimes during clonidine treatment. Fluid retention may be responsible for this phenomenon.

A serious disadvantage of clonidine therapy is the appearance of withdrawal or rebound hypertension, upon abrupt termination of administration of the drug. The condition is characterised by a rapid increase in blood pressure in excess of pre-treatment levels. Some of the symptoms seen in this condition are nausea, sweating, tachycardia and headache which are symptoms of adrenergic overactivity.

1.4.1(c). Clinical uses

Clonidine has its principal use as an antihypertensive agent. Presently, it is not the treatment of choice in hypertension and this is mainly due to its toxicity. Clonidine may be given either orally or intravenously in the treatment of hypertension. The oral maintenance dose is between 0.3 to 1.2 mg daily but may be increased as required. In hypertensive crises, clonidine may be given by slow intravenous injection at a dose of between 150 to 300 μg and up to 750 μg over 24 hours. Efficacy may be increased by the addition of a diuretic(Kawasaki *et al*, 1991).

Clonidine has also been used in the treatment of glaucoma. Intraconjunctival instillation of isotonic solutions containing 0.125 - 0.5% of clonidine reduces intraocular pressure by decreasing aqueous humor formation and increasing outflow(Huber *et al*, 1991). Clonidine because of its selective stimulation on α_2-adrenoceptors causes a pronounced ocular hypotensive response.

Clonidine has also been used in the prophylaxis of migraine, recurrent vascular headaches and menopausal flushing. Other reported uses of clonidine are in the

treatment of nervous systems disorders, alcohol and opiate withdrawal syndromes(Martindale, 1989).

1.4.2. Clonidine analogues

The chemical synthesis of compounds with a basic structure resembling that of clonidine, have provided agents that have found usefulness in both therapeutics as well as experimental research. The tritiated form of 4-amino-clonidine, is used to label α_2-adrenoceptors (Ernsberger, *et al*, 1987). Xylazine or 2-(2,6-dimethylphenylamino)-4H-5,6-dihydro-1,3-thiazine, has actions similar to clonidine(Schmitt, 1977). It is relatively more selective for α_2 over α_1-adrenoceptors. Xyalzine is used as a veterinary anesthetic.

1.5. Structure-activity relationships of clonidine.

Clonidine exhibits a potential for tautomerism but has been shown to exist predominantly in the imino form(Jen *et al*, 1972). The molecule may be divided into three parts consisting of the aromatic ring, the bridge nitrogen and the imidazolidine moiety. Modifications have involved each or a combination of anyone of these parts.