THE INTERACTION OF RENIN-ANGIOTENSIN AND SYMPATHETIC SYSTEMS IN HYPERTENSION AND HEART FAILURE

BY

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DEDICATION

To

My loving Father,

Mother,

My Husband, My Son 'Qusay'

And

My Brother

For their prayers, patience, devotion and encouragement throughout the entire time spent in completing this thesis.

REHAB

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The following abbreviations have been used

AD	Adrenaline
NA	Noradrenaline
PE	Phenylephrine
ME	Methoxamine
Ang I	Angiotensin I
Ang II	Angiotensin II
AT_1	Angiotensin II receptor subtype I.
AT_2	Angiotensin II receptor subtype II
AV node	Atrioventricular node
SA node	Sinoatrial node
CNS	Central nervous system
SNS	Sympathetic nervous system
RAS	Renin–angiotensin system
ACE	Angiotensin converting enzyme
SHR	Spontaneously hypertensive rat
MAP	Mean arterial pressure

INTERAKSI SISTEM RENIN-ANGIOTENSIN DAN SIMPATETIK DALAM KEADAAN HIPERTENSI DAN KEGAGALAN JANTUNG

ABSTRAK

Klonidin (2-[(2, 6-diklorofenil)-amino]-2-midazolin), merendahkan tekanan darah melalui tindakannya ke atas α_2-adrenoseptor untuk mengurangkan aliran luar simpatetik. Klonidin telah ditunjukkan mempunyai kesan hipotensif berpusat dan dimediasi oleh tindakan terus ke atas otak, membawa kepada penurunan ton simpatetik. Perindopril menurunkan tekanan darah dengan menghalang aktiviti enzim penukar angiotensin di dalam subjek manusia dan haiwan. Angiotensin II adalah satu vasokonstriktor periferal yang poten di mana ianya mengaruh rembesan aldosteron oleh adrenal korteks dan menyediakan tindakbalas negatif ke atas rembesan renin.

Objektif kajian ini ialah untuk menilai peranan drug bertindak pusat, klonidin dan perencat enzim penukar angiotensin II, perindopril di dalam perubahan respon presor kepada pelbagai agonis adrenergik dan angiotensin II eksogenus di dalam tikus normal dan hipertensif dengan atau tanpa kegagalan jantung. Hasil kajian ini berkemungkinan boleh digunakan untuk mengenalpasti interaksi antara sistem renin-angiotensin dan simpatetik di dalam keadaan penyakit-penyakit ini.

Kegagalan jantung diaruhkan dengan kombinasi rawatan kafein (40 mg/kg) dan isoprenalina (5 mg/kg) selama tujuh hari. Haiwan-haiwan diagihkan kepada lapan kumpulan. Empat kumpulan pertama terdiri daripada tikus Wistar, tikus Wistar dengan kegagalan jantung, SHR dan SHR dengan kegagalan jantung, diberi klonidin (50 µg/kg). Kumpulan kedua terdiri daripada tikus Wistar, tikus Wistar dengan kegagalan

jantung, SHR dan SHR dengan kegagalan jantung, diberi perindopril (0.2 mg/kg). klonidin dan perindopril diberi secara oral selama 6 hari. Kumpulan-kumpulan kawalan diberi samaada salin biasa atau tween 80 mengikut kaedah yang sama. Di dalam kajian akut, haiwan-haiwan ini diberi anastesia (natrium pentobarbiton, 60 mg/kg i.p), dan trakeotomi dilakukan. Arteri karotid kiri dikanulat untuk pengukuran tekanan darah, vena jugular kiri dikanulat untuk membenarkan infusi berterusan anastesia (12.5 mg/kg/h) dan untuk pemberian dos bolus agonis, noradrenalina (NA) (200, 400 dan 800 ng/kg), fenilephrina (PE) (2, 4 and 8μg/kg), metoksamina (ME) (2, 4 dan 8μg/kg) and angiotensin II (Ang II) (5, 10 dan 20 ng/kg).

Perubahan tekanan darah disebabkan oleh agonis-agonis ini direkod sebagai respon pressor. Data, mean ± s.e.m dibandingkan dengan kaedah ANOVA dua-hala diikuti dengan ujian pos-hok Duncan dengan tahap signifikan 5%. Keputusan-keputusan yang diperolehi menunjukkan NA dan PE menghasilkan respon pressor yang dos dependen di dalam semua kumpulan dirawat dengan klonidin. Respon-respon pressor ini berbeza dengan signifikan berbanding dengan kumpulan kawalan masing-masing untuk tikus Wistar, tikus Wistar dengan kegagalan jantung dan SHR. Walaubagaimanapun, tiada perubahan signifikan di dalam respon pressor ditunjukkan oleh kumpulan SHR dengan kegagalan jantung bila dibandingkan dengan kumpulan kawalan. ME menghasilkan respon pressor yang dos dependen di dalam tikus Wistar dan SHR yang dirawat dengan perindopril dan respon-respon ini adalah berbeza secara signifikan bila dibandingkan dengan kumpulan kawalan, manakala, tiada perbezaan signifikan di dalam respon yang diperhatikan di dalam Wistar dengan kegagalan jantung dan SHR dengan kegagalan jantung bila mana ME diberikan.

Berdasarkan keputusan yang diperolehi di dalam kajian ini, boleh disimpulkan di dalam tikus Wistar yang normal dan tikus Wistar dengan kegagalan jantung, pemberian

klonidin tidak memberi perubahan ke atas respon pressor dengan pemberian Ang II tetapi perubahan signifikan terhadap agonis adrenergik diperhatikan. Ini menunjukkan bahawa penghalangan sistem simpatetik periferal oleh klonidin tidak memberi kesan ke atas sistem renin-angiotensin di dalam tikus Wistar yang normal dan tikus Wistar dengan kegagalan jantung. Di dalam SHR dan SHR dengan kegagalan jantung yang dirawat dengan klonidin, respon pressor kepada pemberian Ang II diubah tetapi tidak kepada agonis adrenergik. Penemuan ini menunjukkan berkemungkinan wujudnya satu interaksi kompleks di antara sistem simpatetik dan renin-angiotensin di dalam kumpulan-kumpulan haiwan ini. Di dalam kumpulan tikus Wistar normal dan tikus Wistar dengan kegagalan jantung, pemberian perindopril menyebabkan perubahan pressor respon kepada pemberian Ang II dan agonis adrenergik. Ini menunjukkan sistem simpatetik periferal oleh perindopril dikompromasi sistem renin-angiotensin di dalam tikus Wistar normal dan tikus Wistar dengan kegagalan jantung. Namun, di dalam SHR dan SHR dengan kegagalan jantung yang dirawat dengan perindopril, tiada perubahan signifikan dalam respon pressor kepada agonis adrenergik dan Ang II diperhatikan. Kesemua penemuan dari kajian ini secara kolektifnya mencadangkan berkemungkinan wujudnya satu interaksi antara sistem renin-angiotensin dan simpatetik di dalam proses kawalatur tekanan darah di dalam model-model haiwan yang digunakan di dalam kajian ini.

ABSTRACT

Clonidine (2-[(2, 6-dichlorophenyl)-amino]-2-imidazoline), reduces blood pressure via its action on α_2-adrenoceptors to decrease sympathetic outflow. Clonidine has been demonstrated to exert its hypotensive effect centrally and is mediated by direct action on the brain, leading to a decrease of sympathetic tone. Perindopril lowers blood pressure by inhibiting ACE activity in human subjects and animals. Angiotensin II is a potent peripheral vasoconstrictor which stimulates aldosterone secretion by the adrenal cortex and provides negative feedback on renin secretion.

The objective of this study was to assess the role of centrally acting drug, clonidine and an angiotensin II converting enzyme inhibitor, perindopril in the modification of pressor responses to various exogenous adrenergic agonists and angiotensin II in normal and hypertensive rats with or without heart failure. The outcome of this study could then be used to establish the interaction of the renin-angiotensin and sympathetic systems in these disease states.

Heart failure was induced by combined treatment of caffeine (40 mg/kg) and isoprenaline (5mg/kg) for seven days. The animals were divided into eight groups. The first four groups composing of the Wistar rats, heart failure Wistar rats, SHR and heart failure SHR, these groups were given clonidine (50 μg/kg). The second four groups consisted of the Wistar rats, heart failure Wistar rats, SHR, and SHR with heart failure and given perindopril (0.2 mg/kg). Clonidine and perindopril were administered orally for six days. Control groups received either normal saline or tween 80 in the same manner respectively. In acute studies, the animals were anaesthetized (sodium pentobarbitone, 60 mg/kg i.p), and a tracheostomy done. The left carotid artery was cannulated for measurement of blood pressure, the right jugular vein was cannulated for

a continuous infusion of anaesthesia (12.5 mg/kg/h) and to inject bolus doses of agonists i.e, NA (200, 400 and 800 ng/kg), PE (2, 4 and 8μg/kg), ME (2, 4 and 8μg/kg) and Ang II (5, 10 and 20 ng/kg).

The changes in blood pressure due to the agonists were recorded as pressor responses. Data, means ± s.e.m were compared with two way ANOVA followed by Duncan's post-hoc test with the significance level of 5%. The results obtained indicated that the NA and PE produced pressor responses that were dose dependent in all groups of animal treated with clonidine. These pressor responses were significantly different as compared to their respective control groups in Wistar rats, heart failure Wistar rats and SHR. However no significant changes in the pressor responses were seen in the heart failure SHR group as compared to control group. Methoxamine produced dose dependent pressor responses in Wistar rats and heart failure Wistar rats treated with clonidine. The pressor responses were significantly different as compared to their control groups. No significant changes in the pressor responses were observed when the ME was administered in SHR and heart failure SHR group as compared to control groups.

Angiotensin II produced dose dependent pressor responses in the SHR and heart failure SHR treated with clonidine. These pressor responses were significantly different as compared to control groups. No significant difference in the pressor responses was observed when the Ang II was administered in the Wistar rats and heart failure Wistar rats treated with clonidine as compared to their control groups. Noradrenaline, PE and Ang II produced dose dependent pressor responses in all groups of animals treated with perindopril, which were significantly different as compared to their respective control groups in Wistar rats and heart failure Wistar rats. However, no significant changes in the pressor responses were seen in SHR and SHR with heart failure as compared to

control groups. Methoxamine produced dose dependent pressor responses in Wistar rats and SHR rats treated with perindopril and these responses were significantly different as compared to control groups where as no significant changes in the responses were observed in the heart failure Wistar and SHR heart failure rats when ME was administered.

Based on the results obtained in this study, it is concluded that in the normal Wistar rats and heart failure Wistar rats, the administration of clonidine did not cause any pressor response changes to the administration of Ang II but significant change to the adrenergic agonist was seen. This indicates that the blockade of the peripheral sympathetic system by clonidine did not compromise the RAS in normal Wistar rats and heart failure Wistar rats. In the SHR and heart failure SHR treated with clonidine, the pressor responses to the administration of Ang II were altered but no significant changes to the adrenergic agonist were seen. This finding may indicate that a complex interaction between the sympathetic system and RAS in these groups of animal. In the normal Wistar rats and heart failure Wistar rats, the administration of perindopril caused pressor response changes to the administration of Ang II and adrenergic agonists. This indicated that the blockade of peripheral sympathetic system by perindopril compromised the RAS in normal Wistar rats and heart failure Wistar rats. However, in the SHR and heart failure SHR treated with perindopril, no significant changes in the pressor responses to the adrenergic agonist and Ang II were seen. The findings from this study collectively suggested that there is a possible interaction between the RAS and the sympathetic system in the regulation of blood pressure in the animals used in this study.

CHAPTER ONE

INTRODUCTION

1.1 Hypertension

Hypertension is defined as an elevation of arterial blood pressure above an arbitrarily defined normal value (Beard *et al.*, 1992; Guidieral *et al.*, 1996 and Helge *et al.*, 2001). Blood pressure is the product of peripheral vascular resistance and cardiac output. An increase in cardiac output or peripheral resistance results in an increase in blood pressure. However, if one of these factors increases whilst the other decreases, the blood pressure may not be affected.

The level of resting arterial pressure varies considerably from person to person, and these variations may be related to factors such as age, sex, temperature and emotional state (Folkow and Svanberg, 1993; Mulvany *et al.*, 1991 and Guyton, 1990). An arbitrary level for hypertension is frequently set at 140/90 mm Hg (systolic/ diastolic), and those with arterial pressures consistently higher than these at rest are considered to be hypertensive. Obviously, there are degrees of hypertension, from very mild to very severe. In the latter, pressure may rise to 240/140 mm Hg, or even higher. In some cases, only the systolic or the diastolic pressure is elevated, although in most cases both are increased but not necessarily equally (Bianchi and Ferrari, 1992 and Creigh *et al.*, 1992).

In more than two-thirds of patients with hypertension, a specific disturbance in a particular organ system cannot be found as the cause of elevated blood pressure. These patients are said to have primary or essential hypertension. In the remaining cases a definite cause can be established. These patients have non-essential, or secondary hypertension. The most common diseases in which there is an associated hypertension are those involving the heart, central nervous system, endocrine system, and kidney (Olson, 1998 and Helge *et al.*, 2001).

1.1.1 Cardiovascular hypertension

Cardiovascular hypertension occurs when systemic arterial pressure is elevated by an increase either in cardiac output or peripheral resistance (Cowley, 1992). High output states, such as hyperthyroidism will increase pressure unless accompanied by peripheral vasodilatation. Usually, only moderate increases are seen due to high outputs. An increased cardiac output may also occur in adrenal disease. More commonly, the vessels lose their dispensability with age, which tends to increase systolic pressure. If the smaller vessels are involved, mean pressure may be markedly increased, as seen in generalized arteriosclerosis. High pressure itself, if maintained for a sufficient time (weeks to months), may cause a permanent change in the blood vessels that will prevent the return of peripheral resistance to normal even though the primary cause of the hypertension has been cured or removed (Olson, 1998).

1.1.2 Neurogenic hypertension

In neurogenic hypertension there is an increase in sympathetic tone resulting from a disturbance of cerebral blood flow. This may occur during cerebral ischemia, in cases of hemorrhage within the cranium, or related to a space-occupying mass, e.g., a

brain tumor within the cranial vault. Blood pressure will become elevated in an attempt to maintain cerebral flow. Thus a raised blood pressure after a head injury is often a sign of intracranial bleeding. In some cases the carotid sinus or aortic arch baroreceptors are unable to respond to changes in blood pressure. This might occur, for example, with traumatic injury to the carotid sinus nerves, arteriosclerosis changes in the wall of the internal carotid, or a tumor in the baroreceptor areas (Dampey, 1994 and Shaohua *et al.*, 2002).

1.1.3 Endocrine hypertension

Endocrine hypertension refers to several diseases of the endocrine system associated with an elevation of blood pressure. Disease of the adrenal medulla, which secretes the catecholamines adrenaline (AD) and noradrenaline (NA), may result in excessive production of these hormones. Since NA is the major transmitter for the SNS, and AD has similar actions in larger doses, an increase in vasomotor tone and myocardial force will occur. This occurs, for example, in the presence of a pheochromocytoma, an adrenal medullary tumor that secretes excessive quantities of catecholamines, thereby causing an increase in peripheral resistance and cardiac output. Removal of the tumor is usually curative. Tumors of the adrenal cortex associated with increased production of corticosteroids may also cause high blood pressure. One such tumor produces aldosterone in large amounts; which acts on the kidney to cause sodium and water retention results in an increase in blood volume. In Cushing's syndrome, glucocorticoids are produced in excessive quantities by the adrenal cortex. They apparently also influence the kidney to retain fluid and result in mild to moderate hypertension (Retting *et al.*, 1990).

1.1.4 Renal hypertension

Damage to the kidney itself may result in renal hypertension. Reduction of renal blood flow by arterial occlusion or renal artery compression consistently produces hypertension regardless of whether or not renal nerve fibers are present. The kidney produces a humeral agent known as renin, an enzyme that acts on a circulating plasma protein to produce the polypeptide angiotensine I (Ang I). Angiotensine I is inactive but may be readily converted to an active from angiotensine II (Ang II), Ang II that produces widespread arterial vasoconstriction and resultant hypertension. It may also stimulate the production, release, or both, of aldosterone from the adrenal cortex. The amount of renin released may be greatly increased in conditions of renal ischemia. Kidney disease is therefore an important cause of hypertension. Removal of the diseased kidney, in cases in which only one kidney is involved, may reverse the hypertension (Lifton, 1996).

1.1.5 Essential hypertension

Thus far we have discussed disease states known to produce hypertension. In most cases of hypertension, however, no abnormality in cerebral blood flow, cardiac output, renal function, or the endocrine system can be demonstrated (essential hypertension). The reasons are unclear and the mechanisms involved not well understood, but a number of theories have been advanced to explain such cases (Levy *et al.*, 1996).

One of these postulates swelling of the smooth muscle cells that make up the arterioles due to an increase in sodium and water content. The consequent encroachment of these swollen cells on the lumen of the arteriole results in an increase

in arteriolar resistance. The basic disorder producing the swollen cells is unknown. However, it may not be the fundamental process and may only reflect disease elsewhere for example in the kidney. An increase in blood volume often occurs also for reasons that are not apparent. Some investigators have postulated that the baroreceptors are reset to a higher-than-normal level. Alternatively, the control centers in the CNS could be set to a higher level. The sensitivity of the carotid sinus might be reduced by arteriosclerosis changes in the wall of the carotid artery (Manunta *et al.*, 2001).

1.2 Neural control in blood pressure

There are two important contributors to the control of blood pressure, the sympathetic nervous system (SNS) and the renin–angiotensin system (RAS) (Borchard 2001; Rupp and Jager, 2001). The autonomic nervous system is important in regulating cardiovascular homeostasis as it modifies both cardiac output and the diameter of resistance vessels. Blood vessels, however, are innervated almost exclusively by fibers of the SNS, which regulate vasomotor tone. Increased SNS activity results in increased vasomotor tone and, therefore, is causally related to the development and maintenance of high blood pressure (DiBona *et al.*, 1996).

The RAS has become established as an endocrine system that plays important roles in the physiological regulation of cardiovascular, renal, and endocrine functions. It contributes to the development and persistence of various forms of hypertension (Cody *et al.*, 1983). Activation of this system leads to the formation of renin in the kidney, which converts angiotensinogen to the inactive peptide, Ang I. Ang I is then converted by angiotensin converting enzyme (ACE) to Ang II a potent vasoconstrictor which also stimulates aldosterone secretion and fluid retention (Rupp and Jager, 2001).

Pharmacological interventions that inhibit the RAS have been proven to be efficient antihypertensive and, more recently, have been shown to be beneficial in congestive heart failure (Rupp and Jager, 2001). The RAS is often considered to exert its effect on blood pressure in an independent manner. It is now clear, however, that extensive interactions occur between the RAS and other blood pressure control system, in particular the SNS (Reid, 1992).

The SNS controls the process by which Ang II is produced through the release of renin from the kidneys. As the rate of renin release by the kidneys is crucial for the formation of Ang II, the SNS is a key determinant of circulating Ang II levels. Circulating Ang II itself then interacts with the SNS at various sites and appears to amplify sympathetic activity. It may act on the brain to increase sympathetic outflow, on the sympathetic ganglia and adrenal medulla to increase catecholamine release, and at presynaptic sympathetic nerve endings to facilitate sympathetic neurotransmission through an enhanced NA release (De Jonge *et al.*, 1984; Reid, 1992 and Rupp and Jager., 2001).

1.3 Adrenergic receptors

Adrenergic receptors (adrenoceptors) mediate the central and peripheral actions of primary sympathetic neurotransmitter, NA and the primary adrenal medullary hormone (and central transmitter), adrenaline. Adrenoceptors are found in most of the peripheral tissues and on many neural tissues within the central nervous system. These adrenoceptors mediate a variety of functions such as blood pressure, myocardial contractile rate and force, airway reactivity, and an array of metabolic functions. Several types of neuronal varicosities also have prejunctional (presynaptic) adrenoceptors

serving as auto or heteroreceptors that inhibit or modify nerve-evoked release of several neurotransmitters (Bylund *et al.*, 1994).

There are multiple, closely related adrenoceptor subtypes, although their exact number and the appropriate mode of grouping into major families is still controversial. Generally, current knowledge classifies the adrenoceptors into three major subtypes, called α_1, α_2 and β-adrenoceptors (Bylund *et al.*, 1994).

These subclassifications are based on several functional, molecular and radioligand binding studies after several considerations. Firstly, that the different major types of adrenoceptors affinity for selective drugs are 3 to 4 orders of magnitude (i.e. α_1, α_2 and β), and the affinity ratio for each major subtype is only between 10 to 100. Secondly, the second-messenger responses of each major subtype is different and finally, that the predicted amino acid sequences of the adrenoceptors are more consistent with three rather than two major type (Bylund *et al.*, 1992). These three subtypes are further subclassified in to several subtype, α_{1A}, α_{1B}, α_{1D} and α_{2A}, α_{2B}, α_{2C} and α_{2D} and β_1, β_2 and β_3 (Bylund *et al.*, 1994; Cooper *et al.*, 1996 and Zhong and Minneman, 1999).

1.3.1 α_1-adrenoceptors

α_1-adrenoceptors exist as heterogenous family. More than a decade age, pharmacological studies indicated the existence of α_{1A} and α_{1B}-adrenoceptors (Han *et al.*, 1987; and Minneman *et al.*, 1988). The development of adrenoceptor researches, based on pharmacological and molecular studies have indicated that these cloned subtypes correspond to native α_{1A}, α_{1B} and α_{1D}-adrenoceptor subtypes (Ford *et al.*, 1994;

Hieble *et al.*, 1995 and Bylund *et al.*, 1998). Functionally, these receptors were characterized by their high affinity for prazosin and low affinity for yohimbine (Byund *et al.*, 1994).

α_1-adrenoceptor subtypes are widely expressed in different neonatal and adult rat tissues. High levels of α_{1A} and α_{1D}-adrenoceptors were detected in brain and heart whereas similar levels of α_{1B}-adrenoceptors in liver and heart of neonatal rats by immunoreactive mechanism. In adult rat tissues, α_{1A}-adrenoceptors protein were most marked in the brain, intermediate in heart, aorta, liver, vas deferens and adrenals, and minimal in the kidney and prostate as compared to other tissues. The expression of α_{1B}-adrenoceptors was higher in the brain and heart but the expression of α_{1D}-adrenoceptors in brain was most prominent (Shen, *et al.*, 2000). α_1-adrenoceptor subtypes are localized in different parts of the cell. α_{1A}-adrenoceptor subtypes for example, are localized in perinuclear fashion, whereas α_{1B}-adrenoceptor subtype was detected throughout the entire border of the cell (Hirasawa *et al.*, 1997). Further studies on the vascular smooth muscle did not indicate that the α_{1A} and α_{1D}-adrenoceptor subtypes were defined on the cell but that the α_1-adrenoceptor subtypes were found in the intercellular compartment (Hrometz *et al.*, 1999).

All α_1-adrenoceptor subtypes are activated by the sympathetic neurotransmitters, noradrenaline, adrenaline, even though none of these catecholamines exhibit selective affinity to any of these adrenoceptor subtypes. α_1-adrenoceptor mediated responses are blocked by prazosin and show a low affinity for selective α_2-adrenoceptor antagonists such as yohimbine (Morrow and Greese 1986 and Bylund *et al.*, 1994). α_1-adrenoceptor-induced vasoconstrictions appear to be caused both by release of

intracellular calcium and by the transmembranous influx of extracellular calcium (Caufin and Malik, 1984; Bylund *et al.*, 1994; and Zhong and Minneman, 1999).

α_1-adrenoceptors involve rapid processes such as sequestration and slower processes such as receptor downregulation (Garcia-Sainz, 1993 and Cotecchia *et al.*, 1995). The slower downregulation of these receptors may be related to the pathophysiological processes which occur in disease states such as cardiac failure and chronic renal failure (Packer, 1992a). According to Dong and Han (1995), α_{1B}-adrenoceptor mediated vasoconstriction is easier to be desensitized, while α_{1A}-adrenoceptor mediated constriction is easier to be hypersensitized. Furthermore, both α_{1A} and α_{1D}-adrenoceptor subtypes are functionally upregulated in spontaneously hypertensive rat (SHR) muscle vascular bed. This may provide some clue for the possible role of α_1-adrenoceptor subtypes in maintenance of elevated blood pressure (Ye and Colquhoun, 1998). Functional expression of α_{1D}-adrenoceptor in the rat resistance vessels increase with age; α_{1A} but not α_{1B} or α_{1D}-adrenoceptors, which seem to predominate in immature animals. This represents the first evidence that age-related changes in functional α_1-adrenoceptor subtypes occur in the systemic vasculature *in vivo* (DeMey 1997; DeOliveira *et al.*, 1998 and Ibarra *et al.*, 1999). In addition, all the three receptor subtypes increased with age in the brain cortex, whereas the density of α_{1B}-adrenoceptor increased in the heart but decreased in the liver. Furthermore, α_{1A}-and α_{1D}-adrenoceptor population in liver, kidney and heart of rats were not affected by age (Shen *et al.*, 2000).

1.3.2 α_2-adrenoceptors

The α_2-adrenoceptors can be pre and postjunctional. Some of the prejunctional are neuroinhibitory in their action (Bylund, 1988; Akers *et al.*, 1991 and Oriowo *et al.*, 1991). Prejunctional inhibitory α_2-adrenoceptors are predominantly of the α_{2A}-adrenoceptors subtype. Furthermore, α_{2C}-adrenoceptors may also occur prejunctionally (Docherty, 1998). α_2-adrenoceptors play an important role in mediating the sympathetic nervous system effects on blood pressure. α_2-adrenoceptor can be pharmacologically divided to α_{2A}, α_{2B}, and α_{2C}-adrenoceptors, all of which mediate contractile responses (Cooper *et al.*, 1996; Docherty, 1998 and Zhong and Minneman, 1999). They are activated to variable extents by catecholamines, exerting different effects depending of their localization (Irena *et al.*, 2000). The α_{2A}-adrenoceptor subtype are located in the central nervous system and concentrated in the brainstem (Tavares *et al.*, 1996) which is known to be the center of cardiovascular control, and is responsible for the tonic regulation of the sympathetic nervous system. The α_{2B}-adrenoceptors, on the other hand are thought to be the only subtype located in the vascular smooth cell of the arterial wall, and having a role in the vasoconstriction action (Link *et al.*, 1996; MacMillan *et al.*, 1996 and Altman *et al.*, 1999).

1.4 Specific sympathomimetic drugs

Drugs which partially or completely mimic the effect of sympathetic nerve stimulation or adrenal medullary discharge are termed as sympathomimetics. A wide variety of drugs have sympathomimetic activity and they may be classified into drugs which act:

1- Directly on adrenoceptors e.g. the catecholamines, adrenaline (AD) and noradrenaline .

2- Indirectly, causing release of noradrenaline from the adrenergic nerve ending e.g amphetamine.

3- By both mechanisms e.g. dopamine.

1.4.1 Catecholamines

The brain contains separate neuronal systems that utilize three different catecholamines i.e dopamine, NA, and AD. Each system is anatomically distinct and serves separate, but with similar functional roles within their fields of innervations. Much of the original mapping was performed in rodent brains (Hokfelt *et al.*, 1976, 1977; Foote, 1997 and Lewis, 1997). Catecholamines induce direct vasoconstriction mediated by postsynaptic α-adrenergic receptors of both α_1 and α_2 type (Irena *et al.*, 2000).

1.4.1.1 Noradrenaline (NA)

In contrast to AD, NA acts almost exclusively on α-adrenoceptors, although it is less potent at these receptors sites than adrenaline. Infusions of all doses of NA increase both systolic and diastolic arterial pressure by vasoconstriction of arteriolar and venous smooth muscle. Despite some stimulatory effects on cardiac contraction, the intense vasoconstriction leads either to no change or to a decrease in cardiac output at the cost of increased myocardial oxygen demand. In high dosage the universal vasopressor effect reduces renal blood flow and glomerular filtration rate (Aitkenhead and Smith, 1998).

Noradrenaline effects on the β_1 receptors in the heart are with a similar potency as on α-receptors. In contrast it has relatively little effect on β_2 receptors.

Consequently, NA increases peripheral resistance and both diastolic and systolic blood pressure. Compensatory vagal reflexes tend to overcome the direct positive chronotropic effect of NA but the positive intropic effects on the heart are maintained (Bertam and Katzung, 1995). The cardiovascular effect of an intravenous infusion of NA is that the systolic and diastolic pressure and usually pulse pressure are increased. Cardiac output is unchanged or decreased, and total peripheral resistance is raised (Ruffolo and Hieble 1999). Compensatory vagal reflex activity slows the heart, overcoming a direct cardioaccelerator action and stroke volume is increased. The peripheral vascular resistance increases in most vascular beds, and blood flow is reduced to kidneys. Noradrenaline constricts mesenteric vessels and reduces splanchnic and hepatic blood flow. Coronary flow is usually increased probably owing both indirectly induced coronary dilation as with epinephrine, and to elevated blood pressure (Grossman *et al.*, 1993).

1.4.2 Phenylephrine (PE)

Phenylephrine is a synthetic α_1-selective agonist (Summers, 1984 and Nishimatsu *et al.*, 1999), but having a better affinity for the α_{1A} and α_{1D} as compared to α_{1B}-adrenoceptors (Goetz *et al.*, 1995). It activates β-adrenergic receptors only at much higher concentrations. Phenylephrine causes marked arterial vasoconstriction during intravenous infusion. Phenylephrine also is used as a nasal decongestant and as a mydriatic in various ophthalmic formulations (Bertam and Katzung, 1995).

1.4.3 Methoxamine(ME)

Methoxamine has been used as a prototype of α-adrenoceptor agonists in pharmacological experiments as well as in a clinical setting. While it has been

established that ME acts preferentially on α-adrenoceptors, methoxamine has additional effects on β-adrenoceptors and a direct, non specific action on ion channels, and thus, it has stimulatory as well as inhibitory effects on cardiac contraction (Scholz, 1980 and Endoh, 1982). In the rabbit ventricular myocardium, ME stimulates the hydrolysis of phosphoinositide with an efficacy identical to that of PE, whereas the potency of ME is approximately ten times lower than that of PE (Yang and Endoh, 1994). It is also remarkable that, in contrast to PE, ME elicits a much less pronounced positive inotropic effect upon cumulative administration than upon single administration. Moreover, ME inhibits the positive inotropic effect of PE over a range of concentrations at which it causes the acceleration of the hydrolysis of phosphoinositide (Yang and Endoh, 1994).

Methoxamine is a predominantly directly acting α_1- receptor agonist or, more specifically, by α_{1A}-adrenoceptors (Huang *et al.*, 1996). It may cause a prolonged increase in blood pressure due to vasoconstriction. It also causes a vagally mediated bradycardia. Methoxamine is available for parenteral use, but clinical applications are rare and limited to hypotensive states (Kuo, 1998).

1.5 Renin-angiotensin system (RAS)

The renin-angiotensin system is an important participant in both the short-and long-terms regulation of arterial blood pressure (Dzau 1987; Jin *et al.*, 1987; Seyer *et al.*, 1991; Mickaelle, 1993; Inagami, 1994 and Gary *et al.*, 2001). Factors that decrease arterial blood pressure, such as a decrease in effective blood volume (caused by diuretics, blood loss, congestive heart failure, liver cirrhosis, or nephritic syndrome) or reduction in total peripheral resistance (caused by for example vasodilators), activates renin release from the kidneys (Blaufarb and Sannenblick, 1996).

Renin is synthesized and stored in an inactive form called prorenin in the juxtaglomerular cells of kidneys, which are modified smooth muscle cells located in the walls of the afferent arterioles immediately proximal to the glomeruli (Everet *et al.*, 1990). When the arterial pressure falls, intrinsic reactions in the kidneys themselves causes many of these prorenin molecules to split and release renin. Most of the renin enters the blood and leaves the kidneys to circulate throughout the entire blood stream, although a small amount remains in the local fluids of the kidney (Gary *et al.*, 2001 and Dinh, 2001).

Renin is an enzyme, not a vasoactive substance itself it acts enzymatically on another plasma protein, a globulin called renin substrate (or angiotensinogen) to release a 10 - amino acid peptide, Ang I. Ang I has mild vasoconstrictor properties but not enough to cause significant functional changes in circulatory function. The renin persists in the blood for 30 minutes to an hour and continues to cause formation of Ang I. During few seconds after formation of the Ang I, two additional amino acids are split from it to form the 8-amino acid peptide Ang II. This conversion occurs almost entirely in the small vessels of the lungs, catalyzed by the ACE that is present in the endothelium of the lung vessels (Dinh, 2001).

1.5.1 Actions of angiotensin II (Ang II)

Angiotensin II acts at several sites in the body, including vascular smooth muscle, adrenal cortex, kidney, and brain (Goodfriend *et al.*, 1996); actions the RAS plays a key role in the regulation of fluid and electrolyte balance and arterial blood pressure. Ang II receptors are located on the plasma membrane of target cells

throughout the body. Two distinct receptor subtypes, termed AT_1 and AT_2, have been identified (Goodfriend *et al.*, 1996).

Ang II is a potent pressor agent, considerably more potent than NA. The pressor response to Ang II is rapid in onset (10 - 15 seconds) and sustained during long-term infusions of the peptide. A large component of the pressor response to intravenous Ang II is due to direct contraction of arteriolar smooth muscle mediated by AT_1 receptors. However, Ang II also increases blood pressure through actions on the brain and autonomic nervous system. In particular, it acts centrally to increase sympathetic outflow and peripherally to facilitate sympathetic transmission by increasing the release and reducing the reuptake of NE at adrenergic nerve terminals (Reid, 1992). It also has a less important direct positive inotropic action on the heart. The pressor response to angiotensin is usually accompanied by little or no reflex bradycardia because the peptide acts on the brain to reset the baroreceptor reflex control of heart rate to a higher pressure (Reid, 1992). Ang II acts on the zona glomerulosa of the adrenal cortex to stimulate aldosterone biosynthesis. Aldosterone in turn increases sodium reabsorption in the distal tubule and acts on the kidney to cause renal vasoconstriction, increase proximal tubular sodium reabsorption, and inhibit the secretion of renin (Timmermans *et al.*, 1993).

1.5.2 Function of the renin-angiotensin system

As mentioned earlier the RAS plays a major role in regulating arterial blood pressure over both the short and long term (Dzau, 1987; Jin *et al.*, 1987; Seyer *et al.*, 1991; Mickaelle *et al.*, 1993; Inagami, 1994 and Gary *et al.*, 2001). Modest increases in plasma concentration of Ang II acutely increase blood pressure. On molar basis, Ang II

is approximately 40 times more potent than NA in this regard. Where Ang II is injected interavenously, systemic blood pressure begins to rise within seconds, rapidly reaches maximum, and returns to normal within minutes (Gary *et al.*, 2001).

This rapid pressor response to Ang II is due to a swift increase in total peripheral resistance, a response that helps maintain arterial blood pressure in the face of an acute hypotensive challenge (e.g. blood loss, vasodilation). Although Ang II directly increases cardiac contractility (via opening voltage-gated ca^{2+} channels in cardiac myocytes) and indirectly increases heart rate (via facilitation of sympathetic tone) enhanced noradrenergic neurotransmission and adrenal catecholamine release. The rapid increase in arterial blood pressure activates a baroreceptor reflex that decreases sympathetic tone and increases vagal tone. Thus, Ang II may increase, or not change cardiac contractility, heart rate, and cardiac output, depending on the physiological state. Therefore, changes in cardiac output contribute little if at all to the rapid pressor response induced by Ang II (Ferguson and Washburn, 1998).

Angiotensin II also causes a slow pressor response that helps stabilize arterial blood pressure over the long term. A continuous infusion of initially suppressor doses of Ang II gradually increases arterial blood pressure, with the maximum effect requiring days to achieve (Brown *et al.*, 1981). Angiotensin II induces the stimulation of endothelin-I (Laursen *et al.*, 1997) and super oxide anion (Rajagopalan *et al.*, 1997) production mediates, in part, the slow pressor response. In addition, to buffering short and long-term changes in arterial blood pressure, Ang II significantly alters the morphology of the cardiovascular system i.e., it causes hypertrophy of vascular and cardiac cells (Fink, 1997).

1.5.3 Angotensin-converting enzyme inhibitors

1.5.3.1 Perindopril

Perindopril is a long acting angiotensin converting enzyme (ACE) inhibitor which displays similar effect as captopril (Mancini, 1998). It prevents the conversion of Ang I to Ang II by inhibition of angiotensin-converting enzyme. This results in reduced peripheral vascular resistance and decreased aldosterone production. It also reduces pre-load and after-load in congestive heart failure, and reduces tissue concentration of Ang II leading to arterial and venous dilation (Flather *et al.*, 2000 and Mancini 2000).

Oral administration of perindopril produces dose dependent inhibition of plasma ACE activity in normotensive and hypertensive animals (Laubie *et al.*, 1984; Moursi *et al.*, 1986 and Lo *et al.*, 1990) healthy human subjects (Lees & Reid *et al.*, 1987; Waeber *et al.*, 1989), hypertensive patients (Plouin *et al.*, 1988) and patients with congestive heart failure (Thuillez *et al.*, 1990). As consequence of this ACE inhibition, a decrease in plasma Ang II levels occurs, as well as increases in plasma renin activity and plasma Ang I levels mediated by a negative feedback. Furthermore, a somewhat variable but frequent decrease in plasma aldosteronel levels it also observed. Further evidence of inhibition of ACE following oral perindopril administration is provided by blockade of the pressor response to exogenously administered Ang I in animals (Laubie *et al.*, 1984; DiNicolantonio and Doyle, 1986 and Doyle *et al.*, 1986) and humans (Waeber *et al.*, 1989).

1.6 Congestive heart failure

1.6.1 Autonomic innervation of the heart

The medulla, located in the brainstem, receives sensory input from different systemic and central receptors (e.g., baroreceptors and chemoreceptors) as well as signals from other brain regions (e.g., cortex and hypothalamus). Autonomic outflow from the brainstem is divided principally into sympathetic and parasympathetic (vagal) branches. Efferent fibers of these autonomic nerves travel to the heart and blood vessels where they modulate activity of these target organs.

The heart is innervated by both vagal and sympathetic fibers. The right vagus nerve primarily innervates the sinoatrial node (SA node) while the left vagus innervates the atrioventricular node (AV node). However, there can be significant overlap in the anatomical distribution. Atrial muscle is also innervated by vagal efferents where as the ventricular myocardium is only sparsely innervated by vagal efferents. The sympathetic efferent nerves are present throughout the atria (especially in SA node) and ventricles, and in the conduction system of the heart (Richard *et al.*, 1993).

1.6.2 Congestive heart failure

Congestive heart failure is defined as a pathophysiologic state in which an abnormality of cardiac function is responsible for the failure of the heart to pump blood at a rate to commensurate with the requirements of the metabolizing tissues during stress or exercise (Folkow and Svanberg, 1993 and Herny and Wilson, 2001). Congestive heart failure occurs when the cardiac output is inadequate to provide the oxygen needed by the body. Although it is believed that the primary defect in heart

failure resides in the excitation-contraction coupling machinery of the heart, this clinical condition also involves many other processes and organs, including the baroreceptor reflex, the sympathetic nervous system, the kidneys, the RAS and vasopressin (Bertam and Katzung, 1995). The primary signs and symptom of all types of congestive heart failure includes tachycardia, decreased exercise tolerance and shortness of breath, peripheral and pulmonary edema, and cardiomegaly. Decreased exercise tolerance with rapid muscular fatigue is the major direct consequence of diminished cardiac output. The other manifestations result from the attempts by the body to compensate for the intrinsic cardiac defect (Bertam and Katzung, 1995).

1.6.3 Sympathetic activation in heart failure

The sympathetic nervous system is activated in heart failure, via low and high pressure baroreceptors, as an early compensatory mechanism which provides inotropic support and maintains cardiac output. Chronic sympathetic activation, however, has deleterious effects, causing a further deterioration in cardiac function (Grossman 1991and McDonagh *et al.*, 1998). The earliest increase in sympathetic activity is detected in the heart, and this seems to precede the increase in sympathetic outflow to skeletal muscle and the kidneys that is present in advanced heart failure. Sustained sympathetic stimulation activates the RAS system and other neurohormones, leading to increased venous and arterial tone (and greater preload and afterload respectively), increased plasma NA concentrations, progressive retention of salt and water, and oedema. Excessive sympathetic activity is also associated with cardiac myocyte apoptosis, hypertrophy, and focal myocardial necrosis (Packer, 1992a and Manolis *et al.*, 1995).

In the long term, the ability of the myocardium to respond to chronic high concentrations of catecholamines is attenuated by a down regulation in β receptors although this may be associated with baroreceptor dysfunction and a further increase in sympathetic activity. Indeed, abnormalities of baroreceptor function are well documented in chronic heart failure, along with reduced parasympathetic tone, leading to abnormal autonomic modulation of the sinus node. Moreover, a reduction in heart rate variability has consistently been observed in chronic heart failure as a result of predominantly sympathetic and reduced vagal modulation of the sinus node, which may be a prognostic marker in patients with chronic heart failure (Grossman,1991 and McDonagh *et al.*, 1998). Sympathetic activation during heart failure serves as an important compensatory mechanism, but is also a precipitating factor in worsening heart failure. A common finding in heart failure patients and experimental models is that the sympathetic adrenergic branch of the autonomic nervous system is activated and this results in cardiac stimulation, peripheral vascular constriction and activation of the RAS.

1.6.3.1 Cardiac stimulation

Sympathetic activation of the heart causes an increase in heart rate and inotropy via the release of NA acting primarily upon β_1-adrenoceptors. The increase in inotropy by sympathetic activation however, may not be sufficient to restore normal intropy particularly in ventricles having systolic dysfunction (Cohn *et al.*, 1984 and Kaye *et al.*, 1995). Sympathetic activation has other important effects which can be deleterious, including ventricular hypertrophy, enhanced arrhythmogenesis, and molecular and biochemical changes that lead to further dysfunction over time (Prakash and Deedwania, 1997). Therefore, although sympathetic activation may play some

compensatory role in the failing heart, there is considerable evidence that it actually exacerbates heart failure. For this reason, the use of beta-blockers in some forms of heart failure has been gaining in popularity because of their proven efficacy (Richard *et al.*, 1993 and Jackson *et al.*, 2000).

1.6.3.2 Peripheral Vascular Constriction

Arterial and venous vessels are richly innervated by sympathetic nerves and activation of these nerves causes release of NA that binds primarily to postjunctional α_1-adrenoceptors causing smooth muscle activation and vasoconstriction. Arterial vasoconstriction increases systemic vascular resistance which raises arterial pressure. In heart failure, particularly when cardiac output is significantly reduced, arterial vasoconstriction helps to maintain arterial pressure (Prakash and Deedwania, 1997).

The increased systemic vascular resistance, however, contributes to an increase in the afterload of the heart which can further depress systolic function. Peripheral vasoconstriction, particularly in the smaller arterioles, limits muscle perfusion during exercise thereby contributing to the decrease in exercise capacity. Contractions of venous vessels enhance venous return and preload which helps to maintain stroke volume through the Frank-Starling mechanism. The resulting increase in venous pressure, however, can lead to peripheral edema (Prakash and Deedwania, 1997). Peripheral vasoconstriction caused by enhanced sympathetic activation can be both beneficial and deleterious in heart failure. The deleterious aspects of sympathetic activation can be offset by using arterial and venous vasodilator drugs. This therapeutic approach is very important in the treatment of heart failure (Richard *et al.*, 1993).

The vascular endothelium has an important role in the regulation of vascular tone, releasing relaxing and contracting factors under basal conditions or during exercise. The increased peripheral resistance in patients with chronic heart failure is related to the alterations in autonomic control, including heightened sympathetic tone, activation of the RAS system, increased endothelin concentrations, and impaired release of endothelium derived relaxing factor (or nitric oxide). There is emerging evidence that impaired endothelial function in chronic heart failure may be improved with exercise training and drug treatment, such as ACE inhibitors (Jackson *et al.*, 2000).

1.6.3.3 Activation of the renin-angiotensin system

Enhanced sympathetic outflow to the kidneys causes an increase in renin release. This is mediated by β-adrenoceptors in the kidney. Plasma renin activity, therefore, is often elevated in heart failure patients, in part, because of increased sympathetic activity. Increased renin release causes increased formation of Ang II that has several important effects on volume regulation, blood pressure regulation, and cardiac function and pathology (Colucci and Braunwald, 2000).

Stimulation of renin angiotensin system leads to increased concentrations of renin, plasma Ang II, and aldosterone. Angiotensin II is a potent vasoconstrictor of the renal (efferent arterioles) and systemic circulation, where it stimulates release of NA from sympathetic nerve terminals, inhibits vagal tone, and promotes the release of aldosterone. This leads to the retention of sodium and water and the increased excretion of potassium. In addition, Ang II has important effects on cardiac myocytes and may contribute to the endothelial dysfunction that is observed in chronic heart failure (Jackson *et al.*, 2000).

1.6.4 Pathophysiology of heart failure

Congestive heart failure is a syndrome with multiple causes that involve the right ventricle, the left ventricle, or both. Cardiac output in congestive heart failure is usually below the normal ranges. The causes of the decreased cardiac output that eventually leads to heart failure can be roughly divided into two groups. In patients with conditions such as myocardial infarction or some forms of valvular disease, the ventricle is distorted and less able to contract efficiently (systolic dysfunction) and the end systolic volume is increased. In patients with hypertension and some forms of cardiomyopathy, diastolic compliance is decreased and end-diastolic volume is increased (diastolic dysfunction) (Grossman, 1991 and Ho *et al.*, 1993).

The most important intrinsic compensatory mechanism is myocardial hypertrophy. This increase in muscle mass helps to maintain cardiac performance in the face of adverse effects such as pressure or volume overload, loss of functional tissue (myocardial infarction), or decrease in cardiac contractility. However, after an initial beneficial effect, hypertrophy can lead to ischemic changes, impairment of diastolic filling, and alterations ventricular geometry (Bertam and Katzung, 1995). Briefly, systolic dysfunction results from loss of intrinsic inotropy (contractility), most likely due to alterations in signal transduction mechanisms responsible for regulating inotropy. Global systolic dysfunction results from a loss of intrinsic inotropy (contractility), most likely due to alterations in signal transduction mechanisms responsible for regulating inotropy. Global systolic dysfunction can also result from the loss of viable contracting muscle as following acute myocardial infarction. Diastolic dysfunction refers to the diastolic properties of the ventricle and occurs when the ventricle becomes less compliant, which impairs ventricular filling. Both systolic and diastolic dysfunctions

result in higher ventricular end-diastolic pressure which serves as a compensatory mechanism by utilizing the Frank-Starling mechanism to augment stokes volume. The ventricle dilates as preload pressures increase in order to recruit the Frank- Starling mechanism in an attempt to maintain normal stroke volumes (William *et al.*, 1998)

Most commonly, a primary change in cardiac dysfunction precipitates changes in vascular function, blood volume, and neurohumoral status. To a point, changes in these non–cardiac factors serve as compensatory mechanisms to help maintain cardiac output (primarily by the Frank-Starling mechanism) and arterial blood pressure (by systemic vasoconstriction). However, change in non-cardiac factors can also precipitate or aggravate cardiac dysfunction. Therefore, some of the most effective treatments for chronic heart failure involve modulating non-cardiac factors such as arterial and venous pressures by administering vasodilator and diuretic drugs (Richard *et al.*, 1993).

1.6.4.1 Neurohumoral changes

Neurohumoral responses in heart failure include activation of sympathetic nerve and the renin-angiotensin system, and increased release of antidiuretic hormone (vasopressin) and artial natriuretic peptide. The baroreceptor reflex appears to be at "rest" with a lower sensitivity to arterial pressure, in patients with heart failure. Hance baroreceptor sensory input to the vasomotor center is reduced and even at normal pressure sympathetic outflow is increased whereas parasympathetic outflow is decreased (Packer, 1992b and Jackson *et al.*, 2000). Increased sympathetic outflow causes tachycardia, increased cardiac contractility, and increased vascular tone. Increased arterial tone results in increased afterload and decreased ejection fraction, cardiac output, and renal perfusion. Elevation of venous tone results in increased