

**ADRENERGIC CONTROL OF RENAL
HEMODYNAMICS IN DIFFERENT
PATHOPHYSIOLOGICAL STATES WITH RENAL
IMPAIRMENT: THE ROLE OF α_1 -ADRENOCEPTOR
SUBTYPES**

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Universiti Sains Malaysia

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OF α_1 -ADRENOCEPTOR SUBTYPES**

By

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To
*my beloved mother late Mrs. Halima Khan
and my father-in law late Md. Abdul Latif*

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LIST OF ABBRIVIATIONS

α	Alpha
AMP	Amlodipine
Ang II	Angiotensin ii
ANOVA	Analysis of variance
ANP	Atrial natriuretic peptide
AR	Adrenoceptor
ARF	Acute renal failure
ATN	Acute tubular necrosis
ATPase	Adenosinetriphosphatase
B.Wt.	Body weight
β	Beta
BMY 7378	(-[2-[4-(-methoxyphenyl)-1-piperazinyl]-8-azaspiro[4.5]decane-7,9-dione) dihydrochloride
cAMP	Cyclic 3,5 –adenosine monophosphate
CEC	Chloroethylclonidine
CNS	Central nervous system
CRF	Chronic renal failure
DAG	Diacylglycerol
DM	Diabetes mellitus
EDN	Early diabetic nephropathy
ε	Epsilon
et al	and others
FE _{NA}	Fractional excretion of sodium
G	Gram
γ	Gamma
GSH	Glutathione
Hz/hz	Herz
i.p.	Intraperitoneal
IP ₃	Inositol 1,4,5-triphosphate
JGA	Justaglomerular apparatus
K _d	Dissociation constant
λ	Lambda

MAP	Mean arterial pressure
MeU	5-methylurapidil
Mg/dl	Milligram per deciliter
Mg/kg	Milligram per kilogram
μg	Microgram
ml	Milliliter
ml/min/kg	Milliliter per minute per kilogram
mmHg	Millimeter mercury
mMol/dl	Millimol per deciliter
mRNA	Messenger RNA
ms	Millisecond
MTX	Methoxamine
n	Number of animals
NA	Noradrenaline
ng	Nano gram
ω	Omega
PCR	Polymerase chain reactions
P _{Cr}	Plasma creatinine
PE	Phenylephrine
PIP ₂	Phosphatidylinositol 4,5-biphosphate
PLA	Phospholipase A
PLC	Phospholipase C
P _{Na+}	Plasma sodium
RAAS	Renin-angiotensin-aldosterone system
RAS	Renin-angiotensin system
RBF	Renal blood flow
RNase	Ribonuclease
RNS	Renal nerve stimulation
RT-PCR	Reverse transcriptase-polymerase chain reaction
SHR	Spontaneously Hypertensive Rat
SHR RF	Renal failure spontaneously Hypertensive rat
SNS	Sympathetic nervous system
SPSHR	Stroke-prone hypertensive rat
STZ	Streptozotocin
TGF	Tubuloglomerular feedback
θ	Theta

2KIC	Two kidney one clip
U _{Cr}	Urinary creatinine
U _{Na+}	Urinary sodium
UO	Urine output
V	Volt
vs	Versus
WI	Water intake
WKY	Wistar Kyoto rat
WKY RF	Renal failure Wistar Kyoto rat

**KAWALAN ADRENERGIK KE ATAS HEMODINAMIK GINJAL DALAM
PELBAGAI KEADAAN PATOFISIOLOGI DENGAN KECACATAN GINJAL:
PERANAN α_1 -ADRENOSEPTOR SUBJENIS**

ABSTRAK

Kajian ini menyelidik samada berlaku sebarang perubahan dalam populasi α_1 -adrenoseptor berfungsi dalam mengawalatur vasokonstriksi ginjal diaruh secara adrenergik di dalam model-model haiwan berpenyakit dengan kecacatan ginjal. Keadaan-keadaan patologikal ini termasuk kegagalan ginjal akut, gabungan kegagalan ginjal akut dengan hipertensi, dan nefropati diabetis awal. Juga, usaha dibuat untuk mengenalpasti α_1 -adrenoseptor subjenis berfungsi yang terlibat di dalam mengawalatur hemodinamik ginjal di dalam keadaan-keadaan penyakit ini. Tikus jantan Wistar Kyoto (WKY) dan Spontaneously Hypertensive Rats (SHR) dengan berat 250-300g telah disuntik cisplatin (5 mg/kg i.p.) atau streptozotosin (55 mg/kg i.p.) dan diaruh dengan kegagalan ginjal akut atau nefropati diabetis awal. Dalam kumpulan kegagalan ginjal, 7 hari kemudian, tikus-tikus ini dibius (natrium pentobarbiton, 60 mg/kg i.p.) dan kajian hemodinamik dijalankan. Manakala, di dalam tikus dengan nefropati diabetis awal, kajian hemodinamik dijalankan selepas 4 minggu diberi streptozotosin. Dalam kajian hemodinamik, perubahan dalam pengaliran darah ginjal disebabkan rangsangan saraf elektrik dan pemberian noradrenalin, fenilefrin dan metoksamin secara intra ginjal tertutup ditentukan sebelum dan selepas penggunaan amlodipin, 5-metilurapidil, kloroetilklonidin dan BMY7378. Dalam kegagalan ginjal dan nefropati diabetis awal, ketidakcekapan ginjal disahkan dan ditentukan dengan perubahan fisiologi, iaitu jumlah air diminum dalam 24j, jumlah kencing dalam 24j dan berat badan. Di samping itu,

beberapa parameter fungsi, iaitu penyingkiran kreatinin, pengkumuhan terpecah natrium, kadar penapisan glomerulus, indeks ginjal dan pemerhatian histologi juga diambilkira. Tikus dengan kegagalan ginjal mempunyai ciri-ciri oligourea, penyingkiran kreatinin yang rendah, indeks ginjal yang tinggi, pengkumuhan terpecah natrium yang meningkat dan kadar penapisan glomerular yang berkurangan (kesemua $p < 0.05$). Selain perubahan-perubahan ini, tikus dengan nefropati diabetis awal juga menunjukkan pengaliran darah ginjal yang berkurangan ($p < 0.05$). Di dalam tikus dengan kegagalan ginjal dan nefropati diabetis awal, respon vasokonstriktor ginjal kepada aruhan secara adrenergik (RNS, NA, PE, ME) dipengaruhi secara signifikan ($p < 0.05$) oleh kesemua antagonis adrenergik. Secara umum, AMP, MEU dan BMY 7378 secara signifikan merencat respon vasokonstriktor ginjal di dalam kesemua kumpulan ujikaji (kesemua $p < 0.05$). Bagaimanapun, di dalam kumpulan kajian fungsi normal ginjal, CEC tidak mempengaruhi (kesemua $p > 0.05$) vasokonstriksi ginjal teraruh adrenergik (RNS, NA, PE and ME). Keputusan yang diperolehi dari kajian ini menunjukkan dengan jelas kehadiran α_1 -adrenoseptor subjenis yang pelbagai dalam pengawalatan respon vasokonstriktor ginjal teraruh adrenergik di dalam saluran darah ginjal tikus dengan atau tanpa kecacatan ginjal. Kajian ini juga menunjukkan yang α_1 -adrenoseptor yang terlibat ialah α_{1A} - dan α_{1D} -subjenis. Oleh itu, keputusan-keputusan ini menunjukkan tiada sebarang keadaan patologikal yang digunakan dalam kajian ini menyebabkan perubahan besar di dalam populasi α_1 -adrenoseptor subjenis ginjal berfungsi. Keputusan kami, walaubagaimanapun, buat pertama kalinya melaporkan dengan yakin akan α_1 -adrenoseptor subjenis berfungsi di dalam saluran darah ginjal dengan pelbagai bentuk kecacatan ginjal. Adalah juga dicadangkan di dalam saluran darah ginjal tikus dengan kecacatan ginjal, selain dari α_{1A} - dan α_{1B} -adrenoseptor subjenis, kemungkinan ada jenis-jenis lain α_1 -adrenoseptor subjenis atau α_2 -adrenoseptor yang memainkan peranan. Keputusan-keputusan ini menunjukkan satu kemungkinan penglibatan α_{1B} -adrenoseptor subjenis di lokasi pre- dan pasca-

sinaptik pada saluran darah ginjal tikus dengan kecacatan ginjal. Juga, di dalam tikus dengan kecacatan ginjal, wujud satu interaksi yang kompleks dan juga satu perhubungan di kalangan α_1 -adrenoseptor subjenis pada tahap saluran darah berintangan ginjal di dalam pengawalaturan respon vasokonstriktor ginjal teraruh adrenergik.

**ADRENERGIC CONTROL OF RENAL HEMODYNAMICS IN DIFFERENT
PATHOPHYSIOLOGICAL STATES WITH RENAL IMPAIRMENT: THE ROLE OF
 α_1 -ADRENOCEPTOR SUBTYPES**

ABSTRACT

This study investigated whether there is any alteration in the functional population of α_1 -adrenoceptors in mediating adrenergically induced renal vasoconstrictions in animal models of some pathological states characterized with renal impairment. These pathological states include acute renal failure, combined state of acute renal failure and hypertension and experimental early diabetic nephropathy. Further, attempts were made to characterize the functional α_1 -adrenoceptor subtypes involved in modulating renal hemodynamics in these pathological states. Male Wistar Kyoto (WKY) and Spontaneously Hypertensive Rats (SHR) weighing 250-300g were given either cisplatin (5 mg/kg i.p.) or streptozotocin (55 mg/kg i.p.) to induce with acute renal failure and experimental early diabetic nephropathy, respectively. In renal failure groups, seven days later the rats were anesthetized (sodium pentobarbitone, 60 mg/kg i.p.) and hemodynamic studies were conducted. While, in the rats with experimental early diabetic nephropathy the hemodynamic studies were carried out four weeks post streptozotocin. In the hemodynamic studies, the changes in the renal blood flow (RBF) caused by the electrical stimulation of renal nerves (RNS) and close intrarenal administration of noradrenaline (NA), phenylephrine (PE) and methoxamine (ME) were determined before and after amlodipine (AMP), 5-methylurapidil (MEU), chloroethylclonidine (CEC) and BMY 7378. In renal failure

and experimental early diabetic nephropathy rats, the renal insufficiencies were confirmed and characterized by physiological changes viz. 24h water intake, 24h urine output and body weight. Further, several renal functional parameters viz. creatinine clearance, fractional excretion of sodium, glomerular filtration rate, kidney index and histological observations were also considered in this respect. The renal failure rats were characterized with marked oligourea, severely reduced creatinine clearance, increased kidney index, increased fractional excretion of sodium, reduced glomerular filtration rate and severe reduction of renal blood flow (all $p < 0.05$). While the early diabetic nephropathy rats had severe polyurea, hyperglycemia, moderately decreased creatinine clearance, increased kidney index, fractional excretion of sodium and glomerular filtration rate (all $p < 0.05$). Beside these changes, the early diabetic nephropathy animals also had reduced renal blood flow ($p < 0.05$). In renal failure and early diabetic nephropathy rats, the adrenergically (RNS, NA, PE, ME) induced renal vasoconstrictor responses were significantly (all $p < 0.05$) influenced by all the adrenergic antagonists. In general, AMP, MEU and BMY 7378 significantly attenuated the renal vasoconstrictor responses in all experimental groups (all $p < 0.05$). CEC showed interesting and varied responses in the rats with renal impairment and these responses were either attenuation or accentuation of the adrenergically induced renal vasoconstrictor responses (all $p < 0.05$). However, in the experimental groups with normal renal functions CEC did not influence the adrenergically induced (RNS, NA, PE and ME) renal vasoconstrictions (all $p > 0.05$). The results derived in this study clearly indicated the presence of multiple functional α_1 -adrenoceptor subtypes in mediating the adrenergically induced renal vasoconstrictor responses in the renal vasculature of the rats with or without renal impairment. This study also showed that these α_1 -adrenoceptors were of α_{1A} - and α_{1D} -subtypes. Hence, the results obtained indicated that none of the pathological states used in this study caused any major

shift in the functional population of renal α_1 -adrenoceptor subtypes. Our results, however, for the first time convincingly reported the functional subtypes of α_1 -adrenoceptor in the renal vasculature of rats with different forms of renal impairments. It was further suggested that in the renal vasculature of rats with renal impairment, apart from α_{1A} - and α_{1D} -adrenoceptor subtypes, there could be other α_1 -adrenoceptor subtypes and even the α_2 -adrenoceptors that might play a potential functional role. The results obtained particularly indicated a possible functional involvement of α_{1B} -adrenoceptor subtypes both at the pre- and post-synaptic sites in the renal vasculature of rats with renal impairment. Furthermore, in the rats with renal impairment there was a complex interaction and also a cross-talk relationship between the α_1 -adrenoceptor subtypes at the level of renal resistance vessel in modulating adrenergically induced renal vasoconstrictor responses.

CHAPTER ONE

INTRODUCTION

1.1 The Kidney

1.1.1 Basic anatomy

The kidneys are the primary organs of the urinary system and essential for life. A cell's function depends not merely upon receiving a continuous supply of nutrients and eliminating its metabolic end products but also upon the existence of stable physicochemical conditions in the extra-cellular fluids bathing it or the "internal environment" as mentioned by Claude Bernard's. Maintenance of such stability is the most important function of the kidneys (Vander, 1980).

The kidneys ensure that the body is in a homeostatic balance of fluids and ions essential for most intra- and extra-cellular physiological functions. The kidney processes the plasma portion of the blood by removing the substances from it and in some instances by adding some substances to it. In this effort, they carry out an array of functions. The most important function that kidney plays is the essential role in the regulation of water concentration, inorganic-ion composition and volume of the internal environment. The kidneys excrete the metabolic waste products into the urine as fast as they are produced in the body. The kidney also plays an important role in the excretion of xenobiotics (drugs, pesticide, food additives and their metabolites etc.) in urine. Moreover, the kidneys regulate the level at which the blood pressure is set by means of secreting renin and thus act as an endocrine gland. Being an endocrine gland they are also involved in the synthesis of humoral agents including

1,25-dihydroxyvitamin D, erythropoietin etc. The kidneys also play a pivotal role in the maintenance of blood pressure by virtue of their ability to regulate blood volume and salt excretion. Indeed, there is a well-accepted notion that persistently elevated blood pressure can be resulted due to an inappropriately functioning kidney (Vander, 1980; Sattar, 1994; Applegate, 2000; Vander *et al.*, 2001).

Anatomically, the paired kidneys are located between the 12th thoracic and 3rd lumbar vertebrae, one on each side of the vertebral column. They are located just above the waist between the parietal peritoneum and the posterior wall of the abdomen and hence are known as retroperitoneal organ. In adults, each kidney is about 12 cm long, 6 cm wide and 3 cm thick. It is roughly a bean-shaped organ with indentation on the medial side called hilum. At the hilum, the renal veins leave while the renal arteries enter into the kidney. A frontal section of the kidney shows that it is made of renal cortex and medulla. The cortex and medulla make up the renal parenchyma, which is the functional tissue of the kidney (Applegate, 2000; Vander *et al.*, 2001).

The functional unit of the kidney is nephron. Each kidney consists of over a million of such units in the renal parenchyma. Each nephron is made up of two parts, an initial filtering component known as renal corpuscle and renal tubules that extend out from the renal corpuscle. The renal corpuscle consists of a cluster of capillaries known as glomerulus encapsulated by a double-layered epithelial cup, the glomerular capsule and function as an ultrafilter through which a considerable quantity of cell-free and almost protein free fluid is filtered from the plasma. The renal corpuscles are continuous with the renal tubules that carry fluid away from the glomerular capsules and mainly consist of proximal convoluted tubule, loops of Henlé and distal convoluted tubules (Vander, 1980, 1995; Applegate, 2000; Vander *et al.*, 2001).

1.1.1.1 The glomerulus

The glomerulus consists of a compact tuft of glomerular capillaries and a balloon like fluid-filled capsule known as Bowman's capsule into which this tuft of glomerular capillaries protrudes. The combination of glomerulus and Bowman's capsule constitute the renal corpuscle. Each glomerulus is supplied with blood by an afferent arteriole and as blood flows through the glomerulus, a portion of the plasma filters into the Bowman's capsule. The remaining blood then leaves the glomerulus by the efferent arteriole. The blood in the glomerulus is separated from the fluid in the Bowman's space (the space exists within the capsule) by a filtration barrier that consists of three layers: capillary endothelium, basement membrane and a single celled layer made of capsular epithelial cells. The functional importance of this anatomical arrangement is that the blood, which flows through the glomerular capillary is separated from the Bowman's space by a set of membranes already mentioned. The filtered fluid then flows to the site where Bowman's capsule is joined at the side which is opposite of the glomerular tuft and attached with the first portion of the tubules (Vander, 1980, 1995; Vander *et al.*, 2001).

1.1.1.2 The juxtaglomerular apparatus

The ascending limb of the loop of Henlé comes into contact with the glomerular afferent arterioles of the same nephron in a region where it continues into distal convoluted tubule. In this region of contact the macula densa is formed from the modified cells of the ascending limb whereas juxtaglomerular cells are formed from those in the afferent arterioles. The macula densa and juxtaglomerular cells together form the juxtaglomerular apparatus (Vander, 1980; Applegate, 2000).

1.1.1.3 The tubules

The renal tubule is continuous with the Bowman's capsule and is a narrow hollow cylinder made of a single layer of epithelial cells. The epithelial cells of the tubule differ in structure and function along the tubule's length and 10 to 12 different segments have been recognized so far. The renal tubule consists mainly of three regions, proximal convoluted tubule, loop of Henlé and distal convoluted tubule. Considering the scope of this thesis we will not discuss all these regions in detail, rather a brief description of each will be presented.

Proximal tubule

The segment of the tubule that drains Bowman's capsule is known as proximal tubule. It comprises of the proximal convoluted tubule and the proximal straight tubule. These two parts of proximal tubule differ from each other structurally and functional heterogeneity may also exist. In the proximal tubule, variation in terms of morphology is believed to exist in human and animal. In human, it is observed that the proximal tubule is comprised of the convoluted and the straight portions (Guyton and Hall, 1996). In the rat, rabbit and monkey the proximal tubule consist of three different segments (Maunsbach, 1973; Guyton and Hall, 1996). The first segment is the initial portion of the convoluted tubule (S1), the terminal portion of the pars convulata and the first part of the pars recta made the second segment (S2) while the remainder of the pars recta before its transition into the thin descending limb of the loop of Henlé makes the third segment (S3). Structure of the brush border, the number and size of mitochondria, basolateral invagination and lysosomes distinguished three segments of proximal tubule (Guyton and Hall, 1996).

Loop of Henlé

Following the proximal tubule is a sharp hairpin-like structure known as loop of Henlé, made of a descending limb, straighten from the proximal tubule and an ascending limb leading to distal convoluted tubule. The descending limb is quite thin throughout its entire length. On the other hand, the ascending limb remains thin in the first portion of a long loop and then becomes thick in the upper portion. However, in the short loop the entire ascending limb is thick.

The distal tubule

Three morphologically distinct segments form the distal tubule, the thick ascending limb of loop of Henlé, the macula densa and the distal convoluted tubule. At the end of the ascending limb of Henlé's loop, the tubule passes between the arterioles supplying its glomerulus of origin and this particular segment is known as macula densa. The tubule again becomes coiled just ahead of the macula densa and known as distal convoluted tubule. The distal convoluted tubule on returning to the glomerulus of origin makes a short marginal contact with the afferent arteriole just before it enters into the Bowman's capsule (Vander, 1980, 1995).

The collecting ducts

Urine passes from the nephron into the collecting duct system. In relation to the other part of the renal tubule, fluids from the distal convoluted tubule flows into the collecting tubule. The first part of this collecting duct is the connecting tubule, which is followed by the cortical and then the medullary collecting ducts. Each of the over one million nephron, from the glomerulus to the collecting duct system is completely separated from each other. In fact, multiple initial collecting tubules from separate nephron join to form cortical collecting duct which then turn downward into the medulla

and form the medullary collecting duct. They finally drain urine into the kidney's central cavity known as renal pelvis which is continuous with the ureter draining the kidney (Vander, 1980; Applegate, 2000; Vander *et al.*, 2001).

1.1.2 Renal hemodynamics

The total blood flow to the kidneys in a typical adult is approximately 1.1 L/min. Remarkably, although the combined weight of both kidneys is less than 1% of the total body weight, the kidneys receive almost 20-25% of total cardiac output (5 L/min) i.e. almost 1.1 -1.2 L/minute (Guyton, 1989; Ganong, 1999; Applegate, 2000).

The kidneys are highly vascularized organs abundantly supplied with blood vessels. The right and left renal arteries carry the 20-25% of the resting cardiac output to the kidney. The renal artery typically divides into a larger anterior branch and a comparatively smaller posterior branch just before or immediately after entering into the hilus of the kidney. Several segmental arteries, each supplying a particular segment of the kidney originates from these branches. These segmental arteries are further subdivided into branches and those that enter into the renal parenchyma between the renal pyramids in renal column are known as interlobar arteries. These interlobar arteries arch between the cortex and medulla at the bases of the pyramids and are known as arcuate arteries. The renal arcuate arteries give off branches known as interlobular arteries that extend into the cortex and give rise to the afferent arterioles (Guyton, 1989; Guyton and Hall, 1996; Ganong, 1999; Applegate, 2000).

The blood passes through the capillaries in the glomerulus of the renal corpuscle from these afferent arterioles and then from the glomerular capillaries into the efferent arterioles. Each renal corpuscle receives one afferent arteriole which

divides into the tangled capillary network known as glomerulus and these glomerular capillaries then reunite to form efferent arteriole. Each efferent arteriole of a cortical nephron divides to form an extensive capillary network known as peritubular capillaries around the tubular portion (proximal and distal convoluted tubules) of the nephron. The peritubular capillaries also form from the efferent arterioles of juxtamedullary nephron. The efferent arterioles also form long loop-shaped vessels known as vasa recta or the hairpin loop. These loops deep down into the medulla alongside the loop of Henlé. To supply a number of different nephron, the efferent arterioles from each glomerulus break up into the capillaries (Guyton, 1989; Ganong, 1999; Applegate, 2000). The peritubular capillaries reunite to form peritubular venules and then interlobular veins. The interlobular veins also receive blood from the vasa recta. The blood then drains through the arcuate veins to the interlobular veins running between the pyramids and the segmental veins and then into the renal veins that exist at the hilus of the kidney. The renal veins return the blood into the inferior vena cava (Applegate, 2000; Ganong, 1999; Guyton, 1989).

The blood flow through the kidneys distributed into large number of vascular channels arranged in parallel circuits, each supplied with similar perfusion pressure. The local blood flow is controlled by the vascular resistance of each channel and may therefore show disproportionate changes in various regions of the kidney (Auckland, 1980). The microvessels of each kidney also have the ability to autoregulate the renal blood flow. The intrarenal blood flow distribution is very important for renal functions and hence alterations in the regional blood flow distribution may cause changes in the renal function (Regan *et al.*, 1995). The nerve supply to the kidney derives from the renal plexus of the sympathetic nervous system and accompanies the renal artery and their branches and is distributed to the blood vessels. They control the blood flow through the kidney by regulating the diameter of the arterioles (Guyton, 1989; Ganong,

1999; Applegate, 2000). The sequence of blood flow through the kidney is depicted in the Figure 1.1.

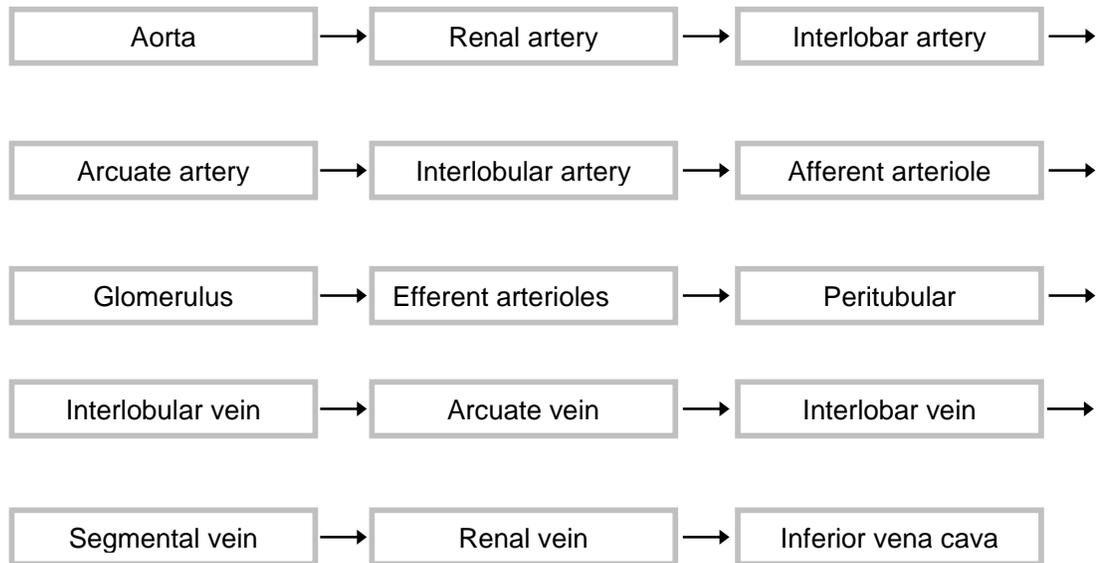


Figure 1.1: The blood flow through the kidney goes in the sequence of the following order (Adapted from Ganong, 1999; Applegate, 2000)

1.1.2.1 Factors influencing renal hemodynamics

The renal blood flow is controlled by a number of physical and humoral factors and these factors are either intrinsic or extrinsic to the kidney. The major intrinsic factors involved in the control of renal blood flow include autoregulatory mechanism, intrarenal renin-angiotensin mechanism, eicosanoids and kinins. The extrinsic factors involved in the regulation of renal blood flow that includes the sympathetic nervous system, angiotensin II, antidiuretic hormones, dopamine and histamine. Beside these, there are some other factors that play important role in causing changes in the renal hemodynamics. These include endothelin, nitric oxide (NO), and atrial natriuretic

peptide (ANP) (Vander, 1995; Unwin and Capasso, 2000; Koeppen and Stanton, 2001; Osborn *et al.*, 2001; Paul and Ploth, 2001).

1.1.2.1.1 Intrinsic factor

Autoregulation

The renal circulation markedly manifests the phenomenon of autoregulation. The autoregulation is an intrarenal system that maintains a constant renal blood flow (RBF) despite a fluctuation of mean arterial pressure (MAP) of about 80-180 mmHg. However, the autoregulation will become dysfunctional if the change in MAP is out of this range (Vander, 1980, 1995). In the autoregulation phenomenon two mechanisms i.e. the myogenic and tubuloglomerular feedback mechanisms are involved. The myogenic mechanism is solely based on the functions of baroreceptors or stretch receptors in the afferent arterioles. In the face of increased MAP, the baroreceptors respond to the increased vascular wall tension and cause increased constriction of afferent arterioles. This constriction prevents transmission of the increased arterial pressure to the glomerulus, hence, maintain the glomerular capillary pressure and glomerular filtration rate (Guyton, 1989; Vander, 1995). In the event of a fall in the MAP, dilation of afferent arterioles occurs to allow for increased blood flow and maintenance of a normal glomerular capillary pressure and glomerular filtration rate. The myogenic mechanism of autoregulatory phenomenon may also be influenced by the increase and decrease in the amount of oxygen and other nutrients (Vander, 1995; Unwin and Capasso, 2000; Osborn *et al.*, 2001; Paul and Ploth, 2001).

The other mechanism of autoregulation which plays an important role in the control of renal hemodynamics is the tubuloglomerular feedback (TGF) mechanism that involves juxtaglomerular apparatus or JGA. In fact, the TGF mechanism related to the

function of macula densa and JGA cells. When there is any increase in RBF or GFR, there will be an increased delivery of NaCl to the macula densa cells in the distal nephron. In response to this NaCl load the cells of macula densa mediate vasoconstriction of the afferent arterioles and that in turn causes an increase in the glomerular blood flow, decrease in the glomerular capillary pressure and finally normalization of GFR. In contrast, when there is a decrease in the delivery of NaCl to the macula densa there will be a decrease in the glomerular blood flow. This change in the glomerular blood flow leads to afferent arteriolar dilatation, increase in glomerular blood flow, increased glomerular capillary pressure and consequently the return of GFR towards normalization. Adenosine has been identified as the possible mediator for TGF mechanism of autoregulation of renal hemodynamics. This mechanism works more slowly than the myogenic mechanism. However, it is critical for the maintenance of GFR in conditions that cause changes in intra-arteriolar pressure (Vander, 1995; Unwin and Capasso, 2000; Osborn *et al.*, 2001; Paul and Ploth, 2001).

Renin-angiotensin

The renin-angiotensin system is an important regulator of intrarenal blood flow. The granular cells of juxtaglomerular apparatus secrete renin in response to several kinds of stimuli like hypovolemia, decreased cardiac contractility and renal artery stenosis. The regulation of renin release is carried out by both intra- and extra-renal mechanisms. The intra-renal mechanism comprises of afferent arteriolar baroreceptors and cells of macula densa while the extra-renal mechanism is the sympathetic nervous system. Renin is released when renal baroreceptors cause constriction of the afferent arterioles in the face of decreased volume. As far as the macula densa is concern, its cells stimulate renin release when they detect a decreased NaCl load being delivered to the distal tubules as a result of decreased RBF. Angiotensin II is produced by the action of renin and angiotensin converting enzyme and is an active hormone which

causes vasoconstriction of both the afferent and efferent arterioles. It decreases RBF through afferent arteriolar constriction. However, simultaneous efferent arteriolar constriction produces resistance to fluid leaving the glomerular capillaries, which maintains glomerular hydrostatic pressure and GFR at normal despite a fall in RBF. The renin-angiotensin system is an intrinsic system triggered by local and systemic stimuli. One of the important functions of this system is to maintain the perfusion of the vital organs like heart and brain in the face of hypovolemia and intrarenal vasoconstriction (Vander, 1995; Navar, 1998; Unwin and Capasso, 2000; Osborn *et al.*, 2001; Paul and Ploth, 2001).

Eicosanoids

The kidney produces a variety of eicosanoids locally in the nephron, interstitial cells and by the glomerular and vascular endothelium. These eicosanoids include prostaglandins, thromboxanes, leukotrienes, and monooxygenase products. Some of these eicosanoids are prostaglandins such as PGE₁, PGE₂ and prostacyclin like PGI₂ which act as vasodilators. Whereas, other eicosanoids like thromboxane, leukotrienes, and some monooxygenase products are vasoconstrictors. The vasodilatory prostaglandins play a major role in maintaining normal RBF in individuals with impaired renal function. On the other hand, the physiological role of the vasoconstrictor prostaglandins is still unclear. The best documented role of vasodilatory prostaglandins, however, is to counteract the vasoconstrictor effects of renal nerve and angiotensin. These prostaglandins primarily act on the afferent arterioles and counteract the effects of angiotensin II and renal nerves. In this way, these prostaglandins maintain the RBF despite systemic arteriolar vasoconstriction. The prostaglandins also counteract the angiotensin II induced mesangial constriction and thereby preserve adequate surface area for filtration. The vasodilatory prostaglandins also protect the kidneys by preventing them from extreme vasoconstriction in response

to angiotensin II or norepinephrine (Vander, 1995; Unwin and Capasso, 2000; Osborn *et al.*, 2001; Paul and Ploth, 2001).

Kallikrein-Kinin

These are inflammatory mediators produced in the kidney. The bradykinin is formed in the kidney by the action of kallikrein secreted from the distal nephron cells. The physiological role of bradykinin in the control of renal hemodynamics is not fully understood, however, they are known as a vasodilator. As far as the vasodilatory effect is concern, bradykinin stimulates the release of nitric oxide that leads to vasodilation. It is also reported that they attenuate the renal ischemic effects of angiotensin II and norepinephrine (Osborn *et al.*, 2001; Paul and Ploth, 2001).

1.1.2.1.2 Extrinsic factors

Several extrinsic factors are known to influence the renal hemodynamics. These substances or systems are mainly based outside the kidneys and can directly or indirectly initiate changes in RBF and GFR. These extrinsic factors include sympathetic nervous system, angiotensin II, nitric oxide, endothelins, atrial natriuretic peptide, antidiuretic hormone (ADH) and dopamine. Considering the importance of these factors in the regulation of RBF and GFR and also the context of the present study, here we will focus only on the effect of sympathetic nervous system and angiotensin II.

Sympathetic nervous system

The kidney is richly innervated with sympathetic nerves that enter the kidney at the hilum. The nerve endings terminate in the smooth muscle cells of the afferent and efferent arterioles. The afferent and efferent arteriolar vasoconstrictor responses are

produced by the direct activation of adrenergic receptors present in the cell surface of smooth muscle cells. Indeed, strong activation of the renal sympathetic nerves result in substantial renal vasoconstriction mediated by α -adrenoceptors (mainly α_1 -adrenoceptors) which leads to a decrease in RBF and GFR. However, the simultaneous efferent arteriolar vasoconstriction helps to maintain the GFR by regulating the glomerular hydrostatic pressure (Vander, 1995; Unwin and Capasso, 2000; Osborn *et al.*, 2001; Paul and Ploth, 2001). The α -adrenoceptors mediated increase in renal vascular resistance also represents a pathway for the α -adrenoceptors that influences the renin secretion through baroreceptors dependent mechanism (Navar, 1998).

Angiotensin II

Angiotensin II mediates multiple actions that act in concert to minimize renal blood fluid and sodium losses and to maintain arterial pressure (Navar, 1998). In addition to its renal vascular effects, angiotensin II also stimulates the release of aldosterone that allows sodium and water reabsorption and increase the circulating volume. These two effects combine for the maintenance of systemic pressure and perfusion of vital organs of the body. Angiotensin II also stimulates the release of vasodilatory prostaglandins that attenuate the vasoconstrictive effects of angiotensin II in the kidneys. Thus they play an important role in the maintenance of renal blood flow and glomerular filtration rate (Navar, 1998; Unwin and Capasso, 2000; Paul and Ploth, 2001).

1.1.3 Innervations of the kidney

1.1.3.1 Intrinsic innervations

The kidney is innervated by intrinsic nerves and comprised of both efferent and afferent innervations. The efferent intrinsic innervations of the kidney represents an expansion of the central nervous system that responds to peripheral and central afferent inputs. A number of techniques have been used for the characterization of intrinsic innervations of the kidney; however, the electron and fluorescent microscopy have helped the most in the final characterization of intrinsic renal innervations. The efferent sympathetic nerves reach all the segments of renal vasculature, entering at the hilus of the kidney. These sympathetic innervations are distributed throughout the renal cortex and outer strip of the medulla having the highest density in the juxtamedullary region of the inner cortex and the lowest density in the tubules (Barajas *et al.*, 1992). These innervations follow the vasa recta into the outer medulla and simultaneously disappear with the disappearance of smooth muscle cells (Dinerstein *et al.*, 1979).

It is observed that an increased net renal venous outflow of norepinephrine derived from renal nerve terminals could result from direct stimulation of renal nerve. Chronic renal denervation could cause significant decrease in the renal norepinephrine concentration, hence, highlighting the importance of norepinephrine as a neurotransmitter (Femandez-Repollet *et al.*, 1985). This notion also came under significant consideration from the increased norepinephrine concentration in the venous blood after stimulation of renal sympathetic nerve (Kopp *et al.*, 1983).

It has been proposed that low level of renal tubular innervation is inadequate to offer a full explanation of tubular effect of such innervations (Luff *et al.*, 1992). It has

also been proposed that how a neurotransmitter would reach the tubular epithelial cells could be explained by a substantial release of norepinephrine into the renal interstitium cells (Barajas *et al.*, 1984).

In a relatively recent report, it is proposed that functionally specific nerve fiber groups separately innervate tubules, juxtaglomerular granular cells and vessels and constituted the sympathetic renal nerves (DiBona, 2000).

The foregoing reports may provide explanation on how the adrenergic renal nerves produce different effects on a variety of renal functional responses including tubular function as it allows for a different extent of renal nerve stimulation of some nerve fiber groups compared to others. The sensory afferent renal nerves are localized in the corticomedullary connective tissues of the pelvic region and in the major vessels (Barajas *et al.*, 1992). These sensory afferent renal nerves project into ipsilateral dorsal root ganglia and the dorsal horn in the spinal cord. These nerves are projected into medullar and hypothalamic sites that also receive afferent nerve fibers from the carotid sinus (DiBona, 1985).

The role of the kidney in the homeostatic regulation of body fluid volume is ensured by peripheral afferent input provided by the sensory innervations. Indeed, the renal sympathetic nerves are increasingly considered as being significant in the control of renal hemodynamics and tubular functions to maintain body's fluid homeostasis, hence, blood pressure (DiBona and Kopp, 1997).

Apart from sympathetic innervations, the intrinsic innervations of the kidney also consist of dopaminergic and parasympathetic nerves. The presence of dopaminergic

nerves in the kidney is a compelling issue (DiBona, 1990). However, dopamine has been found to be present in all adrenergic nerve terminals as a precursor of noradrenaline. Dopamine is released from the adrenergic sympathetic postganglionic nerve terminals together with noradrenaline (DiBona and Kopp, 1997).

In kidney, the parasympathetic innervation is presumed to be the part of the abdominal visceral distribution of the vagus nerve. Acetylcholine is secreted by the postganglionic parasympathetic nerve terminals and their existence has been inferred by the use of histochemical staining technique for acetylcholinesterase. However, there is no conclusive evidence in favor of independent cholinergic innervation in kidney. In studies with retrograde tracing for possible efferent parasympathetic innervation in rat showed that the rat kidney did not have functional cholinergic innervation (DiBona and Kopp, 1997).

1.1.3.2 Extrinsic innervations

Several different labeling techniques have contributed in the understanding of the extrinsic innervations of the kidney. In this effort, particularly in the rat, acetylcholinesterase was utilized in the tracing of efferent and afferent nerve fibers between the kidney and also other nerves like the celiac plexus, splanchnic nerves, the lumbar splanchnic nerves and the inter-mesenteric nerve plexus (Drukker *et al.*, 1987). Indeed, the use of different labeling techniques allowed to trace into the central nervous system (Vollanueva *et al.*, 1991; Barajas *et al.*, 1992). The regulatory centers of the renal sympathetic activity include the rostral ventrolateral medulla (RVLM), A5 area, caudal raphe nuclei of the sympathetic premotor nuclei and paraventricular nucleus of the hypothalamus (Ding *et al.*, 1993). Among these the rostral ventrolateral

medulla is the most important for the cardiovascular reflexes and also in the generation of sympathetic tone (Dampney, 1994).

Anatomically, the extrinsic innervation of the kidney originates from the 12th thoracic and 1st lumbar segments, passes through the celiac ganglia and its subdivisions, the lumbar splanchnic nerves, the superior mesenteric ganglion and along the renal artery towards the kidneys (Lumley *et al.*, 1996). The celiac plexus comprises of the aorticorenal ganglion, celiac ganglion and major splanchnic nerves. The thoracic splanchnic nerves, lumbar splanchnic nerves and vagus nerves partly form the celiac ganglion. The suprarenal ganglion gives off many branches towards the adrenal gland and some of which pass along the adrenal artery to the perivascular neural bundles around the renal artery and entering into the hilus of the kidney, while other branches enter into the outer side of the renal hilar region of the kidney (Lumley *et al.*, 1996; Dworkin *et al.*, 2000).

1.2 Sympathetic control of renal hemodynamics

It is well known that the sympathetic nervous system plays a pivotal role in the regulation of renal hemodynamics. The kidneys receive a rich supply of sympathetic nerves and it is perceived that the renal sympathetic nerve activity plays an important role in the regulation of renin release and tubular sodium reabsorption (Yoshimoto *et al.* 2004). There is large body of evidence indicating the involvement of renal adrenergic nerves in the regulation of renal vascular resistance (Navar, 1998; Salomonsson and Arendshorst, 2001). In renal vasculature, the catecholamines released from nerve terminals and of humoral origin exert their effect by the stimulation of adrenoceptors present in the cell surface of smooth muscle cells in order to produce changes in the cytosolic calcium concentration and subsequent contraction. In this process, the

binding of ligands to the cell surface adrenoceptors lead to the binding of calcium ion with calmodulin followed by a change in calmodulin structure. This change in the structure of calmodulin then cause activation of myosin light chain kinase that in turn cause increased tone of smooth muscle cells (Walsh, 1994).

It is observed that an increase in the sympathetic nervous system activity of the kidney causes direct increase in renal vascular resistance. Marked renal vascular constriction mediated by α -adrenoceptors can be caused by strong activation of renal nerve that leads to decreased renal blood flow and glomerular filtration rate, increased renin release and increased sodium and water reabsorption. Increased renal adrenergic nerve activity can be a part of overall sympathetic response or may be more selective. Decrease in renal nerve activity can be induced reflexively through cardiopulmonary receptors and renorenal reflexes (Navar, 1998; Salomonsson and Arendshorst, 2001). It is observed that low-level of renal nerve stimulation can cause activation of β -adrenoceptors which in turns cause renin release by direct action on the juxtaglomerular apparatus cells. Moderate level of renal nerve stimulation causes comparable increase in afferent and efferent arteriolar resistance and leads to a slightly higher drop in renal blood flow than glomerular filtration rate. However, higher level of renal nerve stimulation causes strong periglomerular resistance that leads to a major drop in the renal blood flow. These observations, indeed, showed an important role of the sympathetic control of the renal hemodynamics (Navar, 1998).

1.3 Role of kidney in hypertension – special reference to the role of renal impairment

The kidney and hypertension were linked decades before the sphygmomanometer had been invented. Richard Bright, a remarkably astute observer of autopsies, noted in 1836 (Bright, 1836) that patients with the disease now known as chronic nephritis also had hypertrophied hearts. Based on his remarkable observations, he postulated that in these patients the quality of the blood was altered in some manner that required heart to work harder in order to force it through the blood vessels. In 1889 after the invention of sphygmomanometer it soon became clear that the quality of the blood Bright was seeking was blood pressure (Rocci, 1896). In relation to these observations, few years later two investigators from Denmark working on the connection between kidney and hypertension shed light on renin as a long-lasting pressor effector substance present in the blood stream and localized in the renal cortex and further strengthened the view that kidney, indeed, played a role in the genesis of hypertension (Ritz *et al.*, 2003).

Kidney is considered as a vital organ of the body that plays a dominant role in the maintenance of volume in the body fluid spaces through its influence on sodium and water excretion. Thus, it is inevitable that kidney must be involved in the pathophysiology of hypertension. This is because of the fact that the renal perfusion pressure is a major determinant of sodium and water excretion and as mentioned by Guyton *et al.* (1974) that hypertension could not be stated unless the relationship between renal perfusion pressure and its output of sodium and water had been altered.

Indeed, in his original hypothesis Guyton (Guyton *et al.*, 1974) had proposed that a disturbance of the blood pressure-natriuresis relationship is responsible for the

elevated blood pressure observed in the case of impaired renal function. This statement does not, however, indicate that hypertension is a renal disease, rather implied that the kidney and more precisely the pressure–natriuresis relationship is the *sine qua non* condition for the development of hypertension (Ritz *et al.*, 2003).

As we have discussed in the foregoing paragraphs, the influence of the kidney on hypertension is, indeed, strong as was first pointed out in the work of Bright and others. In the recent decades, the use of kidney transplantation studies strongly supported the notion that kidney was involved in the genesis of hypertension. It was observed that when a person with end-stage renal diseases received a kidney from a normal donor, the elevated blood pressure often comes to normal, hence strengthening the testimony to the antihypertensive action of a normal kidney and therefore the role of the kidney and/or diseased kidney.

Several experiments of renal transplantation in rats with genetic hypertension and normal animals showed that “blood pressure goes with kidney”. It has been observed that transplantation of the kidney from a spontaneously hypertensive donor rat causes a progressive elevation of blood pressure in a normotensive recipient animal which had been immunologically manipulated to prevent a possible rejection reaction (Bianchi *et al.*, 1974; Rettig *et al.*, 1990; Patschan *et al.*, 1997). These results indicate that the kidney somehow carries the messages of blood pressure and that the kidney can modulate the level of blood pressure within the body. The same phenomenon has been observed in human as recipients of renal graft from hypertensive donors are found to require more hypertensive medication. In all of these reports it was assumed that the hypertensive donors might have damaged kidney caused by the persistent hypertension or at least they had impaired renal function, a common feature of long-lasting genetic hypertension (Curtis *et al.*, 1983).

Considering these observations several studies have been carried out and it has been reported that kidney could be involved in the genesis of hypertension by virtue of its two obvious endocrine cell types. One of these is the juxtaglomerular cell that secretes renin. Renin causes the elaboration of angiotensin I (ANG I) which converted into angiotensin II (ANG II), one of the most potent pressor agents known so far. ANG II constricts arterioles directly and causes the adrenergic nerves to release more norepinephrine. It acts on specific receptors in the brain to cause an increased sympathetic activity. It enhances aldosterone secretion from adrenal zona glomerulosa and also could act on the renal tubules to cause retention of sodium. Another endocrine cell present in the kidney having vasoactive properties is the interstitial cell which is present in the inner medulla of the kidney of all mammals. These cells secrete prostaglandin and neutral lipid and most importantly they have ANG II receptors in the cell wall. These cells are believed to be involved in the regulation of blood pressure by the kidney. The kidney can also control blood pressure by way of sodium excretion. One good example of this event could be someone with renal parenchymal diseases where one-fourth of the nephron still remains intact. Such patients come into sodium balance with a high level of sodium in the body and very apt to have hypertension, contrasting with someone possessing good kidney with no difficulties in sodium excretion. Thus, the capability of the body to excrete sodium is another means by which kidney can be related in the genesis of hypertension (Tobin, 1983).

In relation to the pressure-natriuresis hypothesis mentioned in the forgoing paragraphs, it has also been hypothesized that the baroreceptors in the kidney, which control the release and secretion of renin, are exposed to an inhomogeneous spectrum of perfusion pressure caused by the luminal narrowing of some of the preglomerular vessels (Navar *et al.*, 2002). As a consequence, a proportion of the glomeruli senses inappropriately low perfusion pressure and secretes renin independent of the systemic

blood pressure. Indeed, it has been shown that in patients with renal diseases, the plasma renin activity is elevated and is not adequately suppressed when blood pressure is increased. It is also reported that the local renin-angiotensin system is activated if renal damage has occurred (Kuczera *et al.*, 1991).

In recent years, based on studies carried out in animals and in human it has been reported that intrarenal chemoreceptors and baroreceptors are stimulated in renal diseases. Such stimulatory signals are transmitted through the medulla to the hypothalamus causing increased norepinephrine turnover in the hypothalamus and efferent sympathetic nerve traffic. It has been seen that increased sympathetic nerve traffic as documented by microneurography of the sural nerve, was no longer demonstrable in dialyzed patients after they had undergone bi-nephrectomy. Similarly, after renal transplantation, the sympathetic nerve activity remains high, but is decreased once the non-functional kidney of the patients has been removed. However, it is still unknown to which extent such activation of the sympathetic nervous system by the kidneys contribute in the pathogenesis of hypertension (Converse *et al.*, 1992; Hausberg *et al.*, 2002).

Another pathway through which the renal abnormalities can interfere with the blood pressure control is increased oxidative stress and endothelial cell dysfunction. In various renal damage models, it has been observed that hypertension can be ameliorated by the administration of tempol, a cell membrane permeable agonist of superoxide dismutase. It has been postulated that increased oxidative stress scavenges nitric oxide and diminish the bioavailability of this endogenous vasodilator (Vaziri *et al.*, 2003) and hence causes vasoconstriction leading to the elevated blood pressure.

1.4 Receptors

Receptors are defined as various cell membrane recognition sites with which various drugs, hormones and neurotransmitters interact to exert discrete biological effects (Williams *et al.*, 1995).

In the early 19th century the concept of receptor first evolved from a historical observation of the extraordinary potency and specificity of some drugs that mimicked biological responses. Drugs able to mimic a particular type of biological response were known as agonist while those that inhibited such responses were named antagonists. Later, this phenomenon was further clarified in terms of quantitative characteristic of competitive antagonism between agonists and antagonists in combining with specific receptors in intact cell preparation. In light of these observations, the concept of receptors has been established by the isolation of molecules that truly fit all the criteria of being receptors (Cooper *et al.*, 1996). To date many receptors have been identified for many neurotransmitters as well as for other biomolecules like angiotensin, bradykinin, opioid peptides, histamines and so on (Cooper *et al.*, 1996). Moreover, multiple rather than single receptors have been shown for some molecules. For instance, the biogenic amines, viz. acetylcholine, GABA, histamine, opiates, the amino acid transmitters and others have multiple receptors.

Receptors study is considered one of the most intensively investigated areas of research in vascular pharmacology and neuroscience which has led to the development of new drugs based on the research of adrenergic, dopaminergic, muscarinic, serotonergic and histaminergic receptors. Recent advances in molecular biological techniques have further enhanced this progress by enabling scientists to identify several receptor subtypes, their mRNAs, relative abundance, tissue distribution

and so on and hence led to the development of drugs highly specific for a certain subtype (Cooper *et al.*, 1996).

1.4.1 Adrenoceptors

The adrenoceptors are membrane receptors located in both neuronal and non-neuronal tissues. These heterogeneous groups of receptors constitute a subfamily of the seven transmembrane domains/G-protein couple receptors involved in mediating central and peripheral actions of endogenous catecholamines, adrenaline and noradrenaline. Noradrenaline is released from the noradrenergic post-ganglionic nerve terminals and secreted from the adrenal medulla. These catecholamines are involved in several important function including cardiovascular, respiratory and neuronal functions. They are also involved in digestion, energy metabolism and endocrine functions. The adrenoceptors through which they operate thus constitute immense importance as therapeutic targets in the treatment of a range of ailments including cardiovascular diseases, asthma, prostatic hypertrophy, obesity etc. (Docherty, 1998; Guimarães and Moura, 2001).

1.4.1.1 Classification of adrenoceptors

Different studies started since early eighteenth century have shaped our present knowledge about adrenoceptors and have facilitated their classification. The first step of any systematical approach in the discovery of the adrenoceptor was made in 1905 from the observations of Dale (Dale, 1905). He observed that the pressor effect of adrenaline was reversed by ergotoxine into a depressor effect. In 1937, Cannon and Rosenblueth put forward a hypothesis, which initiated the idea of classification of adrenoceptors. They put forward their hypothesis based on the existence of two neurotransmitters or endogenous mediators in the organism to explain