NEURAL CONTROL OF RENAL HAEMODYNAMICS IN DIABETES AND HYPERTENSION: THE ROLE OF α_1-ADRENOCEPTOR SUBTYPES

By

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α	alpha
ANOVA	analysis of variance
ANP	atrium natriuretic peptide
ATPase	adenosine triphosphatase
β	beta
BW	body weight
CEC	chloroethylclonidine
CGRP	calcitonine gene-related peptide
cNOS	constitutive nitric oxide synthase
DGB	direct granular bodies
DM	diabetes mellitus
DNA	deoxyribonucleic acid
DOCA	deoxycorticosteroneacetate
ECDCDM	Expert Committee On The Diagnosis And Classification Of Diabetes Mellitus
et al.	And others
5-MeU	5-methylurapidil
g	gram
GAD	glutamic acid decarboxylase
GDM	gestational diabetes mellitus
GOD	glucose oxydase
α	alpha
GSH	glutathione
HLA	human leucocyte antigene
HLA-DQ/DR	human leucocyte antigene DQ/DR
HNF	hepatocyte nuclear factor
i.a.	intraarterialy
i.e.	in example
i.m.	intramuscular
i.p	intraperitoneal
i.v.	intravenous
IAA	insulin autoantibody
I-cell	intercalated cells
IDDM	insulin-dependent diabetes mellitus
kg	kilogram
λ	lambda/ wavelength
LINES	local immune-neuroendocrine system
MAP	mean arterial pressure
ME	methoxamine
mg	milligram
mg/dl	milligram/deciliter
mg/kg	milligram/kilogram
μg	microgram

ml	milliliter
ml/min/kg	milliliter/minute/kilogram
mmHg	millimeter mercury
mMol/dl	millimol/deciliter
MODY	maturity-onset diabetes of the young
mRNA	messenger ribonucleic acid
n	number of animals
NA	noradrenaline
NAD	nicotinamide adenine dinucleotide
NDDG	National diabetes data group
ng	nanogram
NIDDM	non-insulin-dependent diabetes mellitus
nm	nanometer
NO	nitric oxides
NOS	nitric oxide synthase
NPY	neuropeptide Y
OGTT	oral glucose tolerance test
θ	omega
P-cell	principal cells
PCr	plasma creatinine
PE	phenylephrine
PG	plasma glucose
PgE_2	prostaglandin E_2
PNa	plasma sodium
RBF	renal blood flow
rDNA	recombinant deoxyribonucleic acid
RNA	ribonucleic acid
RNS	renal nerve stimulation
RTPCR	reverse transcryptase-polimerase chain reaction
s.c.	subcutaneous
SD	Sprague Dawley
SHR	spontaneously hypertensive rat
SP	substance P
SPSHR	stroke-prone spontaneously hypertensive rat
STZ	streptozotocin
2K1C	two kidney one clip
UCr	urine creatinine
UNa	urine sodium
VIP	vasoactive intestinal peptide
WHO	world health organization
WKY	Wistar Kyoto

KAWALAN SARAF TERHADAP HAEMODINAMIK GINJAL DALAM DIABETES DAN HIPERTENSI : PERANAN SUBJENIS ADRENOSEPTOR α_1

ABSTRAK

Diabetes dapat mencetuskan perkembangan kerosakan saluran darah yang berkaliber kecil dan besar serta saraf periferal dan keadaan ini meningkatkan risiko serangan jantung, strok, kebutaan, amputasi dan kegagalan ginjal (Porte dan Schwartz, 1996), dan tekanan darah yang tinggi memburukkan lagi kerosakan ini (Todd *et al.*, 1993). Adrenoseptor-α_1 diketahui berperanan penting dalam vasokonstriksi terhadap rangsangan saraf adrenergik. Akan tetapi, belum ada informasi mengenai fungsi daripada subjenis adrenoseptor-α_1 pada rintangan pembuluh ginjal dalam diabetes. Kajian ini meneliti peranan subjenis adrenoseptor-α_1 dengan cara membandingkan respons vasokonstriksi terhadap pelbagai rangsangan adrenergik dengan dan tanpa kehadiran beberapa antagonis pada ginjal tikus diabetik dan hipertensi.

Dalam penyelidikan ini tikus Sprague Dawley (SD), "spontaneously hypertensive rats" (SHR) dan "two kidney one clip" (2K1C)-Goldblatt hipertensi digunakan. Streptozotocin (STZ) (55 mg/kg, ip) digunakan untuk mengaruh diabetes dan tikus-tikus digunakan 7 hari selepas suntikan STZ.. Berat badan (BW), pengambilan air (WI), pengeluaran urin (UO), paras glukosa, natrium dan kreatinin plasma, ekskresi sodium dan kreatinin selama 24 jam ditentukan.

Setelah tikus dibiuskan (pentobarbiton, 60 mg/kg), tikus dipersiapkan untuk pengukuran tekanan darah. Ginjal kiri didedahkan melalui pembedahan bahagian

tengah abdomen, dan sebuah prob flowmeter electromagnetik dipasangkan pada arteri ginjal untuk mengukur aliran darah. Saraf ginjal dikenalpasti, dan sebuah elektrod perangsang bipolar diletakkan padanya. Aliran darah ginjal (RBF) terhadap rangsangan langsung saraf ginjal (RNS) pada 1, 2, 4, 6, 8 and 10 Hz, 15mV dan 2 millisaat selama 20 saat dan pemberian agonis adrenergik noradrenalina (25, 50, 100 dan 200 ng), fenilefrina (0.25, 0.5, 1 dan 2 μg) and metoksamina (1, 2, 3 dan 4 μg) tanpa dan dengan kehadiran antagonis ditentukan Antagonist yang digunakan ialah nitrendipina (suatu penghambat terusan Ca^{++} jenis-L), 5-metilurapidil (suatu antagonis adrenoseptor-α_{1A}), kloroetilklonidina (suatu agen pengalkilasi adrenoseptor-α_{1B}) and BMY 7378 (suatu antagonis adrenoseptor-α_{1D}). Data dianalisa dengan ANOVA tiga faktor untuk RBF dan ANOVA dua faktor untuk parameter-parameter yang lain, dan nilai purata ± s.e.m. dibandingkan dengan menggunakan "multi range "t" test" dan dianggap signifikan pada paras 5%.

Keputusan menunjukkan bahawa kadar glukosa plasma, "nilai basal" dari pada tekanan darah nilai purata (MAP) and RBF pada tikus SD diabetik adalah 291.5 ± 13.0 mg/dl, 122 ± 6 mmHg and 26.5 ± 3.0 ml/kg/ min dan pada tikus bukan diabetik adalah 118.0 ± 4.8 mg/dl, 122 ± 8 mmHg and 24.6 ± 1.8 ml/min/kg. Pada tikus SHR diabetik, nilai tersebut adalah 316.2 ± 10.5 mg/dl, 144 ± 12 mmHg and 27.0 ± 4.1 ml/kg/min dibandingkan dengan tikus bukan diabetik di mana nilai 112.3 ± 4.7 mg/dl, 156 ± 24 mmHg and 28.1 ± 2.6 ml/kg/min diperolehi. Selanjutnya, pada tikus 2K1C diabetes, nilai kadar glukosa darah, "nilai basal" MAP dan RBF adalah 298.0 ± 11.6 mg/dl, 125 ± 6 mmHg dan 27.7 ± 4.2 ml/kg/min sedangkan pada tikus 2K1C bukan diabetik nilainya adalah

113 ± 5.3 mg/dl, 144.3 ± 3 mmHg dan 28.9 ± 1.2 ml/kg/min berturut-turut. Respons vasokostriktor ginjal menunjukkan bahawa pada tikus SD, SHR dan 2K1C bukan diabetik, respons vasokonstriksi ini dimediasi oleh adrenoseptor subjenis α_{1A}- dan α_{1D}- tetapi dalam tikus SD dan 2K1C diabetik adalah adrenoseptor α_{1A}, sedangkan dalam tikus SHR dan oleh adrenoseptor subtipe α_{1A}- dan α_{1D}- pada tikus SHR diabetes. Terdapat kemungkinan terlibatnya adrenoseptor pre-sinaps (samada α_{1B}, α_{1L} atau α_2) pada tikus SHR dan 2K1C diabetes dalam memediasi vasokonstriksi pembuluh darah di ginjal.

ABSTRACT

Diabetes mellitus could trigger the development of damage of small and large caliber blood vessels and peripheral nerves; wich greatly increasing the risk of heart attack, stroke, blindness, amputation and renal failure (Porte and Schwartz, 1996), and hypertension exacerbate these alterations (Todd *et al.*, 1993). α_1-adrenoceptors are known to play an important role in the vasoconstrictions in response to adrenergic stimulation. However, there is no information about the functional importance of α_1-adrenoceptor subtypes in the renal vascular resistance in diabetes. This study sets out to examine the role α_1-adrenoceptor subtypes by comparing the vasoconstrictor responses to different adrenergic stimulations in the presence and absence of several antagonists in the kidney of diabetic and hypertensive rats.

Sprague Dawley (SD), spontaneously hypertensive rats (SHR) and two kidney one clip (2K1C)-Goldblatt hypertensive rats were used. Streptozotocin (55 mg/kg, ip) was utilized to induce diabetes and rats were used 7 days post treatment. The body weight (BW), water intake (WI), urine output (UO), the plasma glucose, sodium and creatinine levels, 24-hour sodium and creatinine excretion were measured. After anaesthesia (pentobarbitone, 60 mg/kg, i.p.), the rats were prepared for the blood pressure measurements. Left kidney was exposed via a midline abdominal incision, and an electromagnetic flowmeter probe was fitted on the renal artery for blood flow measurements. The renal nerve was identified, sectioned and placed on bipolar stimulating electrodes. The renal blood flow (RBF) to direct renal nerve stimulation (RNS) at 1, 2, 4, 6, 8 and 10 Hz at 15mV

and 2 millisecond for 20 seconds) and administration of adrenergic agonists noradrenaline (25, 50, 100 and 200 ng), phenylephrine (0.25, 0.5, 1 and 2 μg) and methoxamine (1, 2, 3 and 4 μg) in the absence and presence of the antagonists were determined. The antagonists used were nitrendipine (an L-type Ca^{++} channel blocker), 5-methylurapidil (an α_{1A}-adrenoceptor antagonist), chloroethylclonidine (an alpha1B-adrenoceptor alkylating agent) and BMY 7378 (an alpha 1D-adrenoceptor antagonist) were utilized. Data were analyzed using three way ANOVA for RBF, and two way ANOVA for the rest of the parameters, and the means ± s.e.m. were compared with multi range "t" test and Duncan's Post-Hocs test, and the significance taken at the 5% level.

Results showed that plasma glucose, base-line MAP and RBF in diabetic SD rats were 291.5 ± 13.0 mg/dl, 122 ± 6 mmHg and 26.5 ± 3.0 ml/kg/ min respectively and in non-diabetic rats the values were 118.0 ± 4.8 mg/dl, 122 ± 8 mmHg and 24.6 ± 1.8 ml/min/kg respectively. In diabetic SHR these values were 316.2 ± 10.5 mg/dl, 144 ± 12 mmHg and 27.0 ± 4.1 ml/kg/min respectively as compared to non-diabetic SHR 112.3 ± 4.7 mg/dl, 156 ± 24 mmHg and 28.1 ± 2.6 ml/kg/min respectively. In the diabetic 3K1C rats, the values of blood glucose level, base-line MAP and RBF were 298.0 ± 11.6 mg/dl, 125 ± 6 mmHg and 27.7 ± 4.2 ml/kg/min respectively, whereas in non-diabetic 2K1C rats these values were 113 ± 5.3 mg/dl, 144.3 ± 3 mmHg and 28.9 ± 1.2 ml/kg/min respectively. Renal vasoconstrictor responses measurements indicated that in non-diabetic SD, SHR and 2K1C rats, these vasoconstrictions are mediated by α_{1A}- and α_{1D}-adrenoceptors, but in the diabetic SD and 2K1C rats it is mediated by α_{1A}-adrenoceptors and by α_{1A}- and α_{1D}-adrenoceptors in the diabetic SHR.

Furthermore, evidence has been obtained showing the involvement of pre-synaptic (either α_{1B}, α_{1L} or α_2 –) adrenoceptors in diabetic SHR and 2K1C rats in the mediation of the renal vasoconstrictions.

CHAPTER I

INTRODUCTION

1.1. Receptors

To produce biological effects, most drugs, hormones and neurotransmitters interact with receptors. Receptors are described as various recognition sites at which drugs act (Williams *et al.*, 1995). This concept was first described in the earlier nineteenth century based on an observation of the extraordinary potency and specificity of some drugs that mimicked a biological response (agonists) while others inhibited it (antagonists). Later, Clark (1933), independently described the quantitative characteristics of competitive antagonism between agonists and antagonists in combining with specific receptors in intact preparations. This receptor concept has been substantiated by isolation of macromolecule substances that fit all the criteria of being receptors. To date, receptors have been identified for all the proven neurotransmitters as well as for histamines, opioid peptides, neurotensin, bradykinin, angiotensin etc., although all of them have not been cloned (Cooper *et al.*, 1996).

Multiple receptors have been shown to co-exist for all the biogenic amines, acetylcholine, GABA, histamines, opiates, the amino acids transmitters and others. These receptors appear to metastasize at an uncontrollable rate, but it should be viewed doubtfully until a physiological response to the ligand has been shown or a specific gene has been cloned and expressed. Currently, the research for receptors

is amongst the most intensively investigated area in neurosciences. Identification of adrenergic, dopaminergic, muscarinic, serotoninergic and histaminergic receptor subtypes has lead to the synthesis of highly specific drugs that are considerably more specific than their prototypes which were developed after general screening for activity. As in gene cloning and expression, more and more receptor subtypes are being identified, each presumably having its own function. This lead to the design of future drugs that fit with a single receptor subtype, thus precluding what are called the side effects of non-specific drugs. Most of the receptors are located on the surface of the cell, except receptors for steroids and thyroid hormones, which are localized intracellularly (Cooper, *et al.* 1996).

For many secreted transmitters, there are receptors on the presynaptic as well as on the postsynaptic elements. The presynaptic receptors serve as auto-receptors or heteroreceptors. Autoreceptors can facilitate or inhibit the release of neurotransmitters (Langer, 1997; and Ganong, 1999). The inhibition of neurotransmitter release mediated by autoreceptor was originally based on several findings. Firstly, the Ca^{2+} dependent release of neurotransmitter triggered by the action potential was inhibited by receptor agonists. Secondly, antagonists on their own, enhanced neurotransmitter release and finally interaction between agonists and antagonists that modulate transmitter release was of competitive nature. The presynaptic heteroreceptors are the second category of presynaptic receptors that modulate neurotransmitter release, as a response to the presence of chemical signals in the synaptic cleft other than the neurone's own transmitter. These presynaptic heteroreceptors are sensitive to cotransmitter neuropeptides, transmitter releases from adjacent terminals or other substances that are already produced or blood

borne, that either facilitate or inhibit the release of the neurotransmitters (Langer, 1997).

Receptors are dynamic in nature and undergo both ligand and gene related control throughout their life (Hollenberg *et al.*, 1985). The turnover of the receptors occur as a consequence of the growth with a halflife that varies between hours to days (Mahan *et al.*, 1987). Ligand occupancy can also alter the receptor density. Hence, the neurotransmission and neuromodulation processes are under tonic control. The molecular basis of many disease states may thus reflect an increase or a decrease in the stimulation of a given receptor system. This inequity in turn may reflect over or under production of the endogenous ligand, a decrease in receptor density or function, a persistence in activation due to maladaptation of the transduction system etc. (Williams *et al.*, 1995). The accumulation of evidence for the association of specific receptor density changes with specific disease states has been a major goal of biomedical research over the past two decades. Thereby molecular mechanisms involved in the disease process can be identified and used for drug targeting and disease diagnosis (Williams *et al.*, 1995).

1.1.1. Receptor Assays

The interaction of neurotransmitters, hormones and drugs with cells can be determined as follows:

1. By determining the biological responses of an intact isolated organ such as guinea pig ileum to the applied agonists or antagonists. The disadvantage of using this method is that the effect of cascade events beginning with transport,

distribution, metabolism of the agent before it interact with receptors and give responses are avoided (Cooper *et al.*, 1996).

2. By measuring ligand binding to a homogenated or slice preparation using highly specific radioactive ligand with high affinity for the receptors. This can be done directly or indirectly, by incubating receptor preparation with labeled agonist / antagonist. The receptor-ligand complex is then separated from the free ligand by centrifugation, filtration or precipitation by using equilibrium dialysis in which the receptors-ligand and complex is determined by subtracting the ligand concentration in bath from that of the dialysis sac respectively (Cooper *et al.*, 1996).
3. Through molecular biological studies using the recombinant DNA (rDNA) technology. This technique requires the isolated DNA encoding the protein of interest from the library of complimentary DNA sequences. Once cloned and identified, the nucleotide sequence of the receptor gene can be determined and this allows the primary structure of isolated receptor to be deduced. The ability of a drug to alter the structure of cloned receptor specifically through modification of small number of nucleotides is a standpoint of drug discovery (William *et al.*, 1995).
4. By using the electrophysiological techniques in which the receptor subtype(s) is fractionally identified by the intracellular stimulation and recording via microelectrodes which are inserted into brain slice or neurons in culture combined with the application of receptor agonists and antagonists (Cooper *et al.*, 1996).

5. By using macromolecular crystallography technique. This technique is based on the ability of a molecule to scatter the X-ray and the geometry of scattering is governed by Bragg's law $n\lambda = 2d\sin\theta$, where n is a positive interger, λ is the wavelength, d is the spacing of plane in the crystal and the θ is the angle of incidence to the plane. Generally, the macromolecule is highly purified (better than 99%) before determination and then crystallized (Oakly and Wilce, 2000).

1.1.2. Adrenergic Receptors

Adrenergic receptors (adrenoceptors) are receptors that mediate the central and peripheral action of primary sympathetic neurotransmitters noradrenaline and the primary adrenal medullary hormone (and central transmitter) adrenaline. Adrenoceptors are found in most of the peripheral tissues and in many neuronal populations 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). This sub-classifications are based on several functional, molecular and radioligand binding

studies after several considerations. Firstly, the different major types of adrenoceptors affinity for selective drugs is 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 are different and finally, that the predicted amino acid sequences of the adrenoceptors are more consistent with three rather than two major types (Bylund, 1992). These three subtypes are further sub-classified into several subtypes, α_{1A}, α_{1B}, α_{1D}, and α_{1L}, α_{2A}, α_{2B}, α_{2C}, and α_{2D}, and β_1, β_2 and β_3 (Bylund *et al.*, 1994; Cooper, 1996; and Zhong and Minneman, 1999).

1.1.2.1. α_1-adrenoceptors

α_1-adrenoceptors exist as a heterogenous family. More than a decade ago, pharmacological studies indicated the existence of α_{1A}- and α_{1B}-adrenoceptors (Morrow and Greese, 1986; Han, *et al.*, 1987; and Minneman, 1988). More recent studies by molecular cloning technique have exposed the co-existence of α_{1a}-, α_{1b}-, and α_{1d}-adrenoceptors. 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; Bylund *et al.*, 1995; and Hieble *et al.*, 1995). Functionally, these receptors were characterized by their high affinity for prazosin (previously called α_{1H}) and low affinity for yohimbine (Bylund, *et al.*, 1994). Other subtype (if any) with a low affinity to prazosin i.e. the α_{1L}, has been postulated to mediate contractions in some tissues (Flavahan and Vanhoutte, 1986; Muramatsu *et al.*, 1990; Oshita *et al.*, 1991;

Ford *et al.*, 1996; and Zhong and Minneman, 1999), but its relationship to cloned receptors remains to be established (Bylund *et al*, 1998). This receptor is more prominent in the human, rabbit and dog urethra and in the dog and human prostate (Fukasawa *et al.*, 1998; and Langer, 1999), and is also suggested to mediate the contraction of canine pulmonary artery (Flavahan *et al.*, 1998).

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}-adrenoceptor 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 a 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 cells 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 intracellular compartment (Hrometz *et al.*, 1999).

All α_1-adrenoceptor subtypes are activated by the sympathetic neuro-transmitters, noradrenaline and adrenaline, even though none of these catechol-amines exhibit selective affinity to any of these adrenoceptor subtypes. α_1-

drenoceptor mediated responses are blocked by prazosin and showed a low affinity or selective α_2-adrenoceptor antagonists such as yohimbine or rauwolscine. These eceptors can be labeled with (^{3}H) prazosin or (^{125}I)IBE-2254, and activation of ach subtype is associated with an increase in intracellular calcium concentration Bylund *et al.*, 1994). α_{1A}-adrenoceptor has a higher affinity for 5-methylurapidil Ford *et al.*, 1994; Michel and Insel, 1994; Perez, *et al.*, 1994; and Testa *et al.*, 1995), KMD-3213 (Shibata *et al.*, 1995), S(+) niguldipine (Boer *et al.*, 1989; and Ford *et al.*, 1994), benoxathian (Han *et al.*, 1987) and oxymetazoline (Ford *et al.*, 1994), as compared to the other two subtypes. Dapiprazole seems to be moderately selective (approximately 10-fold) for the A and D over the B subtype of α_1-adrenoceptors (Eltze, 1997). BMY 7378 (8-[2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl]-8-azaspiro[4.5]decane-7,9-dionedihydro chloride)(Saussy, 1994; Goetz *et al.*, 1995; and Kenny, 1995) and SKF 105854 (Hieble, *et al.*, 1995) possess a higher affinity for α_{1D} subtype or cloned human α_{1D}-adrenoceptor. The α_{1D}-adrenoceptor is relatively resistant to alkylation by chloroethylclonidine (CEC) and has a low affinity for 5-methylurapidil and (+)niguldipine (Ford *et al.*, 1994). The α_{1B}-adrenoceptor (previously called α_{1b}) subtype is most sensitive to be alkylated by CEC (Minneman *et al.*, 1988; and Bylund *et al.*, 1994), risperidone (Sleight *et al.*, 1993; and Ford *et al.*, 1994) and spiperone (Michel *et al.*, 1987; and Ford *et al.*, 1994). Buspirone, like its close analogs, BMY 7378 and MDL 73005EF, may also be a useful tool for functionally discriminating α_{1D}- from α_{1A}-, α_{1B}- and α_{1L}-adrenoceptors in various tissues. This compound is a weak antagonist without an intrinsic activity towards α_{1A}-adrenoceptors in the rat vas deferens, α_{1B}-

adrenoceptors in guinea-pig and mouse spleen and towards α_{1L}-adrenoceptors in rabbit spleen. Buspirone also caused a partial vasoconstriction in the rat kidney that was attenuated by the α_{1D}-adrenoceptors antagonist BMY 7378, but hardly by the α_{1A}-adrenoceptors selective antagonist, B8805-033 ((+/-)-1,3,5-trimethyl-6-[[3-[4-((2,3-dihydro-2-hydroxy-methyl)-1,4-benzodioxin-5-yl)-1-piperazinyl] propyl] amino] 2,4 1H, 3H)-pyrimidinedione). Furthermore, Buspirone behaved as a partial agonist towards α_{1D}-adrenoceptors in the rat aorta and pulmonary artery (Eltze, 1999).

The new native and cloned classification of α_1-adrenoceptor subtypes and their historical cloned nomenclature are shown in Table 1.1.

Table 1.1. Nomenclature for α_1-adrenoceptor subtypes

Native	Cloned (New nomenclature)	Cloned (historical nomenclature)
α_{1A}	α_{1a}	α_{1c}
α_{1B}	α_{1b}	α_{1b}
α_{1D}	α_{1d}	α_{1a}, α_{1d}, $\alpha_{1a/d}$
α_{1L}	?	?

(Ford *et al.*, 1994; and Hieble *et al.*, 1995)

All α_1-adrenoceptor subtypes activate phospholipase C (Schwinn *et al.*, 1991; and Perez *et al.*, 1993) through the G(q/11) family of G proteins (Wu *et al.*, 1992), release stored Ca^{2-}, and activate protein kinase C (Zhong and Minneman, 1999) and inositol phosphate turnover (Docherty, 1998), although with significant differences in coupling efficiency ($\alpha_{1A} > \alpha_{1B} > \alpha_{1D}$). Other second messenger

pathways are also activated by these receptors, including Ca^{2+} influx (Han *et al.*, 1992; Sayet *et al.*, 1993; Lazou *et al.*, 1994; and Minneman and Esbenshade, 1994), arachidonic acid release, and phospholipase D activation. The α_1-adrenoceptor-induced vasoconstriction appears to be caused both by the 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). The ratio between both of these processes is very different, depending upon the type of α_1-adrenoceptor agonist and on the experimental preparations used (Van-Zwieten and Timmermans, 1987). α_1-adrenoceptors also activate mitogen-activated protein kinase pathways in many cells, although some of these responses are independent of Ca^{++} and protein kinase C and involve small G proteins and tyrosine kinases. Direct interactions of α_1-adrenoceptors with proteins other than G proteins have not yet been reported. However there is a consensus for the binding motive of the immediate early gene Homer in the C-terminal tail of the α_{1D} subtype (Zhong and Minneman, 1999).

α_1-adrenoceptors involve rapid processes such as sequestration and slower processes such as receptor down-regulation (Garcia-Sainz, 1993; and Cotecchia *et al.*, 1995). The slower down-regulation of these receptors may be related to the pathophysiological processes which occur in disease states such as cardiac failure and chronic renal failure (Packer, 1992). 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 clues for the possible role of α_1-adrenoceptor subtypes in the maintenance of elevated blood pressure (Ye and Colquhoun, 1998).

Functional expression of α_{1D}-adrenoceptors in the rat resistance vessels increases with age; α_{1A}-, but not α_{1B}- or α_{1D}-adrenoceptors, seems to predominate in immature animals. This represent the evidence that age-related changes in functional α_1-adrenoceptor subtypes occur in the systemic vasculature *in vivo* (DeOliveira *et al.*, 1998; Ibarra *et al.*, 1999; and Villalobos-Molina *et al.*, 1999). The steady state levels for α_{1A}-, α_{1B}- and α_{1D}-adrenoceptors in aorta declined with maturation and aging. In the renal artery, there was a decrease in mRNA for the α_{1B}-adrenoceptor in aged rats. However, in mesenteric and pulmonary arteries there were no changes in mRNA levels for the three subtypes of α_1-adrenoceptors as a result of maturation and aging (Xu, *et al.* 1997). 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}-adrenoceptors population in liver, kidney and heart of rats were not affected by age (Shen, 2000).

1.1.2.2. α_2-adrenoceptors

α_2-adrenoceptors can be pharmacologically divided into α_{2A}-, α_{2B}-, α_{2C}- and α_{2D}-adrenoceptors, all of which mediate contractile responses (Cooper, 1996; Docherty, 1998; and Zhong and Minneman, 1999). All subtypes can be blocked by yohimbine and rauwolscine and labeled with ^{3}H analog of these antagonists,

although the affinity varies substantially between the subtypes (Bylund *et al.*, 1994). α_{2A}-adrenoceptor subtype is characterized by its low affinity, and conversely, α_{2B}-subtype by its high affinity to prazosin (Bylund and Ray-Prenger, 1989). α_{2A}-adrenoceptors are selectively inhibited by BRL44408, while α_{2B} by ARC 239, spiroxatrine and imiloxan (Young *et al.*, 1989). α_{2C}-adrenoceptor is typically similar to α_{2B}-subtype. It has a relatively high affinity for prazosin, ACR 239 and spiroxatrine, but a higher affinity for rauwolscine. Other antagonists which are selective for α_2-adrenoceptor subtype are BAM 1303 and WB4101 (Bylund, 1994). The α_{2D}-adrenoceptor subtype has a low affinity for (^{3}H)rauwolscine than the other subtypes, low affinity for prazosin, spiroxatrine and ARC 239, but moderately selective for BAM1303 (Simonneaux *et al.*, 1991). It is presumed that the rat α_{2D}-adrenoceptor subtype is a human α_{2A}-adrenoceptor subtype homologue (Bylund *et al.*, 1994).

The α_2-adrenoceptors can be pre- and postjunctional. Some of the prejunctional α_2-adrenoceptors 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}-adrenoceptor subtype (the α_{2D}-adrenoceptor is a species orthologue). Furthermore, α_{2C}-adrenoceptors may also occur prejunctionally (Docherty, 1998). Although α_2-adrenoceptors are linked to inhibition of adenylate cyclase (Bylund *et al.*, 1994), this may not be the primary signal in causing smooth muscle contraction. Prejunctional inhibitory actions probably involving the restriction of Ca^{2+} entry or the opening of K^+ channels (Docherty, 1998).

1.1.2.3. Beta Adrenoceptors

Previously, two β-adrenoceptor subtypes were known to exist i.e. β_1- and β_2-adrenoceptors (Lands *et al.*, 1967). The subtype-selective agonists, antagonists and therapeutic application of several of these pharmacological classes have been developed. Further and later studies have successfully identified β_3-adrenoceptor subtype using selective agonists and recombinant receptor expression (Bylund *et al.*, 1994). The pharmacological characteristics of the recombinant receptors appear to correspond well with those of the three receptor subtypes identified in native tissues, although there are some differences in the case of the β_3-adrenoceptors.

Endogenous catecholamines, adrenaline and noradrenaline are equipotent to β_1-adrenoceptor with adrenaline having a 100-fold selectivity for β_2-adrenoceptors. Conversely, noradrenaline is more potent than adrenaline as a β_3-adrenoceptor agonist. Propranolol and many of its analogs are potent antagonists of β_1- and β_2-adrenoceptors. However, β_3-adrenoceptors seem to be less sensitive to these antagonists (Bylund *et al.*, 1994). Both β_1-adrenoceptor and β_2-adrenoceptors can be labeled with (^{3}H)dihydroalprenolol or (^{125}I)iodopindolol and its analogs, while β_3-adrenoceptors, although can be labeled with (^{125}I)iodocyanopindolol, has a 10-fold lower affinity compared to β_1- and β_2-adrenoceptors (Emorine *et al.*, 1992). All of the three β-adrenoceptor subtypes activate adenylyl cyclase as a primary mechanism for signal transduction (Bylund *et al.*, 1994).

β_1-adrenoceptors mediate the increases in cardiac rate and force of contraction, stimulation of renin secretion, relaxation of coronary arteries and relaxation of gastrointestinal smooth muscles. β_2-adrenoceptors mediate smooth

muscle relaxation at many sites, including airways, most blood vessels and uterus. The prejunctional β_2-adrenoceptors modulate noradrenaline release from the sympathetic nerve terminals (Bylund *et al.*, 1994).

1.1.3. α_1-adrenoceptors in Vasculature

The α_1-adrenoceptors, component of the sympathetic nervous system, is involved in the regulation of cardiovascular function, and its subtypes are heterogeneously distributed in various vessels (Kohno *et al.*, 1994). The differences in α_1-adrenoceptor population and distribution depend on the blood vessel and the pathological state (LeTran and Forster, 1997). Arterial vasoconstriction in the rat is primarily mediated by α_1-adrenoceptors (Ohyanagi *et al.*, 1991; Vargas and Gorman, 1995). The genes encoding the α_1- adrenoceptors are widely expressed in the heart and peripheral arteries (Perez *et al.*, 1994; Piascik *et al.*, 1995; Guarino *et al.*, 1996) and this has been shown by a variety of functional studies (Han *et al.*, 1990; Elhawary *et al.*, 1992; Bylund *et al.*, 1995; Kenny *et al.*, 1995; Piascik *et al.*, 1995; Testa *et al.*, 1995; Leech and Faber, 1996; Zhou and Varga, 1996). As discussed earlier, α_1-adrenoceptors comprises of α_{1A}-, α_{1B}-, α_{1D}- and α_{1L}-adrenoceptor subtypes and recent studies indicate that the rat vascular smooth muscle can express mRNAs for the α_{1A}-, α_{1B}- and α_{1D}-adrenoceptors besides α_{2B}-, and α_{2D}-adrenoceptors (Leech and Faber, 1996).

Although α_1-adrenoceptors are widely distributed, certain receptors are not link to mediating contraction in a majority of arteries in which they are expressed. In other words, receptors can be expressed but may not necessarily participate in the

contractile regulation (Hrometz *et al.*, 1999). As an example, the p53 knockout mice aortic smooth muscle line contains predominantly α_{1B}-adrenoceptors and a small population of α_{1D}-adrenoceptors. However, phosphoinositide/Ca^{2+} signaling of this cell line is mainly mediated through the minor population of α_{1D}-adrenoceptors (Ohmi, 1999).

According to Zhou and Vargas (1996), α_{1D}-adrenoceptors mediate vascular smooth muscle contraction *in vivo,* but further studies indicated the involvement of different α_1-adrenoceptor subtypes in a variety of vessels. According to Stassen *et al* (1998), there is a reversible positive relationship between the presence of adrenergic nerves and that of α_{1A}-adrenoceptor in rat arteries.

The guinea pig and rabbit aorta contractions are mediated by α_{1L}-adrenoceptors (Oshita *et al.*, 1993; Kenny *et al.*, 1995; Brackner *et al.*, 1996; and Yamamoto *et al.*, 1999) and α_{1D}-adrenoceptors (Piascik *et al.*, 1995), while the rat aorta contraction are mediated by α_{1B}-adrenoceptors (Testa *et al.*,1995) and α_{1D}-adrenoceptors (Hussain *et al.*, 1997). However, Satoh *et al.* (1999) reported that there is no indication of α_{1D}-adrenoceptors involvement in either thoracic or abdominal aorta and in the aortic smooth muscle line (Ohmi, 1999).

The contraction of bovine carotid and vertebral arteries are mediated by α_{1B}-adrenoceptors (Kohno *et al.*, 1994), whereas that of rat femoral artery is mediated by α_{1B}-adrenoceptors (Hrometz *et al.*, 1999) or α_{1L}-adrenoceptor (Kohno *et al.*, 1994). The contraction of rat iliac artery is mediated by α_{1D}-adrenoceptors (Kenny *et al.*, 1995; Piascik *et al.*, 1995; and Satoh *et al.*, 1999), and that of the rat small mesenteric artery by α_{1L}-adrenoceptors (Stam, 1999; and Yamamoto *et al.*, 1999),

or α_{1A}-adrenoceptors (Kong *et al.*, 1994; and Zhu *et al.*, 1999). Furthermore, the contraction of rat and human vas deferens is mediated by α_{1A}-adrenoceptors (Fukuwara, *et al.*, 1995; and Honner and Docherty, 1999), porcine coronary artery by α_{1A}-adrenoceptor (Yan *et al.*, 1998), the hind limb and skeletal muscle by α_{1A}-adrenoceptors (Zhu *et al.*, 1997).

The rabbit's ear microvasculature has a heterogenous distribution of α_1-adrenoceptor subtypes. The α_{1A} and α_{1D}-adrenoceptor subtypes appear to have a greater influence on constrictive function in the rabbit ear arterioles, whereas the α_{1D}-adrenoceptor is the dominant constrictor-of arteriovenous anastomoses (Li *et al.*, 2000). The creamaster skeletal muscle arterioles constriction are suggested to be mediate by α_{1D}-adrenoceptor subtypes while the venules by α_{1B}-adrenoceptors (Leech and Faber, 1996).

1.1.4. α_1-adrenoceptors in the kidney

Multiple adrenoceptor subtypes have been shown to exist in the kidney (Minneman *et al*, 1988; Han *et al*, 1990; and Feng *et al.*, 1991). The density of α_1-adrenoceptors is highest in the cortex and decreases from the cortex to papilla. The proportion of α_{1A}- and α_{1B}-adrenoceptors is almost equal in the cortex and outer stripes of the outer medulla, but with the α_{1B}- subtype predominating in the inner stripe of the outer medulla, whilst at the proximal tubules they are expressed at approximately equal levels (Feng *et al.*, 1991).

A recent study by Kurooka *et al.* (1999) described that three α_1-adrenoceptor subtype mRNAs were recognized in human renal cortex and detected

particularly in the smooth muscles of the arteries. There were more α_{1A}-adrenoceptors subtype in human renal cortex than the other subtypes. Expression of the three α_1-adrenoceptor subtypes mRNAs were confirmed in the arteries of the renal cortex (arciform, interlobular, arteriole), but among the three subtypes, the α_{1B} was less apparent by *in situ* hybridization. Intense α_1-mRNA staining was apparent especially in the smooth muscle of the arterial walls of the kidney. In both proximal and distal renal tubules, each of the α_1-mRNAs was less marked in cytoplasm than in the arteries. In the glomeruli, weak staining was detected in the endothelium but there was no obvious staining in the vein. Reverse transcryptase-polymerase chain reaction (RT-PCR) showed all three subtypes of α_1-adrenoceptor in the rat renal cortex. Similarly, Moriyama *et al.* (2000) studies using the RNase protection assay showed that the predominant α_1-adrenoceptor subtype mRNA in human renal cortex was α_{1A}. Furthermore, these authors described that the mean amount of α_{1A} mRNA was much greater than that of α_{1B} or α_{1D} mRNAs in both the main and branched renal arteries using RNase protection assay. *In situ* hybridization showed that all α_1 subtype mRNAs were localized in the smooth muscle cells of the tunica media of the artery, and the distribution pattern of these three mRNAs in the main artery was the same as in the branched artery. However, the intensity of signals for α_{1D}- and α_{1B}- antisense RNAs probes were lower than that for the α_{1A}- probe. Furthermore, functional study demonstrated that the $\alpha_{1L/A}$-adrenoceptor mediates primarily the responses to noradrenaline in this artery (Moriyama *et al.*, 2000).

Vasoconstrictor responses to noradrenaline in the isolated perfused kidney of the rat are mediated by α_1-adrenoceptors (Schmitz *et al.*, 1981). Further studies

by Blue *et al.* (1995) using the same preparation demonstrated that α_{1A}-adrenoceptor is the predominant α_1-adrenoceptor subtype mediating vasoconstrictor responses to exogenous administration of noradrenaline. In the intact rats, activation of α_{1A}-adrenoceptors caused constriction of the renal vascular resistance bed (afferent and efferent arterioles) and induces antinatriuresis and antidiuresis. This situation was seen in the Wistar, Stroke-Prone Spontaneously Hypertensive rats (SPSHR), two kidney one clip (2K1C) and deoxycorticosterone acetate-Salt (DOCA-Salt) hypertensive rats (Sattar and Johns, 1994a and b). Moreover, these authors showed that the α_{1B}-adrenoceptor subtype seems to play a lesser role in mediating similar responses. These findings are supported by a more recent study showing that the α_{1A}-adrenoceptor is the major subtype in renal resistance arterioles (Zhu *et al.*,1997). It has also been reported, that the renal adrenergic vasoconstrictor responses are mediated by α_{1A}- (Blue *et al.*, 1992; Elhawari *et al*, 1992; Eltze and Boer, 1992; Blue *et al*, 1995; Eltze *et al.*, 1994; and Villalobos-Molina and Ibarra, 1997) and or via α_{1D}-adrenoceptor subtypes (Villalobos-Molina and Ibarra, 1996 and 1997), although Piascik *et al.* (1995), provided evidence showing that the α_{1D}-adrenoceptors did not play a role in mediating the contractions in this vessel. However, a later study by Eltze (1999) and Satoh *et al.*, (1999) indicated the presence and involvement of α_{1D}-adrenoceptors in mediating the rat renal vasoconstriction.

1.2. The Kidney

1.2.1. Anatomy

The kidneys are a pair of encapsulated organs located in the retroperitoneal area and consists of two parts i.e., the cortex and medulla. Renal medulla is made up of outer and inner medulla that protrudes towards the renal pelvis (Muirhead, 1984). The cortex forms the renal columns through which passes the renal vessels, (Lumley *et al.*, 1996) i.e., interlobar and arcuate arteries (Basar and Weis, 1981) (Figure 1.1). The functional unit of the kidneys is the nephron, which comprises of glomerulus and tubule and each human kidney contains approximately 1.3 million nephrons. The size of the kidney is determined largely by the number of nephrons they contain (Ganong, 1999).

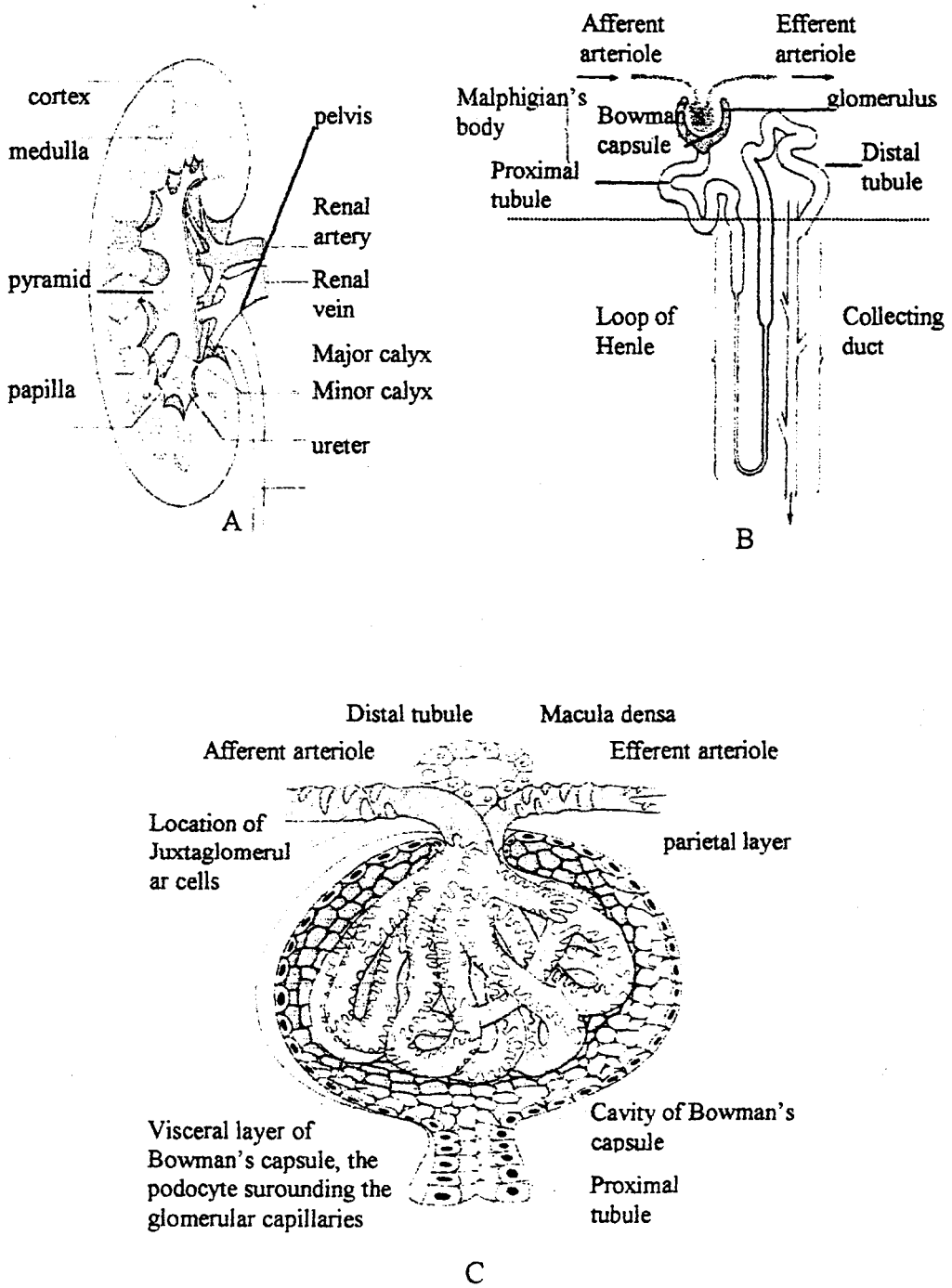

Figure 1.1. Structure of the kidney (A), nephron (B) and glomerulus (C) (Adopted from Folkow and Neil, 1971)

1.2.1.1. Glomerulus

The glomerulus is about 200 μm in diameter and formed by an invagination of a tuft of capillaries into the dilated, blind end of the nephron i.e. the Bowman's capsule. The capillaries are supplied by an afferent arteriole and drained by an efferent arteriole. In Bowman's capsule, the blood from the glomerular filtrate is separated by two cellular layers, called the capillary endothelium. These two layers are separated by a basal lamina. Between the basal lamina and the endothelium there are stellate cells known as mesangial cells which are common between two neighbouring capillaries, and forms a sheath shared by both capillaries (Ganong, 1999; and Junqueira *et al.*, 1999). These cells are contractile (Ganong, 1999) and play a role in the regulation of glomerular filtration (Ganong, 1999; and Junqueira *et al.*, 1999). They also secrete a number of substances and are involved in glomerular diseases (Ganong, 1999) such as glomerulonephritis, IgA nephropathy, diabetic nephropathy etc. (Lingappa, 1997).

The endothelium of the glomerular capillaries is fenestrated with pores that are 70 – 90 nm in diameter. Epithelium cells (podocytes) have numerous pseudopodia that integrate to form filtration slits which are approximately 25 nm wide along the capillary wall (Leaf and Cotran, 1976; and Ganong, 1999;). In the vessels, endothelial cells are sensitive to shear stress. When the shear stress exceeds a critical values, the cell begin to proliferate (Davies, 1989). This will lead to an increase in the diameter of the vessel and thus decreasing the shear stress (LaBarbera, 1990).

1.2.1.2. Proximal Convoluted Tubule

The proximal convoluted tubule is about 15 mm long and 55 μm in diameter. The walls of this tubule are made up of a single layer of cells that are interdigitated with one another and are united by apical tight junction. Between the bases of these cells, there are extensions of the extracellular space called lateral intracellular spaces. The luminal edges of the cells have a striated brush border due to the presence of innumerable microvilli. The convoluted portion of the proximal tubule (pars convoluta) drains into the straight portion (pars recta) and terminate at the thin segment of the beginning part of the loop of Henle. The cortical nephrons have short loop of Henle while the juxtamedullary nephrons have longer loops. In humans, only 15% of the nephrons have long loops. The total length of the thin segment of the loop varies between 2 – 14 mm. It ends with the thick segment of the ascending limb, with the length of about 12 mm. The thick ascending limb of the loop of Henle reaches the glomerulus of the nephron from which the tubule arise and passes close to its afferent arteriole. The walls of the afferent arterioles contain the renin-secreting juxtaglomerular cells. At this point, the tubular epithelium is modified histologically to form the macula densa. The juxtaglomerular cells, the macula densa and the lacis cells near by are collectively known as the juxtaglomerular apparatus (Ganong, 1999)

1.2.1.3. Distal Convoluted Tubule

This tubule is about 5 mm long, its epithelial cell is lower than that of proximal tubule, and although there are few microvilli, there is no distinct brush

border. The distal tubules are joint together to form collecting ducts that are about 20 mm long and pass through the renal cortex and medulla to empty into the pelvis of the kidney at the apexes of the medullary pyramids. The epithelial cells of the collecting ducts are predominantly the principal cells (P-cells) and small number of intercalated cells (I-cells). The P cells are involved in Na^+ reabsorption and vasopressin stimulated water absorption, while I cells are concerned with acid secretion and $HCO3^-$ transport. The total length of the nephrons include the collecting ducts ranging from 45 to 65 mm. Type I medullary interstitial cells contain lipid droplets and are associated with the prostaglandin PgE_2 secretion (Ganong, 1999).

1.2.2. Renal Circulation

The kidneys are highly vascularized organs, which are supplied with a renal artery and the renal vein that enters and exits at the hilum respectively. The renal artery arises from the abdominal aorta and the venous tributaries unite to form the single wide renal vein, which drains into the inferior vena cava (Lumley *et al.*, 1996).

The afferent arterioles are short and straight branches of interlobular arteries. Each divides into multiple capillary branches to form a tuft of vessels in the glomerulus. The capillaries coalesce to form the efferent arteriole, which in turn break up into capillaries that supply the tubules (peritubular capillaries) before draining into the interlobular veins. The arterial segments between glomeruli and tubules are thus technically a portal system and the glomerular capillaries are the

only capillaries in the body that drain into the arterioles. However, there is a little smooth muscle in the efferent arterioles (Ganong, 1999).

The capillaries draining the tubule of the cortical nephron form a peritubular network, whereas the efferent arterioles from the juxtamedullary glomeruli drain not only into the peritubular network, but also into vessels that form hairpin loops (the vasa recta). These loops dip into the medullary pyramids along side the loops of Henle. The efferent arteriole from each glomerulus breaks up into the capillaries that supply a number of different nephrons (Ganong, 1999).

Approximately 25% of cardiac output goes to the kidneys, and the blood is filtered to remove waste and to regulate extracellular electrolytes and intravascular volume. Blood flow through the kidney is approximately 1,200 - 1,300 ml/min and is distributed into a large number of vascular channels arranged in parallel circuits, each supplied by a similar perfusion pressure. The vascular resistance of each channel thus governs local blood flow which may therefore show disproportionate changes in various regions of the kidney, a so-called "redistribution of the renal blood flow" (Aukland, 1980). The microvessels in the kidneys have the ability to show autoregulatory activity, i.e., to contracts upon pressure rise and dilates upon pressure drop (Baez, 1968; and Johnson and Intaglietta, 1976) (Figure 1.2). The intrarenal blood flow distribution is very important for renal functions and hence alterations in regional renal blood flow distribution will alter renal function (Regan *et al.*, 1995).