MODIFICATION OF RENAL HAEMODYNAMICS IN CYCLOSPORINE A – INDUCED RENAL FAILURE RATS BY TEMPOL

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MODIFICATION OF RENAL HAEMODYNAMICS IN CYCLOSPORINE A - INDUCED RENAL FAILURE RATS BY TEMPOL

By

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LIST OF ABBREVIATIONS

2K,1C	2 kidney, 1 clip model
ACE	Angiotensin converting enzyme
Ang I	Angiotensin I
Ang II	Angiotensin II
Ang III	Angiotensin III
Ang IV	Angiotensin IV
ANS	Autonomic nervous system
ARB	Angiotensin receptor blocker
AT_1	Angiotensin II type 1 receptor
AT_{1b}	Angiotensin II type 1b receptor
AT_2	Angiotensin II type 2 receptor
AT_3	Angiotensin II type 3 receptor
AT_4	Angiotensin II type 4 receptor
ATP	Adenosine triphosphate
Ca^{2+}	Calcium
CKD	Chronic kidney disease
CNS	Central nervous system
CrCl	Creatinine clearance
CRF	Chronic renal failure
CsA	Cyclosporine A
DBP	Diastolic blood pressure
DNA	Deoxyribonucleic acid
eNOS	Endothelial nitric oxide synthase

eNOS	Endothelial NOS
FE_{K}^{+}	Fractional excretion of potassium
FE_{Na}^{+}	Fractional excretion of sodium
GFR	Glomerular filtration rate
GPCRs	G protein-coupled receptors
GSH	Oxidized glutiathione
GSSH	Reduced glutiathione
H_2O	Water
H_2O_2	Hydrogen peroxide
HR	Heart rate
iNOS	Inducible NOS
KCl	Potassium chloride
KI	Kidney index
L-NAME	Nω-nitro-L-arginine methyl ester
MAP	Mean arterial blood pressure
MDA	Malondialdehyde
mRNA	Messenger ribonucleic acid
N_2O_3	Dinitrogen trioxide
NaCl	Sodium chloride
NADPH	Nicotinamide adenine dinucleotide phosphate
NaOH	Sodium hydroxide
NIBP	Non-invasive blood pressure
nNOS	Neuronal NOS,
NO•	Nitroxide radical
NO_2•	Nitrogen dioxide radical

NOO•	Nitrogen dioxide radical
NOS	Nitric oxide synthase
NOS 2	Nitric oxide synthase 2
NOx	Nitric oxide
O^-	Singlet oxygen
O_2	Molecular oxygen
O_2^-	Superoxide anion
O_3	Ozone
OH^-	Hydroxyl ion
$ONOO^-$	Peroxynitrite anion
Pcr	Plasma creatinine
PNS	Peripheral nervous system
PWV	Pulse wave velocity
RAAS	renin-angiotensin aldosterone system
RAS	renin-angiotensin system
RCBP	Renal cortical blood perfusion
R-NO+	Oxammonium cation
R-N-OH	Hydroxylamine
RNS	Reactive nitrogen species
RO^-	Alkoxyl
ROO^-	Peroxyl
ROS	Reactive oxygen species
RSNA	Renal sympathetic nerve activity
SBP	Systolic blood pressure
SNS	Sympathetic nervous system

SOD	Superoxide dismutase
T-AOC	Total antioxidant capacity
TBARS-MDA	Thiobarbituric acid reactive substances-malondialdehyde
Tempol	4-hydroxy-2,2,6,6-tetramethyl piperidine-N-oxyl
TGF	Tubuloglomerular feedback
TGF-β	Transforming growth factor-β
T-SOD	Total superoxide dismutase
Ucr	Urine creatinine

MODIFIKASI HEMODINAMIK RENAL TIKUS KEGAGALAN GINJAL CYCLOSPORINE A TERARUH OLEH TEMPOL

ABSTRAK

Peningkatan bukti menunjukkan bahawa tekanan oksidatif yang terlibat dalam patogenesis pelbagai penyakit kardiovaskular termasuk tekanan darah tinggi dan kegagalan ginjal. Tekanan oksidatif mungkin menyumbang kepada perkembangan penyakit ginjal secara tidak langsung dengan meningkatkan kadar atau secara langsung meransang kerosakan glomerular dan iskemia. Kegagalan kardiovaskular yang disebabkan oleh tekanan oksidatif dalam penyakit buah pinggang telah meningkatkan secara mendadak dan konsep ini semakin mendapat banyak perhatian. Tempol-superoxide dismutase (SOD) mimetic, berkesan dalam memerangkap radikal bebas. SOD memangkin penukaran O_2^- kepada H_2O_2, yang kemudian ditukarkan kepada air melalui tindak balas katalase. Kajian ini telah dijalankan untuk mengkaji kesan potensi Tempol ke atas fungsi dan hemodinamik teraruh oleh CsA kegagalan ginjal dan CsA model hipertensi teraruh L-NAME. 64 tikus jantan Sprague-Dawley telah dibahagikan secara rawak kepada lapan kumpulan. Model kegagalan ginjal telah dihasilkan dalam kumpulan yang terpilih menggunakan CsA pada dos 25 mg / kg / hari secara oral. Manakala model hipertensi kekurangan nitrik oksida (NOx) telah dicipta menggunakan L-NAME pada dos 15 mg / kg / hari secara oral dalam kumpulan yang terpilih. Tekanan darah di bawah sedar telah diukur menggunakan kaedah tail cuff plethysmography secara mingguan sepanjang tempoh kajian. Selain itu, data metabolik, parameter fungsi ginjal turut dikaji. Di samping itu, nadi halaju gelombang dan tindak balas vasokontriksi renal kortikal keatas noradrenalin, phenylephrine, methoxamine dan angiotensin II telah diperhatikan semasa kajian akut. Tambahan pula, plasma malondialdehyde,

superoxide dismutase, nitric oxide dan total antioxidant capacity telah digunakan untuk menentukan tekanan oksidatif. Data analisis, mean ± SEM menggunakan one/two-way ANOVA dengan signifikasi pada tahap $p<0.05$. Rawatan CsA, rawatan L-NAME pada tikus normal dan tikus kegagalan ginjal tikus telah menunjukkan hipertensi yang signifikan, kemerosotan fungsi ginjal dan tekanan oksidatif dibanding dengan tikus kawalan. Tambahan lagi, rawatan CsA, rawatan L-NAME pada tikus normal dan tikus kegagalan ginjal menunjukkan penurunan tindakbalas vasokontriksi ginjal keatas noradrenalin, phenylephrine, methoxamine dan angiotensin II dibandingkan dengan tikus SD kawalan normal. Rawatan Tempol menjadikan tekanan darah normal, memperbaiki kegagalan gingal dan meningkatkan pertahanan antioksidan dalam tikus rawatan CsA dan L-NAME dibanding dengan tikus yang tidak menerima rawatan. Selain itu, rawatan Tempol juga meningkatkan tindak balas vasokontriksi ginjal keatas noradrenalin, phenylephrine, methoxamine dan angiotensin II dibandingkan dengan kumpulan yang tidak dirawat. Penemuan ini menunjukkan bahawa rawatan kronik CsA dan L-NAME mengurangkan kepekaan vaskular pada α_1-adrenergic agonis dan angiotensin II. Di samping itu, sub-unit α_{1A}-adrenoceptor dianggap tidak terlibat dalam pengantaraan kegagalan ginjal CsA. α_1-adrenoceptors dan angiotensin II memainkan peranan penting dalam kawalan hemodinamik ginjal pada tikus kegagalan ginjal CsA dan hipertensi teraruh L-NAME. Kesimpulannya, SOD adalah predominant superoxide scavenger jika dibandingkan dengan NOx.

MODIFICATION OF RENAL HAEMODYNAMICS IN CYCLOSPORINE A - INDUCED RENAL FAILURE RATS BY TEMPOL

ABSTRACT

Increasing evidence suggests that oxidative stress is involved in the pathogenesis of a wide range of cardiovascular diseases including hypertension and renal failure. Oxidative stress may contribute to the progression of renal diseases indirectly by aggravating hypertension or directly by inducing the glomerular damage and ischemia. The deleterious cardiovascular effects due to oxidative stress in chronic renal failure are dramatically increasing and this concept is gaining much attention. Tempol is a redox-cycling nitroxide namely superoxide dismutase (SOD) mimetic that promotes the metabolism of many reactive oxygen species (ROS) and improves nitric oxide bioavailability. SOD catalyzes the conversion of O_2^- to H_2O_2, which converts into water by catalase reaction. The present study was undertaken to investigate the potential effect of Tempol on the renal functional and haemodynamics in Cyclosporine A-induced renal failure and L-NAME induced hypertension models. 64 male Sprague-Dawley rats were randomly divided into eight groups (n=8). Renal failure model was produced in selected groups using CsA at a dose of 25 mg/kg/day p.o. Nitric oxide (NOx) deficiency hypertensive model was created using L-NAME at a dose of 15 mg/kg/day p.o in selected groups. Conscious blood pressure was measured using tail cuff plethysmography method weekly throughout the study period. Besides that, metabolic data, renal functional parameters were studied. Moreover, pulse wave velocity and renal cortical vasoconstriction responses to noradrenaline, phenylephrine, methoxamine and angiotensin II were observed during acute study. In addition, plasma malondialdehyde, superoxide dismutase, nitric oxide and total antioxidant capacity levels were used to determine the oxidative stress.

Data, mean±SEM were subjected to one/two-way ANOVA with significance level at $P<0.05$. CsA treated, L-NAME treated normal and renal failure rats developed significant hypertension, deteriorated renal functional parameters and oxidative stress as compared to control. Furthermore, CsA treated, L-NAME treated normal and renal failure rats exhibited reduction in renal vasoconstrictor response to noradrenaline, phenylephrine, methoxamine and angiotensin II compared to normal SD control rats. Tempol treatment normalized the blood pressure, ameliorates the renal insufficiency and up-regulates the antioxidant defenses in both CsA and L-NAME treated rats as compared to non-treated counterparts. Moreover, Tempol treatment also increased the renal vasoconstrictor response to noradrenaline, phenylephrine, methoxamine and angiotensin II as compared to those renal failure control rats. These findings suggest that chronic administration of CsA and L-NAME blunts the vascular sensitivity to α_1-adrenergic agonists and angiotensin II. In addition to that, α_{1A}-adrenoceptor is not the functional subtype that mediates renal vasoconstriction response in CsA-induced renal failure rats. α_1-adrenoceptors and angiotensin II play a pivotal role in regulation renal haemodynamics in CsA-induced renal failure and L-NAME induced hypertension. This study concluded that SOD was the predominant superoxide scavenger as compared to NOx.

CHAPTER 1

INTRODUCTION

1.1 Kidney

1.1.1 The gross anatomy

The human kidneys are paired bean shaped and each kidney is measures about 11.25 cm in length, 5 to 7.5 cm in breadth and more than 2.5 cm in thickness by which it is approximately as big as the fist of an adult human. The exact position of a human kidney is at the posterior part of the abdomen, one on either side of the vertebral column, behind the peritoneum at about the level of the 12^{th} rib just above the waistline, thus it is retroperitoneal (Standring *et al.*, 2005). Each kidney weighs is about 115-170 grams where its combined weight is less than 1% of the total body weight of an adult human.

The cross section of the kidney shows two major regions with the outer layer called the renal cortex which is reddish brown in colour. Whereas, the dark brown inner layer is called the renal medulla and is striped on the its surface (Shier, 2003). The renal medulla is then further subdivided into a number of conical sections called renal pyramid with the papillae tubules called collecting ducts which drain into a single funnel-shaped passage to the initial portion of the ureter called the renal pelvis (Drake *et al.*, 2005).

Deep down to the renal pyramids, there are millions of microscopic subunits called nephrons, which are the functional units for blood filtration and urine formation. Each particular nephron is composed of a renal corpuscle and renal tubule which filter

the blood and form urine respectively. Eventually the filtrate is modified and excreted in the form of urine. The renal corpuscle consists of the inflow subunit called Bowman's capsule and a turf of capillaries called glomerulus which is the original site for blood filtration and tubular fluid formation (Hall & Guyton, 2006a). Blood enters the glomerular capillaries through the afferent arteriole and exit via the efferent arteriole to produce protein free plasma filtrates via a process of glomerular filtration. This filtrate then flows into the first part of the tubular region of the nephron, the proximal convoluted tubule and further empties into the hairpin loop tubules located in the medulla region known as descending and ascending limbs of loop of Henle. From the ascending loop of Henle, the filtrate then enters the distal convoluted tubules which actually resembles the proximal one but this tubule is shorter. Later, the fluid enters a short straight terminal segment of the nephron called the connecting tubule that joins the nephron to the collecting duct. The collecting ducts then empty the filtrates into the calyces and the filtrate is excreted in the form of highly concentrated urine via the urinary system (Martini, 2001).

Despite the size of the kidney which is relatively small, the blood supply to this organ is approximately 22% of the cardiac output. The blood flows into the renal artery and branches into other segmental arteries which in turn flows into another type of artery called interlobar arteries. From here, the blood is further fed into the arcuate arteries and branch into the last segment called interlobular arteries where the blood is supplied to individual nephron. The renal glomerular ultrafiltration process in the renal corpuscle is driven by the Starling forces existing across the walls of glomerular capillaries. After the filtration process, the blood is transported into small capillary beds namely peritubular

capillaries which branch from the efferent arterioles that are located close to the renal tubules and vasa recta which run along the loops of Henle and collecting duct deep into the renal medulla. As in the arteriole distribution, the filtered blood is then drained into the interlobular veins from the peritubular capillaries and vasa recta. At this point, blood is transported away from the nephrons by arcuate veins to the interlobar veins and renal vein eventually returns to inferior vena cava (Meyer *et al.*, 2004).

1.1.2 The physiology of kidney

The principal function of the kidney is to preserve the constancy of the body's interior environment by excreting waste products and regulating the electrolyte contents, volume and pH of the extracellular fluid due to the consequence of the varying internal and external environments as well as dietary intake (Dantzler, 1989).

The normal kidney receives almost a quarter of the cardiac output from more than hundred liters of plasma daily and performs its task by simple mechanisms, i.e. filtration, reabsorption, and secretion that happen in the nephron. Water and solutes are exchanged between fluid and plasma in the renal tubules to regulate the composition of plasma. Filtration occurs in the renal corpuscle which is the mass flow of protein free plasma from the glomerular capillaries into the Bowman's capsule. Reabsorption is done by the selective transport of glomerular filtrate from the lumen of the tubules to the interstitial cells outside the tubules. Whereas, secretion is the reverse mechanism by which molecules from the peritubular fluid is transported back into the lumen of the renal tubules (Guyton & Hall, 1991).

Glomerular filtration rate describes the flow rate of filtered fluid through the kidney. Specifically, it estimates how much blood passes through the tiny filters in the kidneys, called glomeruli, each minute. Under the normal condition, the regulation of the glomerular filtration rate involves three intrinsic control mechanisms which includes myogenic regulation of smooth muscle in the afferent arteriole, tubuloglomerular feedback and mesangial cell contraction. In contrast, extrinsic control includes the sympathetic nervous control of smooth muscle in the afferent and efferent arterioles (Rang *et al.*, 2007).

The kidney works in integration with other systems in the body, i.e. cardiovascular and respiratory systems. The renal and respiratory system regulates acid-base balance which is very important for normal protein function. Likewise, the interaction of renal and cardiovascular system is crucial for the maintenance of blood pressure. Moreover, the kidney interacts with the endocrine system to secrete renin which is required for the activation of angiotensin II. Through these interactions, the kidney is involved in long term regulation of blood pressure, water and electrolyte balance (Germann *et al.*, 2005b).

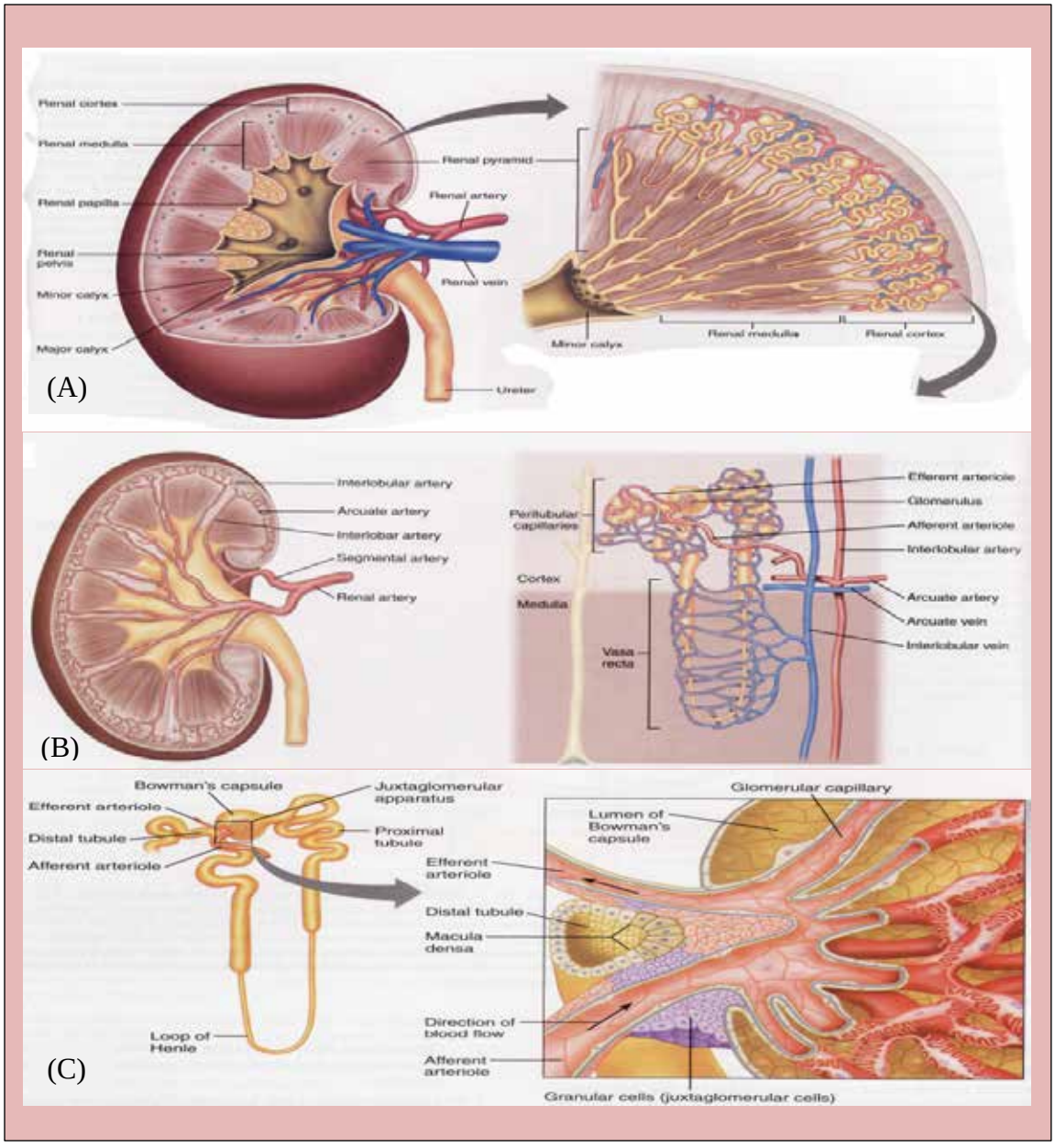

Figure 1.1: Anatomy and blood supply of kidney.

(A) A cross section of a kidney showing the renal cortex and renal medulla with the magnified view of renal pyramid.

(B) Renal arteries supply blood to the kidney with afferent and efferent arterioles lead into one of the two different types of capillary beds: peritubular capillaries and vasa recta.

(C) The magnified view of a juxtaglomerular apparatus.

Diagram adapted from (Principles of Human Physiology, Pearson Education Subscription and End-User License Agreement. Access code: USWSP4-FROMM-STEAK-NEMAN-PLANT-MINES; Expire date: DEC 30, 2013).

1.2 Heart

1.2.1 The gross anatomy

The word “Cardiac” is derived from the Greek word “*Kardia*” which mean “Heart”. The heart is a muscular organ found in all animals whose main function is to generate the force that propel blood for the circulation to the entire body via blood vessels by rhythmic contraction (Cohen, 2004).

Essentially, the exact location of the heart is centrally positioned in the thoracic cavity above the diaphragm that separates the thoracic cavity from the abdominal cavity. In human, the heart is about 300 to 350 grams in males and 250 to 300 grams in females. It is covered by a membranous sac called pericardium that possesses a special fluid that lubricates the heart when it beats. A healthy heart beats about 60 to 80 times per minute (Robb & Robb, 1992).

The human heart can be divided into four chambers. The upper two chambers called atria which receive blood that is transported back to the heart from the vasculature system and two lower chambers called ventricles. The ventricles receive blood from both atria and generate the force to propel the blood away from the heart via the blood vessels. The atria and ventricles are separated by a septum that prevents blood mixing between the left heart and right heart. The septum that separates the left and right atrium is known as interatrial septum whereas the one that separates the left and right ventricle is the interventricular septum. Just like the heart has both left and right sides, there is another pattern called as “top” and “bottom”. The broader upper pole of the heart is

known as the base. In contrast, the lower pole is called as the apex (Dickstein *et al.*, 2008).

Basically, the heart wall is made up of three layers: an epicardium which is the outermost layer of connective tissue, a middle layer called myocardium and an inner layer called endothelium which extends throughout the entire cardiovascular system. The heart pumps by the continuous rhythmic contraction and relaxation of the myocardium because this connective tissue is composed of muscles with contractile properties. When the muscle in the atrium and ventricle walls contracts, the mechanical forces move the wall inward and squeeze the blood into the chamber. As the squeezing increases, the pressure within both atrium and ventricle force the blood out. The atrium and ventricle expands and fill with blood when the muscle relaxes (Anderson, 2000). The heart contracts by a continuous rhythm called cardiac cycle. This event causes pressures in the heart chambers to fluctuate; therefore, it is very crucial that blood flow in the heart is only unidirectional. This is made possible by the presence of the four types of valves that consists of left and right atrioventricular valves called bicuspid valve or sometime referred as mitral valve and a tricuspid valve which allow the blood flow only from the atrium to ventricle; the aortic semilunar valve and pulmonary semilunar valve also perform the same function to permit blood flow forward at the same time preventing it from flowing backward (Marieb & Hoehn, 2007b).

1.2.2 The physiology of heart

The cardiac contractions are governed by an elaborate conduction system that determines the series of excitation of cardiac muscle cells. Normally the heartbeat is modulated by pacemaker in the sinoatrial node which is situated in the upper right atrium where it joins with superior vena cava and the atrioventricular node which is situated near the tricuspid valve in the interatrial septum. Succeeding each action potential, pacemaker cells exhibit slow spontaneous depolarization. This eventually triggers the next action potential which causes the left ventricle to pump oxygenated blood into the aorta whose branches transport blood to capillary beds of all tissue and organs in the systemic circuit. The deoxygenated blood that returned from the systemic tissues then travels back to the heart via the vena cave into the right atrium. From the right atrium, blood flows through the tricuspid valve into the right ventricle and later it is pumps into the pulmonary artery which carry the deoxygenated blood to the lungs for gaseous exchange. At this juncture, blood becomes oxygenated and then travels to the left atrium via the pulmonary veins. From the left atrium, blood flows through the bicuspid valve into the left ventricle where the whole cycle then repeats (Germann *et al.*, 2005a).

The cardiac cycle involves the events of one heartbeat by which a complete cycle involves the ventricular contraction and relaxation that refers to one systole followed by one diastole. However, the duration of this event is not equal. For instance, the heartbeat at the normal resting human is 72 beats per minutes. Thus, one beat or one cardiac cycle is 0.8 second. Systole lasts about 0.3 second whereas diastole lasts for only 0.5 second (Hall & Guyton, 2006b).

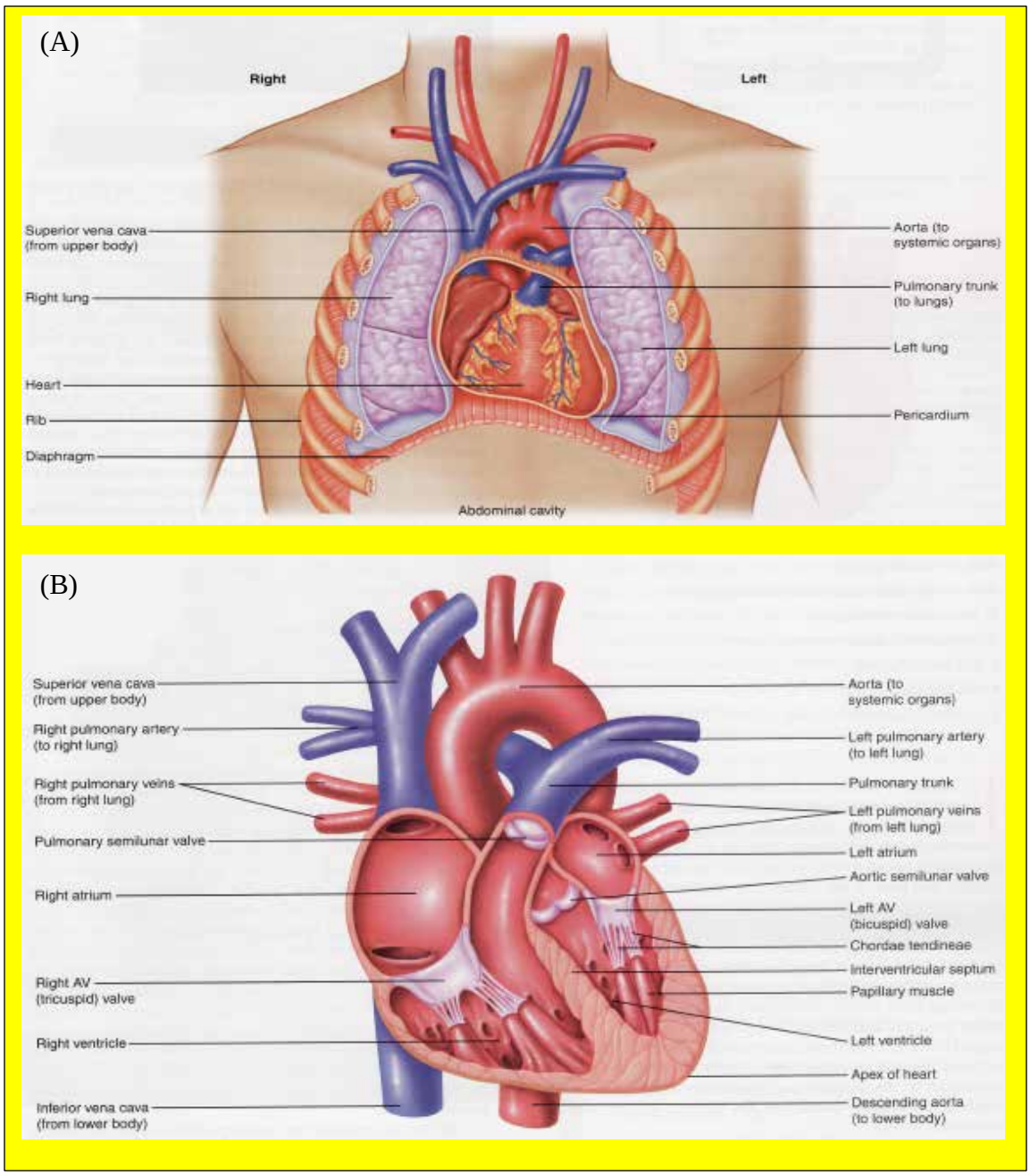

Figure 1.2: Anatomy and location of the heart.

(A) Relative positions of the heart in the thoracic cavity.
(B) A cutaway view of the heart with four chambers and valves connecting to major blood vessels.

Diagram adapted from (Principles of Human Physiology, Pearson Education Subscription and End-User License Agreement. Access code: USWSP4-FROMM-STEAK-NEMAN-PLANT-MINES; Expire date: DEC 30, 2013).

1.3 The blood vessels

1.3.1 The gross anatomy and physiology

The human vasculature systems are the part of the circulatory system that transports blood throughout the body. The blood vessels consist of three major types of blood vessels which are arteries, veins and capillaries. The arteries carry the blood away from the heart and reach the capillaries for the actual exchange of water and chemicals between the blood and the tissues then the veins carry blood from the capillaries back toward the heart (Marieb & Hoehn, 2007a).

Basically, all blood vessels possess a hollow interior called the lumen with its surface lined by a layer of epithelium cells called endothelium. Surrounding the lumen is a wall that has different composition and thickness from one type of vessels to another. The walls of large artery contain large amount of fibrous elastic tissue which enable the artery to withstand high blood pressure. In contrary, vein has approximately the same diameter like artery but has walls about one-half as thick. The thinness of the vein reflects the fact that the blood pressure in the vein is relatively lower than artery. Unlike other blood vessels, the vein is equipped with one way valves to prevent the blood back flow toward organs and tissues. The smallest blood vessel, capillary has only a layer of endothelial cells and basement membrane. The thin wall provide small diffusion distance between blood and surrounding interstitial fluid for gaseous exchange like oxygen, carbon dioxide as well as electrolytes exchange (Kahle *et al.*, 1993).

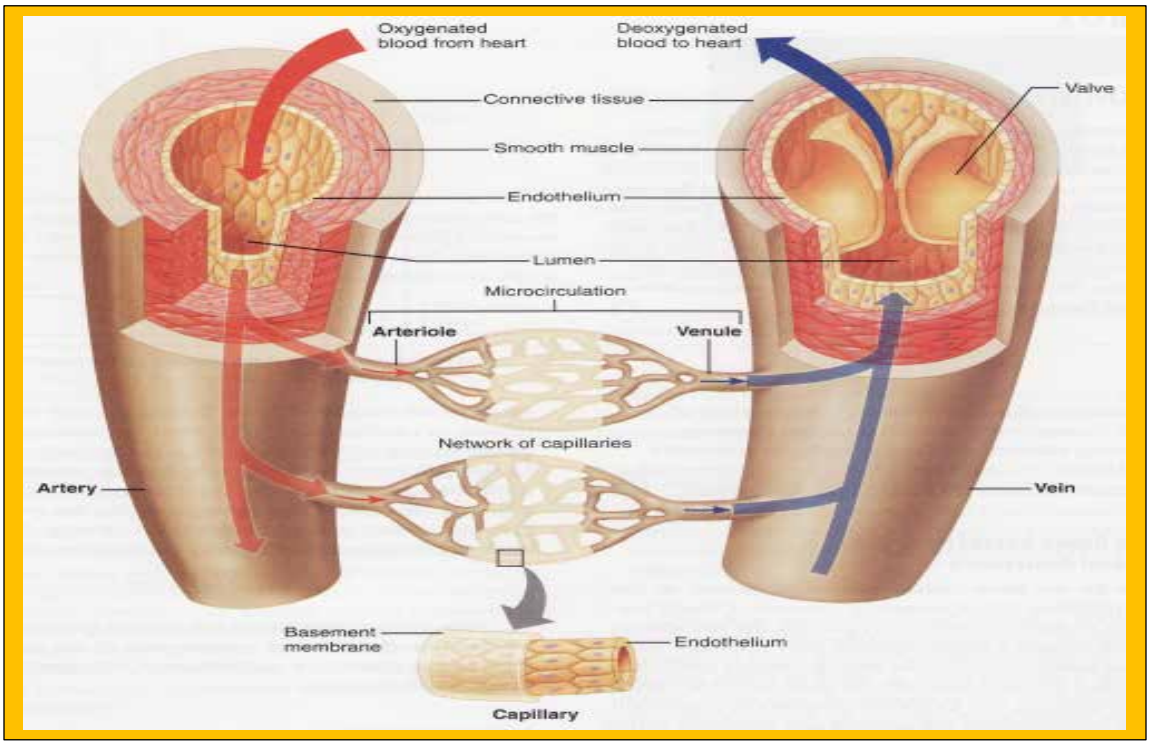

Figure 1.3: Anatomy and structure of blood vessels.

Diagram adapted from (Principles of Human Physiology, Pearson Education Subscription and End-User License Agreement. Access code: USWSP4-FROMM-STEAK-NEMAN-PLANT-MINES; Expire date: DEC 30, 2013).

1.4 The current issue on kidney disease

The study on chronic renal disease (CKD) has recently captured the minds of many medical professionals. It has become a major health burden to the people of many nations either in developed or developing countries. In developing economies, the major concern is on the continuous rising incidence of renal disease as it increases the expenses in a treatment modality that consumes a disproportionate share of the health care budget (Hooi *et al.*, 2005). CKD affects 500 million people worldwide and according to the annual report from Ministry of Health Malaysia, out of 26 million Malaysian populations, 1 in 10 Malaysian suffers from renal CKD. Annually, approximately 2500 people are diagnosed to have renal diseases. In fact hypertension and diabetes are the major culprits of kidney failure that have taken up over 2/3 of kidney failure cases (Malaysia, 2011).

The kidney is affected by many chemicals that are derived from dietary intakes; some of them may even lead to renal diseases (Pfaller & Gstraunthaler, 1998). Undoubtedly, there are several factors that determine the susceptibility of an organ to toxicity. These factors include the pharmacokinetics and metabolic fate of the compound. Other factor such as the capability of the target organ to response to the toxic insult is also important. The kidney is able to metabolize chemicals and reactive intermediates. However, *in situ* metabolic activation of chemicals could lead to selective toxicity to cellular macromolecules via generation of reactive oxygen species. This eventually leads to peroxidative damage of functionally important cellular structure like nucleic acids and plasma membrane (Wirthensohn & Guder, 1986).

Renal failure or kidney failure, formerly called chronic renal insufficiency or renal insufficiency is a medical condition that describes a condition in which the kidneys fail to meet the body's function adequately to excrete the filtered toxins and waste products from blood such as electrolytes, water, acid-base balance and the end product of protein metabolism. Biochemically, it is detected by an elevated serum creatinine level. Physiologically, renal failure is described as a derangement in the glomerular filtration rate. Generally, renal failure can be divided into three categories: acute, chronic and acute on chronic stages. Either form may be due to a large number of other medical problems (Levey *et al.*, 2003). Acute renal failure usually occurs as the result of a sudden interruption in the blood supply to the kidney or as a result of a toxic overload of the kidneys. However, chronic renal failure is more deleterious than acute renal failure. The symptoms may not be prominent until the kidneys are extremely damaged over a period of months or years. As opposed to chronic kidney disease, the acute failure kidneys can often recover as a result of supportive treatment, but they often remain at an increased risk of developing future kidney failure (Bellomo *et al.*, 2004).

1.4.1 In-depth view on chronic renal failure

The progression of chronic renal failure (CRF) is characterized by an irreversible deterioration of renal function that gradually progresses to end-stage renal disease (Yu, 2003). The reason behind the relentless progression of CRF in diverse origins still remains unknown but is thought to be multifactorial. Many diseases can irreversibly injure or damage the kidneys. Therefore, acute renal failure can proceed into chronic stage if the kidney function does not restore after treatment. However, many of them

have claimed that the major causes of CRF are closely related to hypertension, obesity and diabetes (Odigie & Marin-Grez, 2010; Chen *et al.*, 2004; Vanholder *et al.*, 2005).

Essentially, progressive CRF is characterized via histological alterations of tubulointerstitial and glomerular injuries. Indeed, renal dysfunction and outcome in CRF condition correlate better with tubulointerstitial injury than glomerular injury. An experiment in rat model has revealed that the extent of injury in tubulointerstitial region could even exceed that of glomerular region (Kimura *et al.*, 1999). Nevertheless, the causes of CRF are heterogeneous. Furthermore, the locations and mechanisms of the initial injury may also deviate. Several morphological features are prominent during CRF. Fibrosis, loss of normal renal cells via apoptosis and infiltration of leukocytes like monocytes and macrophages are the common observation in the diseased kidney. This accounts for the end result of the constant interplay between vasoactive substances for instance, the growth factors and inflammatory factors activation including cytokines (Yu, 2003). Moreover, tubular epithelial cells are capable of secreting interstitial collagens, proteoglycans and fibronectin, which indicates the degree of transformation of tubular epithelial cells into fibroblasts. Apart from histological changes, alteration in the extracellular matrix composition has also been demonstrated which in turn affect the gene regulation (Kimura *et al.*, 1999). When the renal dysfunction moves from mild to moderately severe, diseased kidneys direct the vasoactive hormones production such as angiotensin II that increase blood pressure (Vaziri *et al.*, 2007). Meanwhile, the participation of renin-angiotensin aldosterone system (RAAS) and activation of renal sympathetic activity that leads to the development of chronic hypertension could also lead to CRF development (Appel *et al.*, 2008). These significant disruptions of the renal

functional processes could trigger a chain of events that culminates in the progression of kidney dysfunction; continuous deterioration of this remnant diseased kidney functions and structure. Moreover this is associated with and largely mediated by profound alteration on renal haemodynamics, inflammation and oxidative stress. This results in the up-regulation of ROS in the kidney and cardiovascular tissue, that eventually advances to renal injury and compound haemodynamic abnormalities (Vaziri *et al.*, 2007).

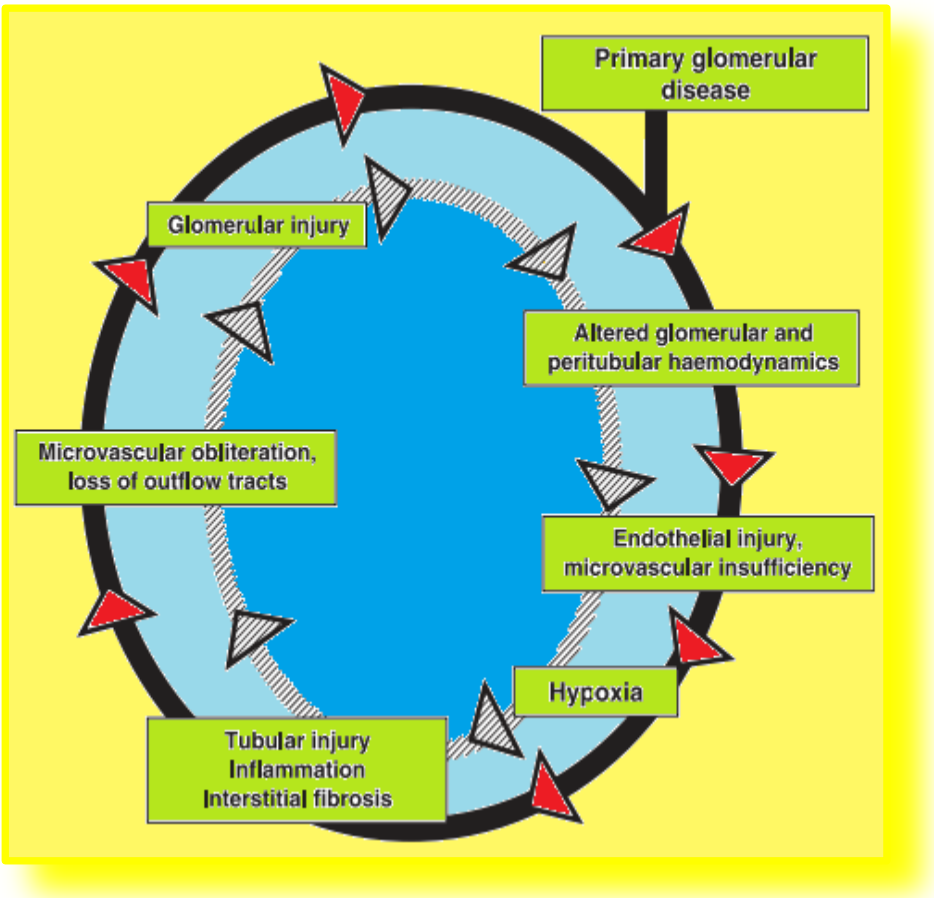

Figure 1.4: Schematic representation of the chronic renal failure hypothesis. Diagram adapted and redrawn with modification from (Norman & Fine, 2006).

1.5 Hypertension

Essentially, hypertension is a condition by which the blood pressure in the arteries is chronically elevated. According to the statistical findings from the Third National Health and Morbidity Survey of 2012, in Malaysia it is now estimated 4.8 million individuals are suffering from hypertension (Sathiabalan, 2012). Moreover, the estimated number in worldwide is almost staggering number of 1 billion individuals. It is however, alarming because hypertension has become the major risk factor for cardiovascular, cerebrovascular and chronic renal diseases (Malaysia, 2009). This condition is associated with the elevation of peripheral vascular resistance and structural alteration in the wall of blood vessels (Folkow, 1990).

Hypertension is medically defined as the persistent elevated resting blood pressure that exceeds 120 mmHg of systolic and 80 mmHg diastolic blood pressures. Fundamentally, there are two main types of hypertension, primary and secondary hypertension. Primary or essential hypertension consists of 90-95% of all the hypertension cases. However till now, the detail cause of primary hypertension remains unknown. In secondary hypertension, the elevated blood pressure happen secondary to another disease. For instances: renal hypertension is associated with kidney disease whereas endocrine hypertension is associated with inappropriate secretion of a hormone (Germann *et al.*, 2005c).

In renal hypertension, the principal cause is the disorder of the kidney function. In renal failure, the kidney fails to excrete normal amounts of salt and water. This leads to water retention and expansion of blood volume. Sometimes, the resistance of blood

flow within a kidney or into the kidney can also be the factor that triggers the inappropriate release of high renin levels. Abnormally high production of renin leads to overproduction of angiotensin II in the plasma which stimulates vasoconstriction. Which in turn increases peripheral resistance and also induces the kidney to retain water and salt (Ausiello *et al.*, 2003). Hypertension cause by endocrine disorder happens when adrenal medulla over secretes vasoactive hormones such as epinephrine, which increases cardiac output and total peripheral resistance. These increases then elevate blood pressure (Douglas, 2000). Other factors that contribute to hypertension include sympathetic activity, endothelin levels, oxidative stress, declined levels of nitric oxide and medullary vasodilation factors (Yu, 2003; Hostetter, 2004; Vaziri & Rodriguez-Iturbe, 2006). However, the elevation of blood pressure is not limited to the basic cause of increased reactive oxygen species (ROS) formation (Vaziri & Rodriguez-Iturbe, 2006). The increase in oxidative stress has also been reported in various experimental models such as Dahl salt-sensitive hypertension, spontaneous hypertensive rat model, angiotensin II-induced hypertension and many more. Other than ROS generation, the increased activation of uncoupling endothelial nitric oxide synthase (Ausiello *et al.* 2003), nicotinamide adenine dinucleotide phosphate (NADPH) oxidase, xanthine oxidase and generation of superoxide also contribute to the development of hypertension (Turrin *et al.*, 1994). The endothelium-mediated vasodilatation factor is also reduced during hypertension due to low bioavailability of nitric oxide (NOx). The interaction of superoxide anion (O_2^-) and NOx lead to the formation of a weak vasodilator molecule called peroxynitrite ($OONO^-$) that has pro-inflammatory properties and impairs the vascular structure (Shihab *et al.*, 2003). Chronic administration of superoxide dismutase (SOD) mimetic and antioxidant may prevent the development of hypertension in animal

models by preventing the occurrence of vascular inflammation and regress vascular remodeling via the improvement of endothelial relaxation function (Schnackenberg & Wilcox, 1999).

Table 1.1: Classification of blood pressure for adults age 18 and older**.**

Category	Systolic (mmHg)	Diastolic (mmHg)
Optimal	<120	<80
Pre-Hypertension	120-139	80-89
Stage 1 Hypertension	140-159	90-99
Stage 2 Hypertension	160-179	100-109
Stage 3 Hypertension	>180	>110

Adapted from (Norman & Fine, 2006; Malaysia, 2009).

1.6 Renin angiotensin aldosterone system

The renin-angiotensin-aldosterone system or renin-angiotensin system (RAS) is a hormonal cascade system that plays a vital role in homeostatic control of blood volume, systemic vascular resistance, tissue perfusion, electrolyte and extracellular volume that influence arterial pressure and cardiac output. It has become a hot issue of research since it turns out to be the backbone in the pathogenesis of many diseases including hypertension, cardiovascular and renal diseases (Siragy & Carey, 2010). As the name indicates, there are three important elements in this system which consists of renin, angiotensin, and aldosterone in order for the system to function (Halter *et al.*, 2009).

The RAS hormonal cascade starts with the biosynthesis of renin by the juxtaglomerular cells located mainly in afferent and some efferent arterioles of the renal glomerulus. The activity of RAS in the circulation is primarily dependent on the activity of the key regulator called enzyme protease renin. Renin is synthesized in the form of preprohormone and the active renin is formed via the proteolytic removal of a 43 amino acid prosegment peptide from the N-terminus of pro-renin or renin precursor. This active renin is then stored in the granules of the juxtaglomerular cells and released through an exocytotic process that involves stimulus-secretion coupling into the renal and the systemic circulation. However, under normal conditions, there is some small quantity of renin remaining in the local fluids of the kidney that can initiate several intrarenal functions in response to different disease condition (Remuzzi *et al.*, 2005).

The regulation of renin secretion is a main determinant of the activity of the RAS. Principally, active renin secretion is regulated by 4 interdependent factors that include:

(1) renal baroreceptor in the afferent arterioles called “polkissen” cells that detect changes in renal perfusion pressure, (2) the “macula densa” cells in the juxtaglomerular apparatus that sense changes in NaCl concentration, (3) stimulation of sympathetic nerves activity by adrenergic receptor and (4) the direct negative feedback action of Ang II on the juxtaglomerular cells (Laragh & Sealey, 1992). The production of renin is regulated by the initial limiting step of the RAS, where the N-terminal portion of a large molecular weight globulin called renin substrate or angiotensinogen which is produced from liver to form the biologically inert decapeptide Angiotensin I (Ang I). The inactive decapeptide Ang I is further hydrolyzed by angiotensin converting enzyme (ACE) through the removal of the C-terminal dipeptide to form the biologically active potent vasoconstrictor octapeptide Ang II. Ang II is metabolized to Angiotensin III (Ang III) and then to Angiotensin IV (Ang IV) by angiotensinases that are located in the vascular beds and red blood cells of most tissues that have presser activity on Ang II (Kobori *et al.*, 2007).

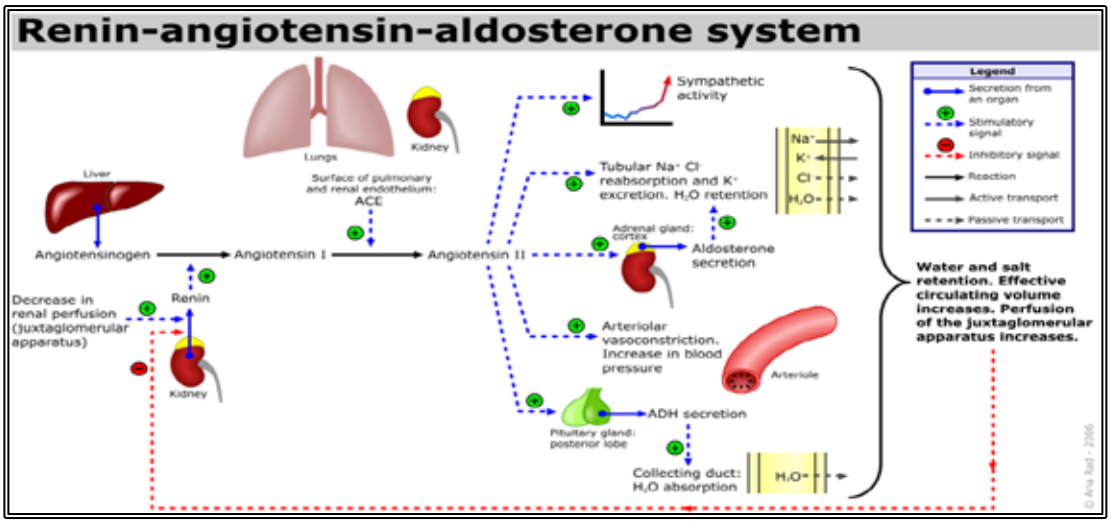

Figure 1.5: Schematic representation of the renin angiotensin aldosterone system. Diagram adapted from http://en.wikipedia.org/wiki/File:Renin-angiotensin-aldosterone_system.png.

1.6.1 Role of angiotensin II

Although there are many angiotensin subtypes like Ang III and Ang IV in the circulation, Ang II is the primary powerful active effector in RAS that can induce several physiological and pathophysiological actions. Physiologically, Ang II enhances myocardiac contractility, increases the sympathetic nerve activity, modulates smooth muscle constriction, stimulates catecholamines, aldosterone production from adrenal medulla and stimulates salt craving and thirst. However, during pathophysiological conditions, Ang II induces inflammation, apoptosis, and cell proliferation that leads to the dysregulation of gene expression in biologically active substances via multiple intracellular signaling pathways that lead to tissue injury (Timmermans *et al.*, 1993). It has been proven in animal studies that increased circulatory Ang II levels could enhance the risk for cardiovascular and renal disorders (Navar, 1997). Moreover, pharmacological investigation with angiotensin converting enzyme (ACE) inhibitor and angiotensin receptor blocker (ARB) has shown that Ang II exerts a cardinal role in pathogenesis of renal injury and hypertension in both preclinical and clinical stages. This is primarily due to the fact that the kidney plays a vital role for hypertension development, as hypertension is both a victim and culprit of renal diseases (Paul *et al.*, 2006).

1.6.2 Angiotensin II receptors

As already mentioned, the major biological actions of Ang II in the kidney are mediated by well characterized receptors. At least 4 types of angiotensin receptor subtypes have been described (Stanton, 2003). Angiotensin II type 1 (AT_1) receptor is a

typical G protein-coupled receptor (GPCR) superfamily that contains 7 trans-membrane spanning sequences, which are widely distributed on many cell types in Ang II target organs. Normally, the AT_1 receptor mediating most of the established physiological and pathophysiological effects of Ang II that includes the vasoconstriction and increase of blood pressure in the cardiovascular system. In the kidney, it enhances renal tubular sodium reabsorption and inhibits the release of renin. Other than this, AT_1 receptor also mediates the effects of Ang II on cell growth and proliferation, inflammatory response, oxidative stress, increase sympathetic activity and stimulate the production of aldosterone in adrenal cortex (Higashi *et al.*, 2002; Carey *et al*., 2000). In rodents, there are two types of AT_1 receptor subtypes. AT_{1a} receptor is predominant in all nephron segments whereas AT_{1b} is more abundant in the glomerular region (Bouby *et al.*, 1997).

The Angiotensin II type 2 (AT_2) receptor is however abundant in fetal life as compared to adult. The main function of AT_2 receptor is to mediate the vasodilation and anti-proliferative and apoptotic effects in vascular smooth muscle and prevent the growth and remodeling in the heart (Brewster *et al.*, 2003). However in kidney, AT_2 receptor may influence proximal tubule sodium reabsorption and stimulate the conversion of renal prostaglandin E_2 to prostaglandin $F_{2\alpha}$ but the importance of these AT_2 mediated action still unknown (Atlas, 2007). When both AT_1 and AT_2 subtypes are co-expressed in the same cell, the action of AT_1 receptor mediated response could be antagonized by AT_2 receptor due to the nature of hetero-dimerization properties between the two receptors (Carey *et al.*, 2000). The type 4 (AT_4) receptor is thought to mediate the action of plasminogen activator inhibitor 1 by Ang II via the N-terminal truncated peptides of Ang III and Ang IV but the exact function of the type 3 (AT_3) receptor

remains unknown (Stanton, 2003). However, others have reported that its function is to stimulate aldosterone secretion and is involved in thirst (Rang *et al.*, 2007a).

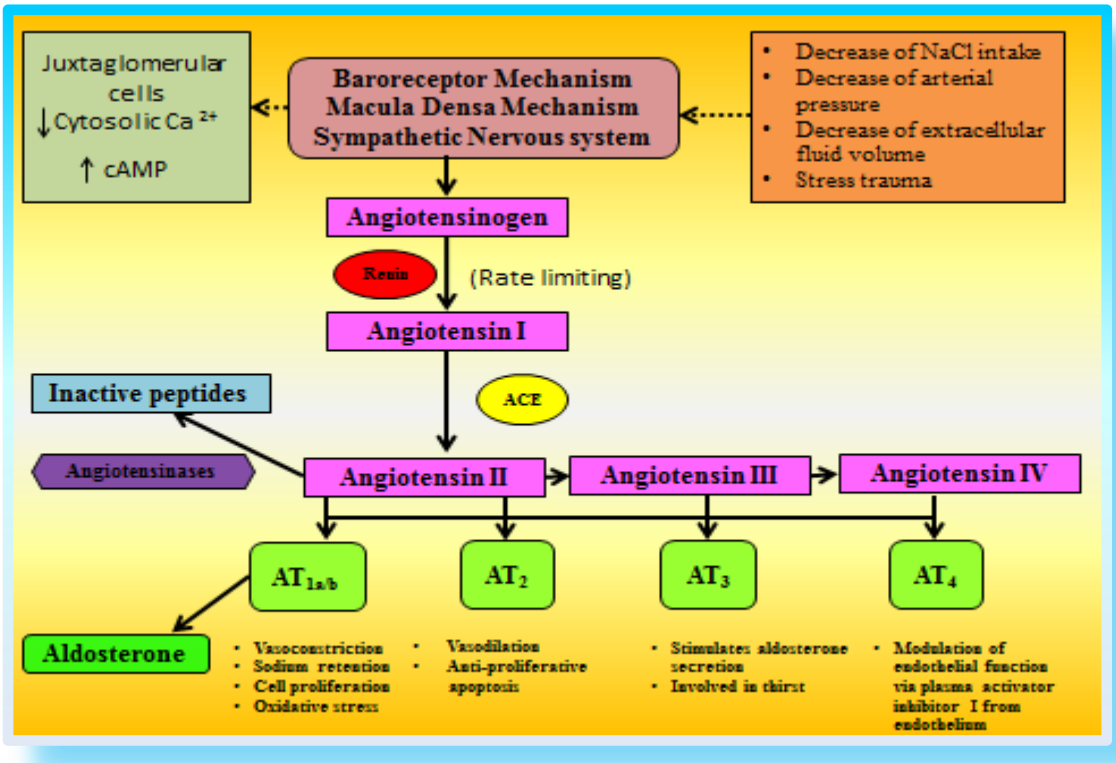

Figure 1.6: Schematic diagram of the renin angiotensin aldosterone system and its subtypes.
Diagram adapted and redrawn with modification from (Brewster & Perazella, 2004; Atlas, 2007).

1.7 Sympathetic nervous system

The nervous system as a whole consists of the central nervous system (CNS), notably the brain and spinal cord, and the peripheral nervous system (PNS), where the nerve fibers connecting all parts of the body with the central nervous system. The PNS is divided into a sensory (afferent) and motor (efferent) with the afferent division that conveys impulses to the CNS and efferent division carrying impulses away from the CNS out to the peripheral organs for an effect or action. The efferent division in PNS is further subdivided into two branches, the somatic nervous system and the autonomic nervous system (ANS). All these nerves are outside the CNS. The somatic nervous system controls musculoskeletal movement, and conducts sensory messages from the body to the CNS. On the other hand, the ANS has two branches namely sympathetic and parasympathetic divisions, which regulate the involuntary processes of the body, the viscera, blood vessels, sense organs and glands. It is called autonomic because it is believed to function autonomously. Autonomic also indicates self-regulating and this is a key principle of all body systems which depend on constant feedback for homeostasis. In standard physiology the two parts of the ANS have been perceived as functioning reciprocally: the sympathetic governing the fight or flight reaction and the parasympathetic involving restorative, relaxation and recuperation function (Germann *et al.*, 2005d).

The sympathetic nervous system is activated by any stimulus over an individual's threshold which can vary enormously, including feelings, and by noise, light, drugs and chemicals (e.g. caffeine). In response to the stimulus an immediate anticipatory state is generated by the release of neurotransmitters such as acetylcholine