α_1-ADRENOCEPTORS AND AT_1 RECEPTORS IN THE MODULATION OF RENAL HEMODYNAMICS IN HIGH SALT AND DOCA MODELS OF HYPERTENSIVE RATS TREATED WITH TEMPOL AND LOSARTAN

by

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LIST OF ABBREVATIONS

Abs.	Absorbance
Ang II	Angiotensin II
AT_1	Angiotensin II type 1 receptor
BP	Blood pressure
CONC	Concentration
DOCA	Deoxycorticosterone acetate
GFR	Glomerular filtration rate
HR	Heart rate
I.P.	Intraperitoneally
K^+	Ion potassium
KI	Kidney index
MAP	Mean arterial pressure
ME	Methoxamine
NA	Noradrenaline
Na^+	Ion natrium
NaOH	Sodium hydroxide
PE	Phenylephrine
PWV	Pulse wave velocity
RCBF	Renal cortical blood flow
ROS	Reactive oxygen species
SBP	Systolic blood pressure
S.C.	Subcutaneously
SD	Sprague Dawley

SHR	Spontaneously hypertensive rat
Tempol	4-hydroxy-2,2,6,6-tetramethyl piperidine-N-oxyl
USM	Universiti Sains Malaysia

α_1-ADRENOSEPTOR DAN AT_1 RESEPTOR MEMODULASI HEMODINAMIK GINJAL DALAM TIKUS HIPERTENSIF MODEL GARAM LEBIHAN DAN DOCA YANG DIRAWAT DENGAN TEMPOL DAN LOSARTAN

ABSTRAK

Hipertensi merupakan penyebab utama morbiditi dan kematian yang berkait rapat dengan kerosakan ginjal. Pengambilan garam lebihan menyumbang kepada pathogenesis hipertensi. Reseptor adrenergik-α_1 menyumbang kepada modulasi nada vaskular ginjal untuk mengawal tekanan darah. Kajian ini telah dijalankan untuk mengkaji kesan tempol dan losartan ke atas fungsi dan hemodinamik ginjal dalam model tikus Sprague Dawley (SD) dengan diet biasa, model tikus SD dengan garam lebihan, model tikus DOCA dengan diet biasa dan model tikus DOCA dengan garam lebihan. Semua tikus model dibahagikan kepada kumpulan kawalan, tempol (3mmol/l), losartan (10mg/kg, 7 hari terakhir) dan tempol bercampur losartan. Semua model tikus telah dikaji selama 42 hari. Kajian hemodinamik ginjal dijalankan pada hari ke-43. Data metabolic, tekanan darah, kelajuan gelombang denyutan dan vaskular ginjal responsif terhadap noradrenalin (NA), phenylephrine (PE), methoxamine (ME) dan angiotensin II (Ang II) telah dikajikan. Data analisis membandingkan min ± SEM menggunakan ANOVA satu/dua hala dengan paras signifikan 5%. Tempol dan losartan tidak mempengaruhi fungsi ginjal dan vasculature ginjal responsif dalam tikus-tikus SD dengan diet biasa. Tikus-tikus SD dengan garam lebihan mengalami kenaikan tekanan darah dan kerosakan ginjal yang terbukti berdasarkan kreatinin plasma yang meningkat, klearans kreatinin yang

menurun dan pengurangan nisbah natrium: kalium. Tikus-tikus DOCA dengan garam lebihan mengalami kenaikan tekanan darah dan ginjal tidak berfungsi yang tersokong berdasarkan kreatinin plasma yang meningkat, klearans kreatinin yang menurun dan penurunan dalam perkumuhan pecahan natrium. Tempol menurunkan tekanan darah dalam model tikus SD dengan garam lebihan, model tikus DOCA dengan diet biasa dan model tikus DOCA dengan garam lebihan. Losartan mengurangkan tekanan darah tinggi dalam tikus-tikus SD dengan garam lebihan. Pengurangan tindakbalas perfusi darah kortikal ginjal terhadap agonis adrenergik membuktikan garam lebihan telah menurun responsif reseptor adrenergik-α_1 ginjal dalam tikus-tikus SD dengan garam lebihan dan tikus-tikus DOCA dengan garam lebihan. Garam lebihan menurun sensitiviti AT_1-reseptor dalam tikus-tikus SD dengan garam lebihan. Tempol memperbaki peratusan penurunan perfusi darah kortikal ginjal terhadap agonis adrenergik dalam tikus-tikus SD dengan garam lebihan. Losartan menaikan reseptor adrenergik-α_1 responsif dan menurunkan kepekaan AT_1-receptor dalam tikus-tikus DOCA dengan garam lebihan. Tempol bercampur losartan memperbaiki peratusan penurunan perfusi darah kortikal ginjal terhadap agonis adrenergic dalam tikus-tikus SD dengan garam lebihan dan tikus-tikus DOCA dengan diet biasa. Dapatan kajian ini mencadangkan bahawa tempol merendahkan tekanan darah dan memperbaiki α_1-adrenoreseptor sensitif yang mampu memberi perlindungan daripada spesies oksigen reaktif dalam model tikus hipertensi.

α_1-ADRENOCEPTORS AND AT_1 RECEPTORS IN MODULATION OF RENAL HEMODYNAMICS IN HIGH SALT AND DOCA MODELS OF HYPERTENSIVE RATS TREATED WITH TEMPOL AND LOSARTAN

ABSTRACT

Hypertension is a major cause of morbidity and mortality from renal impairment. An increase of sodium intake contributes to the pathogenesis of hypertension. α_1-adrenoceptors modulate renal vascular tone in the regulation of blood pressure. This study was undertaken to investigate the effects of tempol and losartan on renal function and haemodynamics in Sprague Dawley (SD) rats fed with normal diet, SD rats fed with high sodium diet, DOCA-salt treated rats fed with normal diet and DOCA-salt treated rats fed with high sodium diet. All models were divided into groups of control, tempol (3mmol/l), losartan (10mg/kg, last 7 days) and combination of tempol and losartan. The animals were studied for 42 days. The acute renal hemodynamic study was performed on day 43. Metabolic data, blood pressure (BP), pulse wave velocity (PWV) and renal vascular responsiveness to noradrenaline (NA), phenylephrine (PE), methoxamine (ME) and angiotensin II (Ang II) were investigated. Data, mean ± SEM were analyzed using one/two-way ANOVA with significance level of 5%. Tempol and losartan did not influence renal function and renal vasculature responsiveness in SD rats fed with normal diet. SD rats given with high sodium diet showed higher BP and renal impairment as evidenced by increased plasma creatinine, creatinine clearance and decreased urinary sodium to potassium ratio. DOCA-salt rats fed with high sodium diet had higher BP and in renal function compromised as supported by increased plasma creatinine, decreased creatinine

clearance and increased fractional sodium excretion. Tempol decreased BP of SD rats fed with high sodium diet, DOCA-salt treated rats fed with normal diet and DOCA-salt treated rats fed with high sodium diet. Similarly, losartan reduced BP in SD rats fed with high sodium diet. High sodium diet decreased the responsiveness of renal α_1-adrenoceptors in SD rats fed with high sodium diet and DOCA-salt rats fed with high sodium diet as supported by decreased renal cortical blood perfusion (RCBP) in response to adrenergic agonists. High sodium diet also decreased sensitivity of AT_1-receptors in SD rats fed with high sodium diet. Tempol increased % drop of RCBP in response to adrenergic agonists in SD rats fed with high sodium diet. Similarly, losartan increased the responsiveness of α_1-adrenergic receptors and decreased the sensitivity of AT_1-receptors in DOCA-salt rats fed with high sodium diet. Tempol and losartan given in combination increased % drop of RCBP in response to adrenergic agonists in SD rats fed with high sodium diet and DOCA-salt rats fed with normal diet. Collectively, the results suggest that tempol lowers MAP and improves sensitivity of α_1-adrenoceptors thus providing protection against ROS in these hypertensive rat models.

CHAPTER 1

INTRODUCTION

Epidemiological and scientific studies have reported that sodium consumption is very high in our daily diets (Brown et al., 2009; Li et al., 2012). Previous experimental and clinical studies mentioned the vital role of sodium in the regulation of blood pressure and the implications of abnormal sodium balance in the development of hypertension (Esteva-Font et al., 2010; Koga et al., 2008; Kuller, 1997; Logan, 2006). Studies have also indicated that a long-term high sodium dietary intake contributes to elevated blood pressure (Johns, 2002) especially when the kidneys have a decreased function of sodium excretion (Ogihara et al., 2003). It can be explained that a decreased function of kidney to excrete sodium would cause sodium and water retention. Then, it leads to an increased extracellular fluid and increased plasma volume resulting into an elevated blood pressure. Restriction in sodium intake has been shown to decrease the blood pressure (Karppanen and Mervaala, 2006). Independent of excess sodium intake effect on blood pressure, it also increases left ventricular hypertrophy as well as fibrosis in kidneys and arteries (Appel et al., 2011).

Experimental work on the pressure natriuresis and diuresis relationship developed by Guyton confirmed kidneys played a role in blood pressure regulation (Guyton, 1991). However, there are also many systems and mechanisms involved in arterial blood pressure regulation such as the baroreceptor mechanism, the chemoreceptor system, the central nervous system ischemic response, the renin-

angiotensin-vasoconstrictor mechanism, the stress relaxation mechanism, the capillary fluid shift mechanism, the renal-body fluid mechanism and the aldosterone mechanism (Guyton et al., 1972). These mechanisms and systems regulate blood pressure by modulating cardiac output, fluid volumes and peripheral vascular resistance.

The decreased function of the kidney to excrete sodium is different from individual to individual. Those require a higher than the normal levels of blood pressure to excrete sodium are called to be salt-sensitive. However, those can excrete excess salt at normal level of blood pressure are known as salt-resistant. Several mechanisms responsible for decreased salt-sensitivity have been reported such as defect in renal function (Campese, 1994), abnormally increasing reactive oxygen species generation leading to impairment in the endothelial derived relaxing factor nitric oxide activity (Lenda et al., 2000) and abnormally high activation of intra-renal renin-angiotensin system (Redgrave et al., 1985).

1.1 Urinary system

1.1.1 Kidney

Both kidneys are reddish and bean shaped organs. They are located in the retroperitoneal cavity of abdomen. The kidneys are located on either side at the level of the twelve thoracic to the third lumbar vertebrae. The right kidney is slightly lower in position than the left because the liver takes up some of the space above the right kidney (Grabowski and Tortora, 2003). Three layers of tissues surround kidneys; renal capsule (deep layer), adipose capsule (middle layer) and renal fascia (superficial layer). A mass of adipose tissue encases each kidney and holds it in its position. The renal capsule and the renal fasciae anchors the kidneys to surrounding structures and helps maintain their positions (Thibodeau and Patton, 2007).

The medial border of the kidney has a concave notch called hilus that allows structures to enter or leave kidney along with blood vessels, lymphatic vessels, and nerves. The frontal section through the kidney reveals two regions that are renal cortex or the superficial region and renal medulla or the inner region. The renal pyramids make up the medullary tissue. The base of each pyramid faces the renal cortex and each renal papilla faces toward the renal hilus (Grabowski and Tortora, 2003). The cortical tissue extends into the medulla between the pyramids to form renal columns. The renal papilla drain into cupslike structure called calyces where urine is received from the renal papilla, drained into renal pelvis and then transported out of the body through the ureters to the urinary bladder and urethras (Thibodeau and Patton, 2007).

The ureters are two tubes which are 25 to 30cm in length and transport urine from the renal pelvis to the urinary bladder (Grabowski and Tortora, 2003). The ureters curve medially from the renal pelvis until reaches through the bladder wall and opens at the lateral angle of the trigone in the urinary bladder (Thibodeau and Patton, 2007). Each ureter is formed of three layers of tissues which are the mucosa or the deepest layer, the muscularis or the intermediate layer and the adventitia or the superficial layer. The muscular layer is composed of smooth muscle fibers which play a major function in peristalsis to propel urine. The peristalsis waves vary in frequency rate and are dependent on how fast the urine is being formed (Grabowski and Tortora, 2003).

The urinary bladder is a muscular and distensible bag organ which is situated directly behind the pubis symphysis and in front of the pelvic cavity (Grabowski and Tortora, 2003). It lies below the parietal peritoneum which covers only its superior surface. The remainder of the bladder surface is covered by a fibrous adventitia whereas the wall of the bladder is made up of smooth muscles (Thibodeau and Patton, 2007). The urinary bladder is lined with mucous transitional epithelium that forms folds called rugae to permit expansion (Grabowski and Tortora, 2003). There are three openings in the bladder, two from the ureters and one into the urethra, which helps in urine drainage from the body (Thibodeau and Patton, 2007). The function of urinary bladder is to store and excrete urine (Andersson and Arner, 2004).

The urethra is a small tube lined with mucous membrane that emerges from the floor of the urinary bladder to the exterior of the body. In both male and female, the urethra is the last part of urinary system for propelling urine out from the body. In males, the urethra extends about 20cm, which passes through the center of the prostate gland just after leaving the urinary bladder (Thibodeau and Patton, 2007). The urethra is joined by two ejaculatory ducts within the prostate. After leaving the prostate, the urethra extends down and enters the base of the penis. The urethra travels through the center of the penis and ends as a urinary meatus at the tip of the penis. In female, the urethra lies directly posterior to the pubis symphysis and anterior to the vagina as it passes through the muscular floor of the pelvis. The urethra extends down and forward from the bladder for about 3cm and ends at the external urinary meatus (Thibodeau and Patton, 2007).

The main functions of the kidneys are to process blood plasma and excrete urine in order to maintain the homeostatic balance of the body. The kidneys are the most important organs in the body for maintaining fluid electrolyte and acid-base balance as well as excreting wastes. They also regulate blood pH, blood volume and blood glucose levels as well as synthesize the active form of vitamin D and certain prostaglandins. The kidneys influence the secretion rate of antidiuretic hormone (ADH) and aldosterone (Grabowski and Tortora, 2003). Kidney plays crucial role in controlling and regulating blood pressure (BP) by handling sodium and water balance (Gu et al., 2008). Kidneys promote natriuresis and diuresis according to different sodium intake in order to maintain normal BP and the development of essential hypertension (De Richelieu et al., 2005).

1.1.2 Nephron

Nephron is the basic structural and functional unit of the kidney. The function of nephron includes blood plasma processing and urine formation by means of three processes. These three processes are filtration, tubular reabsorption and tubular secretion. A hydrostatic pressure gradient drives the filtration of the plasma into the nephron. The filtrate contains materials that the body must save, the walls of the tubules reabsorb these materials back into the blood. As the filtrate begins to leave the nephron, the kidney may secrete a few items into the urine for excretion. There are two general classes of nephrons which are cortical nephrons and juxtamedullary nephrons. Almost all cortical nephrons are located in the renal cortex whereas juxtamedullary nephrons lie near the junction of the cortical and medullary layers (Thibodeau and Patton, 2007). The structure of nephron includes renal corpuscle, Bowman's capsule, proximal convoluted tubule, loop of Henle, distal convoluted tubule and collecting duct.

Filtration is the first step of urine production in which water and small solutes of blood filter out and move across glomerular capillaries into Bowman's capsules (Grabowski and Tortora, 2003). Larger cells and plasma proteins do not filter into the Bowman's capsules. The filtration takes place through the glomerular capsular membrane. Glomerular filtration depends on systemic blood pressure, a decreased blood pressure tends to decrease both glomerular pressure and filtration rate (Grabowski and Tortora, 2003). Increase in blood pressure results in constriction of afferent arterioles in order to maintain the blood flow to the kidney thereby maintaining glomerlar pressure and glomerular filtration (Thibodeau and Patton, 2007).

Reabsorption is the second step in urine formation, which involves passive and active transport mechanisms from all parts of the renal tubules. The proximal tubule reabsorbs all nutrients and major portion of water and electrolytes back into blood stream. The rest of the renal tubule reabsorbs comparatively little of the filtrate.

Tubular secretion means the movement of substances out of the blood and into tubular fluids. The descending limb of the loop of Henle removes urea through diffusion. The distal and collecting tubules secrete potassium, hydrogen, and ammonium ions (Grabowski and Tortora, 2003). They actively transport potassium ions or hydrogen ions out of the blood into tubule fluid in exchange for sodium ions that diffuse back into the blood. Potassium ion secretion increases when the blood aldosterone concentration increases. Aldosterone is a hormone of the adrenal cortex, which targets distal and collecting tubule cells to increase the activity of the sodium-potassium pumps that move sodium ion out of the tubule and potassium ion into the tubule. Hydrogen ion secretion increases when the blood hydrogen ion concentration increases. Ammonium ions are secreted into the tubular fluid by diffusing out of the tubule cells where they are synthesized (Thibodeau and Patton, 2007).

1.1.2 (a) Renal corpuscle and glomerulus

The renal corpuscle is composed of the Bowman's capsule and glomerulus. The renal corpuscle is the nephron's beginning filtering component of the kidney. The Bowman’s capsule is the cup-shaped mouth of a nephron which surrounds the glomerulus. It is formed by two layers of epithelial cells with a space called Bowman’s space. Fluids, waste products and electrolytes are filtered through the capillaries of the glomerulus into the Bowman’s space and constitute the glomerular filtrate which will be processed in the nephron to form urine. At the beginning of the nephron, the glomerulus is a network of fine capillaries that performs the first step of filtering blood. Glomeruli have thin and membranous walls that are composed of a single layer of endothelial cells. Many pores are present in the glomerular endothelium, which are larger than other pores of regular capillaries. This increased porosity is necessary for filtration to occur at the rate required for normal kidney function. Thus, glomerular filtration rate (GFR) is accepted as the measure of the overall kidney function (Stevens et al., 2006).

1.1.2 (b) Proximal tubule

The proximal tubule is the second part of the nephron but the first part of the renal tubule. Its wall consists of one layer of epithelial cells. The luminal surface of the epithelial cells is covered with numerous amounts of microvilli that form the brush border. The proximal tubule regulates the pH of the filtrate and secretes organic acids, such as creatinine and other bases into the filtrate. Sodium ions, water, potassium ions, urea, phosphate ions, citrate ions which enter the proximal convoluted tubule are partly reabsorbed into the peritubular capillaries (Grabowski and Tortora, 2003). Most of filtrate that enters the renal tubule from Bowman's capsule is reabsorbed before it reaches the end of the proximal tubule and only a small volume of filtrate is left to continue to the next portion of the loop of Henle. Sodium ions are actively transported out of the lumen of the tubule and into peritubular blood. As sodium ions accumulate in the interstitial fluid and become temporarily positive with respect to the tubule fluid. This electrical gradient drives the diffusion of negative ions from the filtrate into the interstitial fluid and then into the peritubular blood. The attraction between negative and positive ions is used to drive the passive transport of chloride, phosphate and other negative ions out of the tubule. Ion transports out of the proximal tubules causes water osmosis out of the tubule and into the peritubular blood and makes the two fluids isotonic. Proximal tubules reabsorb nutrients from the tubule fluid, glucose and amino acids into peritubular blood by a type of active transport mechanism called sodium cotransport. Glucose and amino acids passively move out of the tubule fluid by means of the sodium cotransport mechanism (Thibodeau and Patton, 2007).

1.1.2 (c) Loop of Henle

The loop of Henle is the segment of renal tubule just beyond the proximal tubule. It consists of a descending limb and an ascending limb. The descending limb has low permeability to ions and urea but highly permeable to water. The ascending limb is impermeable to water but permeable to ions. The cortical ascending limb drains urine into the distal tubule. The length of the loop of Henle is important in the production of concentrated or diluted urine (Thibodeau and Patton, 2007). The descending loop of Henle receives isotonic fluid from the proximal tubule, then reabsorbs water from tubule fluid and picks up urea from the interstitial fluid. The volume of fluid in the loop of Henle is lesser than the fluid in proximal tubule. The ascending limb of the loop of Henle gets a lower volume of fluid as compared to the descending limb. In contrast, the ascending portion of the loop of Henle becomes impermeable to water but extremely permeable to ions. Sodium and chloride ions are actively reabsorbed from the tubule fluid in the ascending limb. By reabsorbing sodium ions from the ascending limb of the loop of Henle, this can cause the tubule fluid to be diluted. Reabsorption of sodium ions in the ascending limb also creates and maintains a high solute concentration of the medullary interstitial fluid (Thibodeau and Patton, 2007).

1.1.2 (d) Distal tubule and collecting duct

The distal tubule is located between the loop of Henle and the collecting duct system. The juxtaglomerular apparatus is found at the point where the afferent arteriole brushes past the distal tubule. This structure is important in maintaining homeostasis of blood flow because it reflexively secretes renin when blood pressure in the afferent arteriole drops. Renin triggers a mechanism that produces angiotensin, which causes vasoconstriction and the resulting increase in blood pressure. Large smooth muscle cells in the wall of the afferent arteriole called juxtaglomerular cells contain renin granules. These cells are sensitive to increased pressure in the arteriole and function as mechanoreceptors (Thibodeau and Patton, 2007). Modified distal tubule cells in the juxtaglomerular apparatus are crowded together to form a structure called macula densa. Cells in the macula densa are chemoreceptors that can sense the concentration of solute materials in the fluid passing through the tubule. Acting together, both cell types in the juxtaglomerular apparatus contribute to homeostasis of renal function by influencing the ability of the kidney to produce concentrated urine (Grabowski and Tortora, 2003). The function of distal tubule is similar to the proximal tubule, which also absorbs sodium ions by active transport but in smaller amounts. The distal tubule's walls are relatively impermeable to water so that sodium ions can be reabsorbed but not water. As a result, the solute concentration of the tubule fluid continues to decrease. Apart from that, the wall structure of the collecting duct also prevents water from leaving the filtrate by osmosis. The collecting duct conducts the tubule fluid through the hypertonic medullary region (Thibodeau and Patton, 2007). The collecting duct of the kidney consists of a series of tubules and ducts which connect the nephrons to the ureter. The components of the collecting duct include the connecting tubules, cortical collecting ducts and

medullary collecting ducts. The collecting duct system is involved in ion and fluid balance by reabsorption and secretion processes which are regulated by the aldosterone and antidiuretic hormones.

A regulatory mechanism is centered outside the kidney in order to prevent excessive loss of water in the body. ADH is secreted by the posterior pituitary. It causes cells of the distal and collecting tubules to become more permeable to water. Then, water flows out of the tubule by osmosis and goes into the interstitial fluid in order to achieve equilibrium. The more ADH is present, the more water is allowed out of the tubule and the tubular fluid's solute concentration matches that of the surrounding tissues (Thibodeau and Patton, 2007). ADH increases the solute concentration of the urine and decreases water excretion. ADH has a central role in the regulation of urine volume. Control of the solute concentration of urine translates into control of urine volume. If water is not reabsorbed by the distal and collecting tubules, urine volume is relatively high and water loss from the body is high. ADH regulates the body's retention of water by acting to increase water absorption in the collecting ducts of the kidney nephron in order to reduce water loss by the body (Caldwell and Young, 2006). Aldosterone is a hormone that tends to decrease urine volume and conserves water (Hu et al., 2012). Aldosterone is produced from adrenal cortex in the adrenal gland. It increases distal and collecting tubule absorption of sodium, which in turn causes an osmotic imbalance that drives the reabsorption of water from the tubule. The water reabsorption in the distal and collecting tubule portions requires ADH to function. Therefore, the aldosterone mechanism works together with the ADH mechanism in order to maintain the homeostatsis of body.

Urine analysis is often used as an indirect measure of the resulting kidney injury or the health status of a person. Urine contains water (Folin, 1905) along with nitrogenous wastes, electrolytes, toxins, pigments, hormones (Demir et al., 1994) and abnormal constituents. Nitrogenous wastes from protein catabolism are urea, uric acid, ammonia and creatinine (Bingham and Cummings, 1985). Electrolytes mainly present are ions such as sodium, potassium, ammonium, chloride, bicarbonate, phosphate, and sulfate (Shevock et al., 1993). The amounts and types of minerals vary with diet. During diseased state, toxins of the body are also excreted into urine (Le et al., 1994). Pigments like urobilin or urochromes derived from products of the breakdown of old red blood cells in the liver and elsewhere (De Araujo Pantoja et al., 2012). Various types of food and drug (Drayer, 1976) may contain or be converted into pigments that are cleared from plasma by the kidneys into urine. Abnormal constituents such as blood, glucose, albumin and cast or calculi can also be found in urine (Atmani et al., 1996; Fogo and Barakat, 1990).

1.2 Blood vessels

The kidneys are highly vascular organs which process the blood in important ways before returning it to the general circulation (Kang et al., 2001). There are three major types of blood vessels, which are arteries, veins and capillaries. Arteries carry oxygenated blood from the heart to the capillaries from where veins in general return the deoxygenated blood back to the heart (Vito and Dixon, 2003). The actual exchange of water and materials between blood and the tissues occurres in capillaries (Pappenheimer, 1953). The walls of arteries and veins consist of three separate layers which are tunica intima (innermost), tunica media (middle) and tunica adventitia (outer). The tunica intima consists of an endothelial cell monolayer which forms a smooth, flat and low friction surface. This layer is closest to luminal surface that aligns in the direction of blood flow. It also prevents blood cells including platelets, leukocytes and other elements from adhering to the luminal surface. Tunica media is also a layered structure which is made up of smooth muscle cells, elastic connective tissue, collagen and proteoglycans. Smooth muscle cells are responsible for vasoconstriction and vasodilation (Tanaka and Yamada, 1990). Endothelium of blood vessels are in charge of regulating vascular tone via the generation of vasodilator and vasoconstrictor substances (Guzik et al., 2002; Lenda et al., 2000). Medial elastin assists in keeping blood flow by expanding with pressure. Medial collagen prevents excessive dilation (Clark and Glagov, 1985). The tunica adventitia consists of collagen, fibroblasts and some elastin fibers. In some arterial adventitia, there is presence of vasa vasorum which is a vascular network.

1.3 Heart

The heart is a muscular organ which can be found in all animals(Beck, 1935). The main function of the heart is to generate the force for blood circulation throughout the circulatory system by rhythmic contraction and relaxation. In humans, the heart is located at the centre of thoracic cavity above the diaphragm. In addition, the heart is about 310 grams in males and 225 grams in females. The heart rate of a healthy person is 72 beats per minute (Thibodeau and Patton, 2007). It is covered by a membranous sac called pericardium that possesses a special fluid that lubricates the heart when it beats.

The human heart is composed of four chambers. The upper two chambers are called atria which receive blood back from the vasculature system. The two lower chambers called ventricles which receive blood from both atria and generate the force to pump blood away from the heart via the blood vessels. The atria and ventricles are separated by a septum to prevent blood from mixing between left heart and right heart. The interatrial septum separates the left and right atrium, whereas the interventricular septum separates the left and right ventricle. The broader upper pole of the heart is the base and the lower pole is called the apex (Dickstein et al., 2008). The heart wall is made up of three layers, an epicardium (outermost), myocardium (middle) and endocardium (inner). The mechanical force of heart moves the wall inward and squeezes the blood into the chamber when the cardiac muscles in the atrium and ventricle walls contract. As the squeezing gradually increases, the pressure pushes the blood out of both atria and ventricles. When the cardiac muscle relaxes, the atrium and ventricle expand and fill with blood (Anderson, 2000).

Four sets of valves in heart are important to permit the flow of blood in only one direction. Atrioventricular valves are two in number which are mitral valve and tricuspid valve. The pulmonary semilunar valve and aortic semilunar valve are located where the pulmonary artery and the aorta arise from the right and left ventricles, respectively (Thibodeau and Patton, 2007). Cardiac muscle cells play an important role in cardiac contraction. The heartbeat is modulated by pacemaker in the sinoatrial node. One systole followed by one diastole to form one complete heartbeat, which is called as one cardiac cycle.

The left ventricle pumps oxygenated blood into the aorta whose branches deliver blood to capillary beds of all tissues and organs in the systemic circuit. The deoxygenated blood from the systemic tissues transports back to the heart through the superior vena cava into the right atrium. Then, the blood flows from the right atrium into the right ventricle through the tricuspid valve. From the right ventricle, the blood pumps into the pulmonary artery which carries the deoxygenated blood to the lungs for gaseous exchange.

In the lungs, blood becomes oxygenated and travels to the left atrium via pulmonary veins. From the left atrium, blood enters through the bicuspid valve into the left ventricle. When the pressure in the left ventricle is very low during its relaxation, the phase of cardiac cycle is called diastole (Opie, 2004).

1.4 Adrenergic receptors

1.4.1 Classification of adrenergic receptors

Adrenoceptors or adrenergic receptors are a class of G protein-coupled receptors (GPCRs) which are found in cell membrane sites (Rosenbaum et al., 2009). Catecholamines, noradrenaline and adrenaline bind to adrenoceptors which act as important neurotransmitters and hormones in the central nervous system and peripheral nervous system (Guimaraes and Moura, 2001). The adrenoceptors are the targets of many therapeutically important drugs in diseases such as cardiovascular disorders (Guimaraes and Moura, 2001), hypertension (Chen et al., 2007) and renal failure (Khan et al., 2007). There are two major classes of adrenoceptors; α and β adrenoceptors. α-adrenoceptors are further divided into α_1 and α_2 whereas β adrenoceptors are divided into β_1, β_2 and β_3. Furthermore, α_1-adrenoceptors are subdivided into three subtypes: α_{1A}, α_{1B}, and α_{1D}. α_2–adrenoceptors are also classified as α_{2A}, α_{2B}, and α_{2C} subtypes (Gilsbach and Hein, 2012; Guimaraes and Moura, 2001). Specific actions of the α_1 receptors mostly involve smooth muscle contraction. The action of α_1-adrenoreceptor causes vasoconstriction in most of the blood vessels, such as those of the skin (Kenney et al., 1991), renal artery (Schmitz et al., 1981) and brain (Young and Kuhar, 1980). The α_1-adrenoreceptors mediate renal vasoconstriction in rats with hypertension induced by various methods including DOCA-salt and two-kidney one clip models of hypertension (Sattar and Johns, 1996). Specific actions of the α_2–adrenoreceptor include inhibition of insulin release from the pancreas (Nakaki et al., 1981), induction of glucagon release from the pancreas (Hirose et al., 1992) and negative feedback in the neuronal synapses via presynaptic inhibition of noradrenaline (NA) release in central nervous system (Dennis et al., 1987). The β-adrenoceptors mostly perform inhibitory properties except in the heart.

1.4.2 Renal α-adrenergic mechanism

Renal α_1-adrenoceptors mediate the regulation of various renal functions including renal hemodynamic, glomerular ultrafiltration, renovascular tone and tubular electrolyte reabsorption thereby contributing to the regulation of extracellular fluid volume and arterial blood pressure (Dibona and Kopp, 1997). Excessive dietary sodium leads to primary hypertension only in those individuals with heightened sodium sensitivity associated with a strong genetic background (Chen et al., 2007). In hypertension, the primary defect is located in the kidneys. The renal sympathetic nerve which densely innervates all the components of the kidney mediates its actions through renal α- and β-adrenoceptors during the early stages of the hypertension. Renal α-adrenoreceptors are mostly located in proximal tubules and enhance Na^+ reabsorption. An increased sodium intake affects the expression of the renal adrenergic system in normotensive and few hypertensive animal models, which contributes to the pathogenesis of hypertension (Tanoue et al., 2002). Previous findings have shown that high sodium diets can cause an elevation of renal adrenoceptor density (Saiz et al., 1987). High sodium intake associated with increased renal adrenoceptor density causes smooth muscle contractility and sodium reabsorption resulting in enhancement of renal vasocontraction and sodium retention (Weinberger, 1996). These actions result in blood pressure to increase. This increase in blood pressure in turn might cause renal injury.

1.5 Hypertension

1.5.1 Definition

Hypertension is defined as mean systolic blood pressure ≥140 mmHg and mean diastolic blood pressure ≥90 mmHg (Chobanian et al., 2003; Mancia et al., 2013). Hypertension is a major public health issue and its prevalence is high in many developing countries as well as in the developed world. The prevalence of hypertension in Malaysia remains common and high (Ramli et al., 2012; Rampal et al., 2008). Hypertension is a major risk factor for a variety of problems especially cardiovascular problems (Krause et al., 2011; Levy et al., 1996). Furthermore, hypertension is a cause of chronic kidney disease (Foley et al., 1996). The development of unmanaged hypertension leads to mortality among the population. Drug treatment, dietary and lifestyle changes can improve blood pressure management and lower the risk of hypertension complications. Normal blood pressure is within the range of 120-129 mmHg systolic and 80-84 mmHg diastolic. High normal blood pressure is within the range of 130-139 mmHg systolic and 85-89 mmHg diastolic (Mancia et al., 2013). High blood pressure is known as stage 1 hypertension if it is within the range of 140-159 mmHg systolic and 90-99 mmHg diastolic (Burt et al., 1995; Mancia et al., 2013). On the other hand, stage 2 hypertension occurs when systolic blood pressure exceeds 160 mmHg and diastolic blood pressure over 100 mmHg (Burt et al., 1995; Mancia et al., 2013).

1.5.2 Types of hypertension

Hypertension can be classified into essential hypertension and secondary hypertension. The essential hypertension is developed by no obvious underlying medical causes (Carretero and Oparil, 2000). However, the secondary hypertension is caused by other conditions such as increasing age with coexisting atherosclerosis (Anderson et al., 1994). There are many interrelated factors that can cause hypertension and they vary among different individuals. High sodium intake is one of the factors, other factors like obesity, insulin resistance, impaired renin-angiotensin system and sympathetic nervous system, genetic disorders, endothelial dysfunction and neurovascular anomalies can also cause hypertension (Beevers et al., 2001b). Cardiac output, peripheral resistance, renin-angiotensin-aldosterone system, autonomic nervous system, bradykinin, endothelin and nitric oxide (NO) play a role in the development of essential hypertension (Zhu et al., 2004). The pathophysiology of hypertension is still much unknown (Beevers et al., 2001b). ROS has been suggested to impair endothelium-dependent vasodilation and reduce NO production during elevated sodium intake (Zhu et al., 2004). In DOCA-salt treated animals, the mechanism of induction of endocrine hypertension is due to retention of sodium and water, this increases circulating blood volume and results in hypertension. Renin angiotensin system is suppressed in DOCA salt hypertension model and treatment with AT_1-receptor antagonist has no effect on the blood pressure. Normal kidney has the physiologic ability to excrete the daily sodium load effectively without allowing a significant rise in the extracellular volume. Chronic administration of excess sodium can lead to the production of hypertension in rats, which is a good research model to mimic hypertension in human subject.

1.5.3 Renin-angiotensin-aldosterone system

Renin-angiotensin-aldosterone system (RAAS) plays an important role in long-term maintenance of arterial blood pressure despite extremes in dietary sodium intake. Blood pressure is regulated by sodium excretion (Stolarz-Skrzypek et al., 2011). The release of renin is stimulated when sodium intake is low. Renin release is inhibited when sodium intake is high. When the RAAS is dysregulated, changes in sodium intake affect the blood pressure (Weir and Dzau, 1999). Furthermore, hypertension in response to high sodium intake could be partially due to the result of insufficient renal vasodilation which is attributed by RAAS blockade (van Paassen et al., 1996).

Renin is secreted from the juxtaglomerular apparatus of the kidney in response to glomerular underperfusion or a condition when the sodium intake is reduced. Renin is responsible for converting renin substrate (angiotensinogen) to Ang I, which is a physiologically inactive substance and is rapidly converted to Ang II in the lungs by angiotensin converting enzyme (ACE). Ang II is a potent vasoconstrictor and thus causes a rise in blood pressure. It stimulates the release of aldosterone which results in a further rise in blood pressure related to sodium and water retention (Beevers et al., 2001a). Nevertheless, previous studies have reported local participation of the RAAS in cardia vascular system and kidney during sodium loading (Navar et al., 2006; Nickenig et al., 1998). This suggested that despite of suppressed RAAS with salt loading, cardiac and RAAS could be locally stimulated to generate Ang II (Matavelli et al., 2007; Varagic, 2006).

1.5.4 Sympathetic nervous system

The nervous system consists of the central nervous system (CNS); brain and spinal cord as well as peripheral nervous system (PNS) (Nieuwenhuys et al., 2007). The PNS is divided into sensory (afferent) division and motor (efferent) division. The afferent division conveys impulses to the CNS whereas efferent division brings impulses out from the CNS to peripheral organs for taking action (Levine et al., 1986). In PNS, the efferent division is subdivided into somatic nervous system (SNS) and autonomic nervous system (ANS). The SNS regulates skeletal muscles for body movement (Nurmikko et al., 1991) while the ANS controls heart rate and urination (De Groat, 1975; Gabella, 2001; Sztajzel, 2004). The ANS is composed of sympathetic division and parasympathetic division (Mathias and Bannister, 2013).

The general action of the sympathetic nervous division is to mobilize the function of internal organs as diverse as pupil diameter (Wilhelm et al., 2001), gut motility (Straub et al., 2006) and urinary output (Schlaich et al., 2010). It is known for mediating the neuronal and hormonal stress response commonly called the fight-or-flight response and is immediatetly active at a basic level to maintain homeostasis (Jansen et al., 1995). The parasympathetic system controls the function of organs and glands in the body (Quigley, 2010).

A high sodium intake increases the activity of the sympathetic nervous system (Carlson et al., 2000). Moreover, salt sensitivity in essential hypertension has been associated with increased sympathetic activity which is supported by increased renal sympathetic nerve activity (Campese, 1994). The renal sympathetic nerve plays an important part in the modulation of renal function which in turn can affect renin

release, extracellular fluid volume and blood pressure (Katholi, 1983).

The sympathetic nervous system plays an important role in controlling arterial pressure under different conditions by modifying cardiac output, peripheral vascular resistance and renal functions. Kidney increases tubular sodium reabsorption, renin release and renal vascular resistance when its sympathetic nerves are activated (Grisk and Rettig, 2004).

Any disturbed renal sympathetic nerve activity could directly affect the kidney. A mishandling of sodium and water, decreased filtration rate and decreased glomerular renal blood flows can lead to renal vasoconstriction and substantial renin production (Dibona and Kopp, 1997). Ang II activates NADH/NADPH oxidase that increases O_2^- in vascular tissues (Campese et al., 2005). Previous reports have documented that ROS could also mediate the effects of Ang II on sympathtic nerve activity (Ye et al., 2006) and elevation of O_2^- can decrease sodium excretion thus leading to hypertension.

Abnormal increase in the renal sympathetic nerve activity in hypertension can alter renal function. The changing of sodium and water retention will increase blood volume. An increased blood volume associated with an elevated arterial blood pressure enhance urinary sodium excretion and water excretion in order to maintain homeostasis (DiBona, 2004).

High sodium intake activates the central sympathoecitatory mechanisms by increasing brain angiotensin activity and sodium concentration in cerebrospinal fluid (Huang and Leenen, 1998). The increased Ang II in brain due to high sodium intake can alter the brain in regulating renal sympathetic nerve activity and renal excretory function (Johns, 2002).

The arterial baroreceptor reflex activity can modulate the arterial pressure and sodium homeostasis through the renal sympathetic nerve system (Osborn and Hornfeldt, 1998). An increased mean arterial pressure due to high sodium intake can stimulate the sensitivity of arterial baroreceptor. The stimulation can lead to heart relaxation, peripheral blood vessels dilation and reduced stimulation on kidney. These actions reduce renal tubular sodium reabsorption and produce natriuresis, which normalize the arterial pressure (Guyton et al., 1972).

1.6 Oxidative stress

Oxidative stress is an imbalance between oxidants and antioxidants in favour of the oxidants which potentially leads to damage (Sies, 1997). Oxidative stress can cause toxic effects through the production of peroxides and free radicals that damage cells. Oxidative stress plays a role in many clinical conditions such as hypertension, diabetes, and renal failure (Banday et al., 2007; Dobrian et al., 2003; Maritim et al., 2003; Massy and Nguyen-Khoa, 2001).