# EFFECTS OF HIGH SALT LOAD ON RENAL HEMODYNAMICS AND FUNCTION IN NORMOTENSION AND HYPERTENSION: ROLE OF ALPHA 1-ADRENOCEPTOR

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**by**

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***This thesis is dedicated to my lovely daughter***

*Muznah fathimah*

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| | |
|---|---|
| α | alpha |
| ANOVA | analysis of variance |
| BMY7378 | 8-[2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl]-8-azaspiro[4.5]Decane-7,9-dione dihydrochloride) |
| B.Wt | Body weight |
| BPU | blood perfusion unit |
| CEC | Chloroethyylchlonidine |
| $FE_{Na}$ | Fractional sodium excretion |
| GRF | Glomerular filtration rate |
| MAP | Mean arterial blood pressure |
| Mg/kg | Milligram per kilogram |
| Mg/dl | Milligram per deciliter |
| ml/min/kg | Mililiter per minute per kilogram |
| mMol/dl | millimol per deciliter |
| mmHg | millimeter mercury |
| µg | microgram |
| ME | methoxamine |
| ηg | Nano gram |
| NA | Noradrenaline |
| PNa | Plasma sodium |
| PE | Phenylephrine |
| RCP | Renal cortical perfusion |
| RAP | Renal arterial pressure |

| | |
|---|---|
| SHR | Spontaneously hypertensive rats |
| SHRNNa | Spontaneously hypertensive rats normal sodium diet |
| SHRHNa | Spontaneously hypertensive rats high sodium diet |
| 5-MeU | 5- methylurapidil |
| $U_{Na}V$ | Absolute sodium excretion |
| UFR | Urine flow rate |
| UV | Urine volume |
| UNa | Urine sodium |
| WKY | Wistar Kyoto rats |
| WKYNNa | Wistar Kyoto rats normal sodium diet |
| WKYHNa | Wistar Kyoto rats high sodium diet |
| WI | Water intake |
| UO | Urine output |

# KESAN PENGAMBILAN GARAM YANG TINGGI PADA HEMODINAMIK DAN FUNGSI GINJAL PADA NORMOTENSI DAN HIPERTENSI: PERANAN ADRENOSEPTOR-$\alpha_1$

## ABSTRAK

Hipertensi merupakan penyebab utama morbiditi dan kematian yang berkait rapat dengan penyakit jantung koroner, kegagalan ginjal dan strok. Natrium memainkan peranan patofisiologi yang penting di dalam pembentukkan hipertensi. Sistem adrenergik ginjal menyumbang kepada kesan hipertensi daripada pengumpulan natrium. Tujuan kajian ini adalah untuk mengetahui apakah kesan pengambilan diet makanan yang mengandungi kandungan natrium tinggi terhadap purata tekanan darah arteri (MAP) dan juga reaktiviti vaskular kortikal ginjal terhadap rangsangan adrenergik di dalam hal hubungannya dengan mekanisme reseptor adrenergik-$\alpha_1$. Selanjutnya kajian ini bertujuan untuk mengenal pasti sumbangan jenis-jenis reseptor adrenergik-$\alpha_1$ ($\alpha_1$-ARS) di dalam regulasi fungsi kortikal tubul ginjal dan hemodinamik ginjal, baik di dalam tikus normotensif WKY (Wistar Kyoto) mahupun di dalam SHR (tikus hipertensi spontan) apabila diberi kandungan garam yang tinggi. Kedua-dua SHR dan tikus WKY dipelihara dengan diet yang normal (WKYNNa & SHRNNa) dan diet bernatrium tinggi (WKYNNa & SHRNNa) selama enam minggu dan pengumpulan data metabolisme dimulakan. Haiwan-haiwan itu dikurung secara individu di kandang metabolik besi-tidak-berkarat yang diubahsuai sendiri; data asas dikumpulkan diikuti dengan pengumpulan data eksperimental selama enam minggu berturut-turut. Darah dan sampel urin dikumpulkan setiap minggu, dan berat badan, data pengambilan air dalam masa 24 jam dan data pembuangan air kencing dalam masa 24 jam diukur. Kemudian, kajian akut hemodinamik dan fungsional ginjal pada

akhir tempoh 6 minggu pengumpulan data metabolisme dilakukan terhadap tikus-tikus tersebut. Dalam kajian hemodinamik ginjal, perubahan perfusi korteks ginjal (RCP) yang disebabkan oleh penyempitan arteri berhampiran ginjal disebabkan oleh noradrenalin (NA), phenylephrine (PE), dan methoxamine (ME) ditentukan di dalam ketiadaan dan juga di dalam kehadiran 5-MeU, chloroethylclonidine (CEC) dan BMY7378 pada WKY dan SHR berdiet natrium biasa dan juga tinggi. Parameter fungsi tubular ginjal iaitu kadar filtrasi glomerulus (GFR), kadar aliran urin (UFR), pecahan ekskresi natrium dan ekskresi natrium mutlak (UNaV & $FE_{Na}$) terhadap PE dalam ketiadaan dan kehadiran 5-MeU, CEC dan BMY7378 dinilai sebagai saiz inulin klearan. Data-data yakni purata ± sem, dianalisis dengan satu dan dua cara analisis varians diikuti dengan Bonferroni post hoc dengan tahap siknifikan 5%. Keputusan menunjukkan bahawa MAP di dalam kumpulan diet SHRHNa & WKYHNa dan pada kumpulan kawalan diet SHRNNa & WKYNNa tidak menunjukkan sebarang perbezaan statistik. Terdapat ($p < 0.05$) peningkatan signifikan pada pengambilan air, pembuanagn air kencing, kandungan natrium urin di dalam WKY dan SHR diet natrium tinggi. Sementara ($p < 0.05$) kenaikan berat badan hanya diperhatikan pada WKYHNa. Plasma natrium tetap tidak berubah di kedua-dua kumpulan diet SHRHNa dan WKYHNa berbanding dengan kumpulan kawalan mereka. Diet pada SHRHNa dan WKYHNa menunjukkan perningkatan kepekaan vaskular ginjal korteks terhadap NA, PE, dan ME. Vasokonstriktor ginjal terhadap NA, PE dan ME nyata (semua $p < 0.05$) dilemahkan oleh 5-MeU dan BMY7378 di SHR dan WKY berdiet natrium biasa dan tinggi. Selain itu, CEC meningkatkan ($p < 0.05$) respons vasokonstriktor ginjal terhadap NA, PE dan ME pada SHRNNa dan WKYNNa. Di samping itu, di dalam kumpulan SHRHNa dan WKYHNa, vasokonstriksi kortikal ginjal terhadap NA, PE dan ME dikurangkan

(semua p <0.05) oleh CEC. SHRHNa dan WKYHNa menunjukkan kenaikan berlebihan di dalam diuresis dan natriuresis. Tanpa mengira perubahan diet natrium, infusi PE menyebabkan signifikan (p <0.05) antidiuresis dan antidiuresis di WKY dan SHR. Respon antidiuresis dan antinatriuretik terhadap PE mununjukkan penurunan yang signifikan (p <0.05) oleh 5-MeU dan BMY7378 di dalam kumpulan diet WKYNNa, sedangkan 5-MeU nyata (p <0.05) dilemahkan respon antidiuresis dan antinatriuretik terhadap PE di SHRHNa. Tidak ada perubahan signifikan yang diamati di RCP, RAP (tekanan arteri ginjal) dan GFR semasa eksperimen fungsi tubular ginjal. Oleh yang demikian, kesimpulannya, peningkatan respon adrenergik-$\alpha_1$ terhadap rangsangan adrenergik berkait rapat dengan peningkatan garam dengan sensitiviti vaskular ginjal pada SHRHNa dan WKYHNa. Tanpa mengira dietari pengambilan natrium, adrenergik-$\alpha_{1A}$ dan -$\alpha_{1D}$ adalah jenis-jenis reseptor fungsional yang terlibat dalam pengaturan vasokonstriksi kortikal ginjal secara adrenergik yang diinduksi pada tikus SHR dan WKY. Tambahan lagi, adrenergik-$\alpha_{1B}$ adalah jenis resepor fungsional yang terlibat dalam pengaturan vasokonstriksi kortikal ginjal adrenergick yang diinduksi pada WKYHNa dan SHRHNa. Selanjutnya, reseptor adrenergik-$\alpha_1$ terlibat dalam pengantaraan antinatriuresis dan antidiuresis di SHR dan tikus WKY berdiet natrium biasa dan tinggi. Selain itu, adrenergik-$\alpha_{1A}$ dan -$\alpha_{1D}$ adalah jenis reseptor fungsional yang terlibat dalam pengaturan antidiuresis dan antinatriuresis adrenergik yang diinduksi di WKYNNa. Di samping itu, reseptor adrenergik-$\alpha_{1A}$ menengahi antidiuresis dan antinatriuresis dalam diet SHRHNa.

# EFFECTS OF HIGH SALT LOAD ON RENAL HEMODYNAMICS AND FUNCTION IN NORMOTENSION AND HYPERTENSION: ROLE OF $A_1$-ADRENOCEPTOR

## ABSTRACT

Hypertension is a major cause of coronary heart disease, renal failure and stroke. Sodium plays an important pathophysiological role in the development of hypertension. The renal adrenergic system contributes to the hypertensive effect of sodium loading. The aim of this study was to investigate whether elevated dietary sodium intake had any effect on the mean arterial blood pressure (MAP) and renal cortical vascular reactivity to adrenergic stimuli in terms of its relation to $\alpha_1$-adrenergic mechanism. Further this study aimed to identify the contribution of $\alpha_1$-adrenoreceptor subtypes in the regulation of renal cortical hemodynamic and renal tubular functions in both normotensive WKY (Wistar Kyoto rat) and SHR (spontaneously hypertensive rats) subjected to high sodium load. Both SHR and WKY rats were kept on normal (WKYNNa & SHRNNa) and high sodium diet (WKYHNa & SHRHNa) for six weeks and the metabolic data collected. The animals were housed individually in custom-built stainless steel metabolic cages; baseline data were determined followed by experimental data collection for six consecutive weeks. Weekly blood and urine samples were collected, and body weight, 24-h water intake and 24-h urine output were measured. The rats were subjected to acute renal hemodynamic and functional studies at the end of the 6-weeks period of metabolic data collection. In the renal hemodynamic study, changes in the renal cortical perfusion (RCP) of the animals caused by close renal arterial

administration of noradrenaline (NA), phenylephrine (PE), and methoxamine (ME) were determined in the absence and presence of 5-MeU, chloroethylclonidine (CEC) and BMY7378. Renal tubular functional parameters namely glomerular filtration rate (GFR), urine flow rate (UFR), absolute and fractional sodium excretion ($U_{Na}V$ & $FE_{Na}$) upon infusion of PE in the absence and presence of 5-MeU, CEC and BMY7378 were assessed as a measure of inulin clearance. Data, mean ± s.e.m., were analyzed with one and two way analysis of variance followed by Bonferroni post hoc with the significance level of 5%. Results showed that MAP in SHRHNa and WKYHNa diet and in the control SHRNNa and WKYNNa diet were not statistically significantly different. There was significant ($p<0.05$) increase in the water intake, urine output, urine sodium of WKYHNa and SHRHNa compared to control groups. Statistically significant ($p<0.05$) increase in the body weight observed only in the WKYHNa verses WKYNNa. Plasma sodium remains unchanged in both SHRHNa and WKYHNa diet as compared to the control. Both SHRHNa and WKYHNa groups expressed significantly enhanced renal cortical vascular sensitivity to NA, PE, and ME compared to control SHRNNa & WKYNNa. Renal vasoconstrictor response to NA, PE and ME was significantly ($p<0.05$ for all) attenuated by 5-MeU and BMY7378 in SHR and WKY on normal and high sodium diet. On the one hand, CEC accentuated ($p<0.05$) the renal vasoconstrictor response to NA, PE and ME in SHRNNa and WKYNNa. On the other hand in SHRHNa and WKYHNa groups, renal cortical vasoconstriction to NA, PE and ME was inhibited (all $p<0.05$) by CEC. SHRHNa and WKYHNa showed exaggerated increase in the diuresis and natriuresis. Irrespective of dietary sodium intake, PE infusion led to significant ($p<0.05$) antidiuresis and antinatriuresis in WKY and SHR. This antidiuretic and antinatriuretic response to PE was significantly ($p<0.05$) inhibited by

5-MeU and BMY7378 in WKYNNa diet, while 5-MeU significantly ($p<0.05$) attenuated the antidiuretic and antinatriuretic response to PE in SHRHNa. There were no significant changes observed in the RCP, RAP (renal arterial pressure) and GFR during renal tubular functional experiments. Thus it is concluded that, augmented $\alpha_1$-adrenergic responses to adrenergic stimuli contribute to salt-related increase in renal vascular sensitivity in SHRHNa and WKYHNa. Irrespective of dietary sodium intake $\alpha_{1A}$ and $\alpha_{1D}$-adrenoceptors are the functional subtypes involved in mediating the adrenergically induced renal cortical vasoconstriction in SHR and WKY rats. On the other hand $\alpha_{1B}$-adrenoceptors are the functional subtype involved in mediating the adrenergically induced renal cortical vasoconstriction in WKYHNa and SHRHNa. Furthermore, $\alpha_1$-adrenoceptors are involved in the mediation of antinatriuresis and antidiuresis in SHR and WKY rats on normal and high sodium diet. In addition, it is proposed that $\alpha_{1A}$ and $\alpha_{1D}$-adrenoceptors are the functional subtypes involved in mediating the adrenergically induced antidiuresis and antinatriuresis in WKYNNa. On the other hand $\alpha_{1A}$-adrenoceptors mediate the antidiuresis and antinatriuresis in SHRHNa diet.

CHAPTER 1

# INTRODUCTION

Hypertension is among the most common health problems and a main cause for cardiovascular related risk factors. Hypertension is known to be a multifactorial disease; whose combined effects produces hypertension, yet the basic cause is not completely understood. High dietary sodium intake has long been associated with high blood pressure. It is suggested that chronic exposure to a high sodium diet appears to be a major pathophysiological factor involved in the frequent occurrence of hypertension and cardiovascular risk factor in humans (Meneton Pierre *et al.*, 2005). The mechanism by which dietary salt increases blood pressure is not completely understood but it is suggested that, it may be due to the inability of the kidney to excrete excess amount of sodium in the body. Human beings are adapted to ingest and excrete less than 1gm of salt per day, at least 10 times less than the average value currently observed in industrialized and urbanized countries. Independent of its effect on arterial blood pressure, high dietary sodium may also increase cardiac left ventricular mass, arterial thickness and stiffness, the incidence of strokes, and the severity of cardiac failure (Meneton Pierre *et al.*, 2005).

The role of kidney in blood pressure regulation has been confirmed by the experimental and conceptual work developed by Guyton on the pressure natriuresis and diuresis relationship (Guyton AC, 1991, Meneton Pierre *et al*., 2005, Raouf A Khalil, 2006). Renal cross-transplantation experiments have documented the role of kidneys in the development of hypertension (Rettig R *et al*., 1990, Morgan DA *et al*., 1990, Heller J *et al*., 1993, Raouf A Khalil, 2006). Transplantation of a kidney from

young hypertensive rat into a normotensive rat leads to an increase in blood pressure. Similarly, when a kidney from normotensive rat is inserted into young hypertensive rat, the blood pressure of the hypertensive rat did not increase. Moreover, the high blood pressure of a patient associated with nephrosclerosis becomes normal when they are transplanted with a kidney from normotensive donor (Meneton Pierre *et al.*, 2005).

A decrease in the capacity of the kidneys to excrete sodium would cause sodium and water retention leading to an increase in the extracellular fluid and plasma volume thus resulting into an increase in arterial blood pressure. Furthermore the ability of the kidney to excrete sodium declines gradually with age, and any small increase in the sodium intake predisposes an individual into a rise in blood pressure response (Raouf A Khalil, 2006). In addition, as the age increases, the GFR is reduced, accompanied by reduction in functional population of the nephrons and also progressive development of glomerulosclerosis. Thus with increasing age, if sodium consumption is not reduced, sodium balance is maintained by raising fractional sodium excretion which requires elevation in the arterial blood pressure. Thus sodium balance is achieved but with the expense of high arterial blood pressure (Corman B and Michel JB, 1987, Raouf A Khalil, 2006).

The kidneys ability to excrete sodium varies from individual to individual, those who require a higher than the normal blood pressure to excrete sodium are said to be "salt-sensitive." Those who can excrete excess salt at normal levels of blood pressure are called "salt resistant." This variation in kidneys ability to excrete sodium is suggested to be due to an inherited defect. The daily ingestion of large

amounts of salt leads to chronically expanded extracellular volumes that result into a considerable stress on the functional capacity of the kidney. As the individual age's renal capacity to excrete sodium decreases leading to rise in the arterial blood pressure (Freis ED, 1992). Thus it is stated that the relationship between high dietary sodium intake and hypertension has evolved from a possible association of specific mutations in blood pressure controlling genes and alterations in the expression/activity of distinct ion channels, transporters, and enzymes (Raouf A Khalil, 2006).

## 1.1 The Kidneys

The kidneys are paired organs situated retroperitoneally on the posterior abdominal wall. In gross terms, a section through the kidney shows it to be made up of cortex and medulla. The cortex is primarily involved in the reabsorption of bulk filtrate, and the medulla which generates a concentrated osmotic interstitium is essential for the conservation of water. Each human kidney contains about 1.2 millions functional units called nephrons. A popular view considers the kidney to be an organ primarily responsible for the removal of metabolic waste from the body, although this is certainly one function of the kidney, there are other functions that are also more important (Douglas CE and John PP, 2004). In general the kidney is involved in

1 Regulation of water and electrolyte balance.
2 Excretion of metabolic waste.
3 Excretion of bioactive substances (hormones and many foreign substances, specifically drugs) that affect body function.
4 Regulation of arterial blood pressure.
5 Regulation of red blood cell production.

6 Regulation of Vitamin D production.

7 Gluconeogenesis.

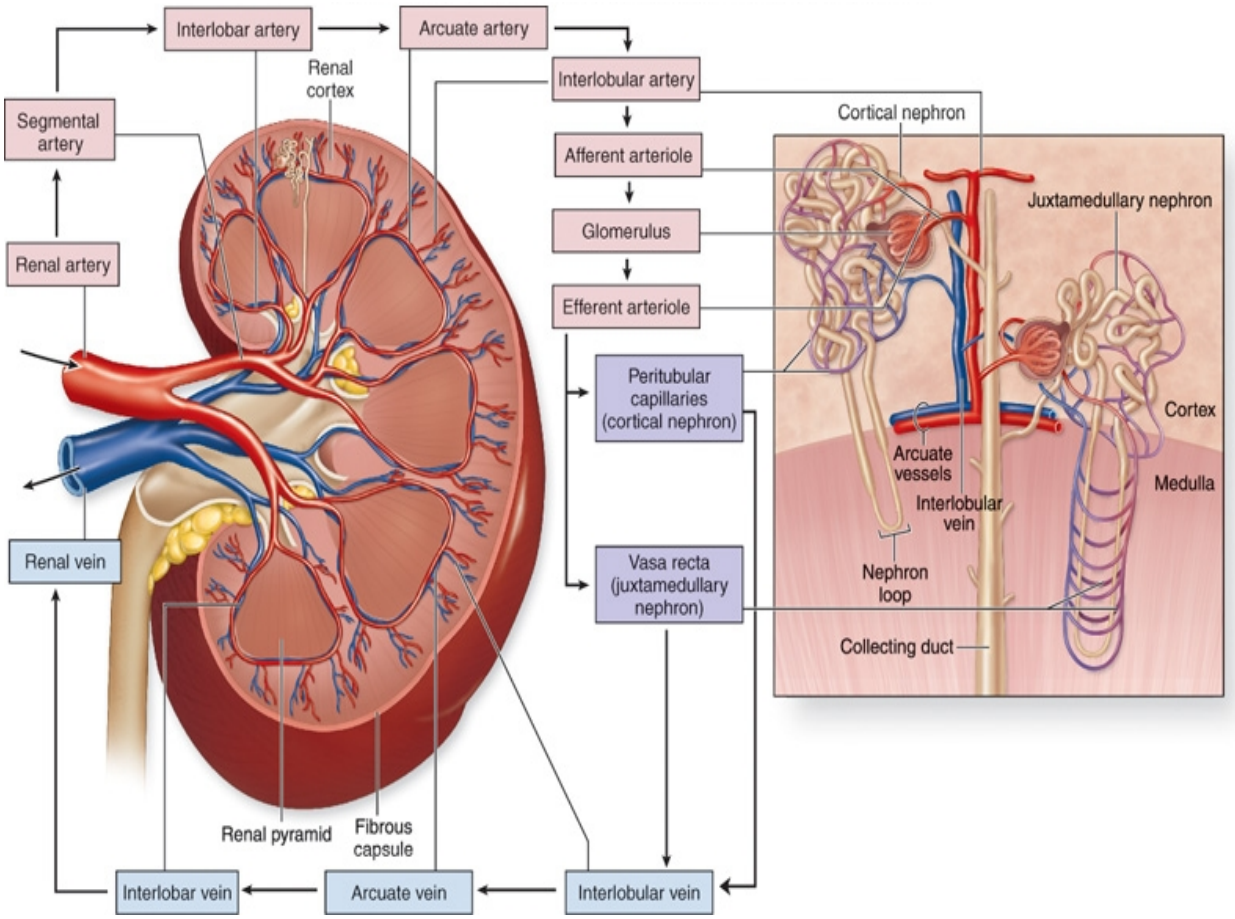


**Figure 1.1** Gross anatomy of the kidney
[Courtesy: http:// academic.kellogg.edu/ ]

### 1.1.1 The Nephron

The nephrons are made up of closely coiled tuft of capillaries, the glomerulus, which serves as an ultrafiltrate through which a considerable quantity of cell free and practically protein free fluid is separated from the plasma. The glomerulus is surrounded by the Bowman's capsule of the tubule. The proximal tubule, which drains Bowman's capsule, consists of a coiled segment, the proximal convoluted tubule followed by a straight segment the proximal straight tubule which descends

toward the medulla, perpendicular to the cortical surface of the kidney. The next segment, into which the proximal straight tubule drains, is the descending thin limb of Henle's loop. The descending thin limb is in the medulla and is surrounded by an interstitial environment that is quite different from that in the cortex. The descending thin limb ends at a hairpin loop, and the tubule then begins to ascend parallel to the descending limb. The loops penetrate to varying depths within the medulla. In long loops nephrons, the thin descending limb continues as the thin ascending limb of Henle's loop. Beyond this segment, in these long loops, the epithelium thickens, and this next segment is called the thick ascending limb of Henle's loop. In short loop nephrons, there is no ascending thin limb but the thick ascending limb begins right at the hairpin loop. The thick ascending limb rises back into the cortex, near the end of every thick ascending limb, the tubule returns to Bowman's capsule, from which it originated, and passes directly between the afferent and efferent arterioles. The cells in the thick ascending limb closest to Bowman's capsule (between the afferent and efferent arterioles) are specialized cells known as the macula densa. The macula densa marks the end of the thick ascending limb and the beginning of the distal convoluted tubule. This is followed by the connecting tubule, which leads to the cortical collecting tubule, the first portion of which is called the initial collecting tubule (Douglas CE and John PP, 2004).

From Bowman's capsule through the loop of Henle to the initial collecting tubules, each of the 1 million nephrons in each kidney is completely separate from the others. However, connecting tubules from several nephrons merge to form cortical collecting tubules, and a number of initial collecting tubules then join end to end or side to side to form larger cortical collecting ducts. All the cortical collecting

ducts then run downward to enter the medulla and become outer medullary collecting ducts and then inner medullary collecting ducts. The latter merge to form several hundred large ducts, the last portions of which are called papillary collecting ducts, each of which empties into a calyx of the renal pelvis.

The pathway taken by fluids flowing within a nephron always begins in the cortex (in Bowman's capsule), descends into the medulla (descending limb of the loop of Henle), returns to the cortex (thick ascending limb of the loop of Henle), passes down into the medulla once more (medullary collecting tubule), and ends up in a renal calyx. Each renal calyx is continuous with the ureter, which empties into the urinary bladder, where urine is temporarily stored and from which it is intermittently eliminated. The urine is not altered after it enters a calyx. From this point on, the remainder of the urinary system serves only to maintain osmotic and solute gradients established by the kidney (Douglas CE and John PP, 2004).

**1.1.1.a Glomerulus**

A glomerulus is formed of the afferent arteriole into an interconnecting capillary tuft surrounded by blind sac called renal or Bowman's capsule in nephrons of the vertebrate kidney. The inner wall of the capsule is made up of a visceral layer of highly specialized epithelial cells called the podocytes and is closely applied to the glomerular capillary network. The outer or the parietal layer of the capsule is made of simple squamaous epithelial cells that lies a short distance from the visceral layer so that an actual space is created between the two layers. This capsule and the contained glomerulus are called the renal corpuscle. It receives its blood supply from an afferent arteriole of the renal circulation. Unlike most other capillary beds,

the glomerulus drains into an efferent arteriole rather than a vein. The resistance of the arterioles results in high pressure in the glomerulus aiding the process of ultrafiltrations, where fluids and soluble materials in the blood are forced out of the capillaries into Bowman's capsule. A glomerulus and its surrounding Bowman's capsule constitute a renal corpuscle, the basic filtration unit of the kidney. The rate, at which blood is filtered through all of the glomeruli, and thus the measure of the overall renal function, is the GFR (Sattar M.A, 1993)

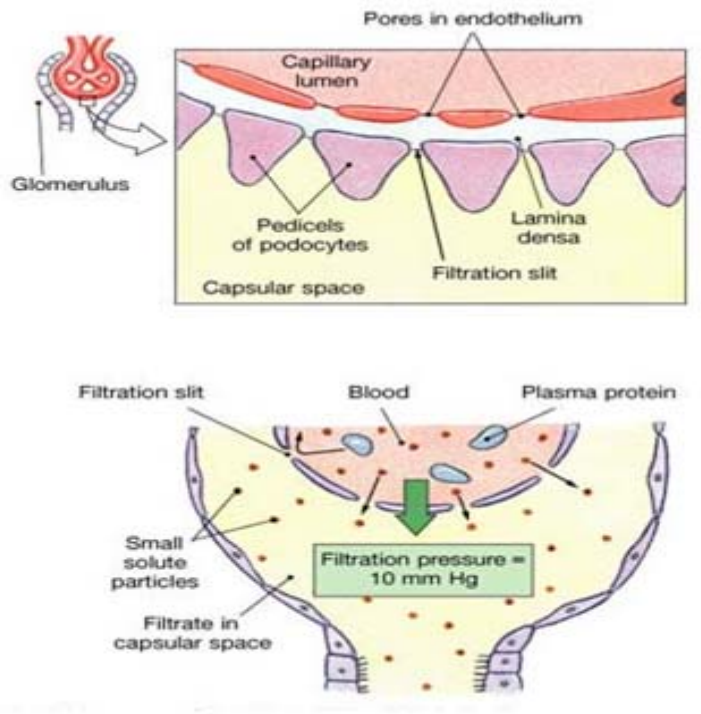


**Figure 1.2** Renal corpuscle and glomerular filtration membrane
[Courtesy: - Benjamin Cummings, an imprints of Addison Wesley Longman, Inc]

### 1.1.1.b Proximal tubule

The segment of tubule that drains the Bowman's capsule is the proximal tubule which initially forms several coils; the proximal convoluted tubule is the longest (14mm) and widest (60μm) part of the nephrons and conveys filtrate from Bowman's capsule to the loop of Henle. The epithelial cells that line the proximal convoluted tubule are columnar cells with large nuclei, a prominent luminal brush border and

abundant mitochondria. The luminal surface of the epithelial cells of this segment is covered with densely packed microvilli. The microvilli greatly increase the luminal surface area of the cells, presumably facilitating their resorptive function. Over 80% of the filtrate is reabsorbed here, including all the glucose, amino acids, vitamins, hormones and 85% of the sodium chloride and water. It is also responsible for secreting many types of medication like para aminohippuric acid, pencilin and organic acids, such as creatinine and other bases, into the filtrate. The proximal tubule regulates the pH of the filtrate by exchanging hydrogen ions in the interstitium for bicarbonate ions in the filtrate (Sattar MA, 1993).

**1.1.1.c Loop of Henle**

The loop of Henle becomes increasingly thin walled as it descend and is called the thin descending limb of loop of Henle that makes a sharp hair-pin bind in the upper third of the medulla for the cortical nephrons and considerably deeper in the medulla for the juxtamedullary nephrons. The thin descending limbs of loop of Henle are 14 to 22μm in diameter, with thin flat epithelial cells; the descending limb has low permeability to ions and urea, while being highly permeable to water. The ascending limb immediately after the bend is thin, but near the cortex it becomes wide, thick and continuous as distal convoluted tubule on reaching its own glomerulus. The thin ascending limb is not permeable to water, but it is permeable to ions, between the outer and inner zones of the medulla, the epithelium of the tubule become columnar and continues as thick ascending limb of loop of Henle. The tubule continuous until it passes between the afferent and efferent arteriole of its own glomerulus and then become the distal convoluted tubule. The main function of this structure is to create a concentration gradient in the medulla by means of a countercurrent multiplier

system, which utilizes sodium pumps; thus creating an area of high osmotic gradient deep in the medulla, near the collecting duct (Sattar MA, 1993).

### 1.1.1.d The distal tubule

The distal tubule is 2 to 9 mm long and up to 50 μm in external diameter and it drains into collecting tubules. The height of the columnar epithelium is lower than of the proximal convoluted tubule. The distal tubule consists of three distinct segments, the thick ascending limb of loop of Henle, the macula densa and the distal convoluted tubule. Distal convoluted tubule is the final segment of the nephrons. It is lined with simple cuboidal cells that are shorter than those of the proximal convoluted tubule. Distal convoluted tubule can be recognized by its numerous mitochondria, basal infoldings and lateral membrane interdigitations with neighboring cells. The point where distal convoluted tubule makes contact with afferent arteriole of renal corpuscle is called macula densa. It has tightly packed columnar cells which display reversed polarity and may monitor the osmolarity of blood. This region is the site of the mechanisms for fine control of salt, water and pH balance of the blood. As the distal segment approaches the collecting tubule it undergoes some cytological differentiation, the prominent feature being the appearance of isolated, large, granulated cells and this portion is called the connecting tubule. It participates in the regulation of water and electrolytes, including sodium, and chloride. The connecting tubule is also sensitive to antidiuretic hormone (less than the cortical collecting ducts), largely determining its function in water reabsorption (Douglas CE and John PP, 2004, Imai, 1979).

### 1.1.1.e The collecting tubule

The collecting duct system of the kidney consists of a series of tubules and ducts that connect the nephrons to the ureter. It participates in electrolyte and fluid balance regulated by the hormones aldosterone and antidiuretic hormone. Anatomically, there are several components of the collecting duct system that include the connecting tubules, cortical collecting ducts, and medullary collecting ducts. Several collecting tubules fuse to form one of the numerous papillary ducts draining into the minor calyx. The tubular epithelium has a one-cell thickness throughout. Before the distal convoluted tubule, the cells in any given segment are homogeneous and distinct for that segment. However, beginning in the second half of the distal convoluted tubule, 2 cell types are found in most of the remaining segments. One type constitutes the majority of cells in the particular segment, is considered specific for that segment, and is named accordingly: distal convoluted tubule cells, connecting tubule cells, and collecting-duct cells, interspersed among the segment-specific cells in each of these 3 segments are individual cells of the second type, called principle and intercalated cells. There are actually several types of intercalated cells; 2 of them are called type A and type B. (The last portion of the medullary collecting duct contains neither principal cells nor intercalated cells but is composed entirely of a distinct cell type called the inner medullary collecting-duct cells (Douglas CE and John PP,2004, Sattar MA, 1993).

## 1.1.2 The Juxtaglomerular Apparatus

A portion of the late thick ascending limb at the point where, it comes in contact with afferent and efferent arterioles at the vascular pole of the renal corpuscle, this entire area is known as the juxtaglomerular apparatus. Each juxtaglomerular apparatus is

made up of 3 cell types: (1) Granular cells, which are differentiated smooth muscle cells in the walls of the afferent arterioles; (2) Extraglomerular mesangial cells; and (3) Macula densa cells, these are the specialized epithelial cells of thick ascending limb. The granular cells (so called because they contain secretory vesicles that appear granular in light micrographs) secrete the hormone renin, a crucial substance for control of renal function and blood pressure. The extraglomerular mesangial cells are morphologically similar to and continuous with the glomerular mesangial cells but lie outside Bowman's capsule. The macula densa cells are detectors of the luminal content of the nephrons at the very end of the thick ascending limb and contribute to the control of GFR and to the control of renin secretion (Douglas CE and John PP, 2004).

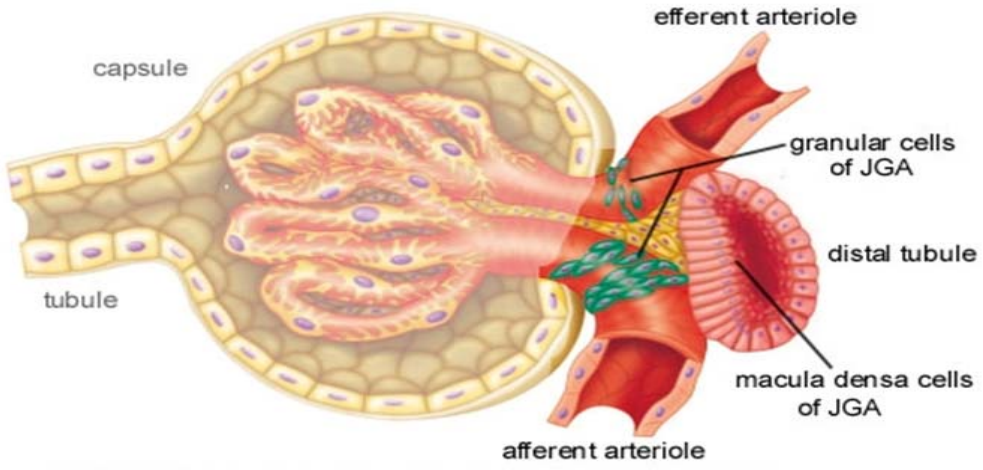


**Figure 1.3** Juxtaglomerular Apparatus
[Courtesy: - Benjamin Cummings, an imprints of Addison Wesley Longman, Inc]

### 1.1.3 Renal circulation

The renal blood flow is relatively very high. The kidneys receive 1.2 to 1.3 L of blood per minute, about 20 to 25% of the cardiac output (Ganong, 2009). Blood

enters each kidney via a renal artery, which then divides progressively into smaller branches: the interlobar, arcuate, and finally cortical radial arteries (also called interlobular arteries). As each of the cortical radial arteries projects toward the outer kidney surface, a series of parallel afferent arterioles branch off at right angles, each of which leads to a glomerulus, the glomerular capillaries recombine to form another set of arterioles called the efferent arterioles.

The efferent arteriole soon subdivides into a second set of capillaries; these are the peritubular capillaries, which are profusely distributed throughout the cortex. The peritubular capillaries then rejoin to form the veins by which blood ultimately leaves the kidney. The vascular structures supplying the medulla differ from those in the cortex, for many of the juxtamedullary glomeruli long efferent arterioles that extend downward into the outer medulla, where they divide many times to form bundles of parallel vessels that penetrate deep into the medulla. These are called descending vasa recta which then continue as ascending vasa recta. The vasa recta, in addition to being conduits for blood, also participate in exchanging water and solutes between plasma and interstitium. The whole arrangement of descending and ascending blood flowing in parallel has major significance for the formation of concentrated urine because plasma constituents can exchange between descending and ascending vessels (Douglas CE and John PP, 2004).

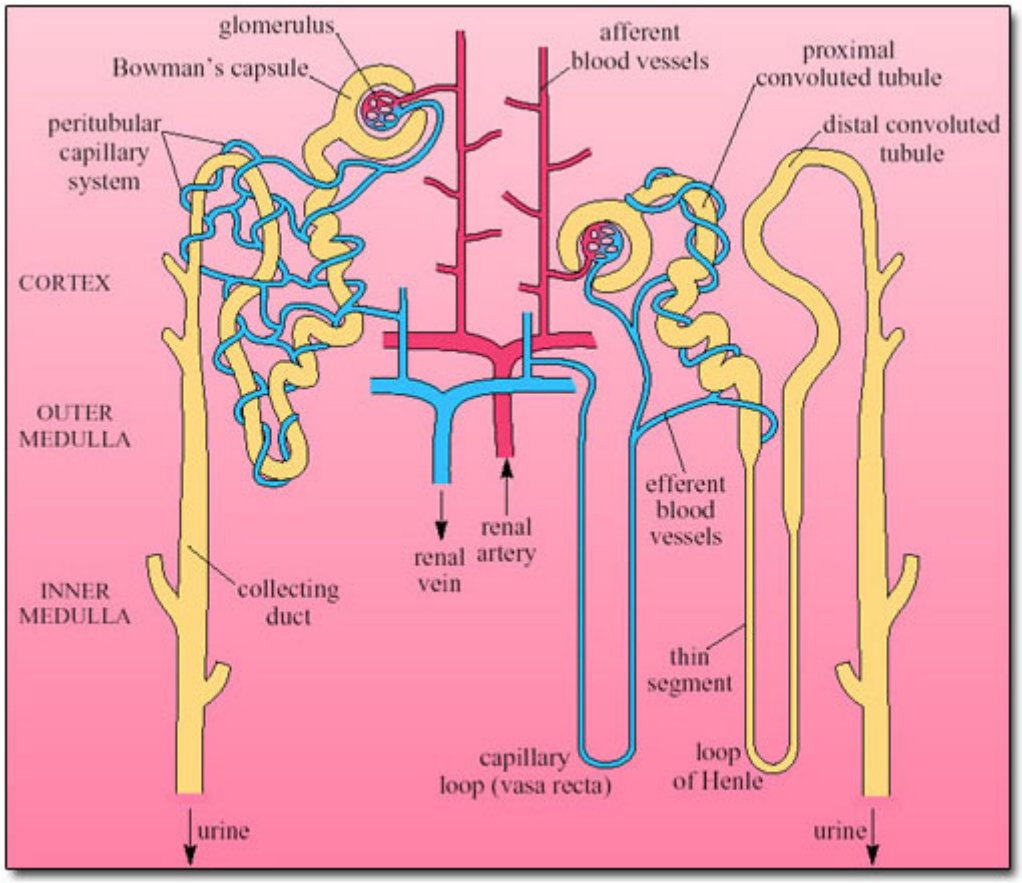


**Figure 1.4** Cortical and Juxtamedullary Nephron along with respective circulation (Adapted from Princeton review in Campbell excretory system)

### 1.1.4 Innervations of the kidney

Autonomic control of the kidney is predominantly mediated by the sympathetic nerve system with nerves extending primarily to all the components (Salomonsson *et al*., 2000). There is less evidence for the parasympathetic innervations (Peter D. Vize *et al*., 2003, Norvell JE and Anderson JM, 1983). The renal nerve are composed of fibers from the celiac plexus, the thoracic plexus and the lumbar braches of the splanchnic nerve, the superior and inferior mesenteric plexus, the intramesentric nerve and the superior hypogastric plexus (Mitchell GA, 1950). These nerve fibers and their interconnection make up renal plexus which lies in the rather constant association with the aorticorenal ganglion (Mitchell GA, 1950). These fibers originate primarily from T5 to L3 of the spinal cord segment and thus

along renal artery and vein enter in to the hilus of the kidney (Takeuchi J *et al.*, 1964). The renal nerve within the kidney spread through the renal parenchyma following the blood vessels (Douglas C.E and John P. P, 2004). The demonstration by (Barajas L *et al.*, 1984) states that all segments of the renal tubule (as well as the Juxtaglomerular apparatus) were innervated by renal sympathetic nerve terminals. The basal discharge rate of renal sympathetic nerve is in the range of 0.5 to 2Hz (Robertson D, 2004).

At the functional level, renal sympathetic nerve stimulation releases adrenaline, noradrenaline and dopamine. Among these, noradrenaline is the dominant neurotransmitter released after stimulation of the renal sympathetic nerves (Gabriela A. Eppel *et al.*, 2004). In addition, dopamine also appears to be present in these nerves as a precursor of noradrenaline synthesis (Gabriela A. Eppel *et al.*, 2004). Moreover the presence of specific dopaminergic nerves within the kidney has also been confirmed (DiBona GF and Kopp UC, 1997). There are reports suggesting that co-transmitters, like neuropeptide Y and ATP are also released from the renal nerve and thus participate in renal sympathetic neurotransmission (DiBona GF and Kopp UC, 1997). They partially mediate renal nerve stimulation induced-reductions in renal blood flow (DiBona GF and Sawin LL, 2001, Pernow J and Lundberg JM, 1989). Other neurotransmitters, like vasoactive intestinal polypeptide and neurotensin have been identified within the renal vasculature, however their role in renal sympathetic neurotransmission and regulation of renal function is not clear (Reinecke M and Forssmann WG, 1988, Gabriela A. Eppel *et al*., 2004). Neuropeptide galanin have also been defined in a proportion of the postganglionic sympathetic neurons innervating the kidney (Longley CD and Weaver LC, 1993).

The binding sites for Neuropeptide Y, vasoactive intestinal polypeptide and neurotensin have been localized to vascular elements of the medullary circulation including juxtamedullary afferent and efferent arterioles (Leys K *et al*., 1987, Reinecke M and Forssmann WG, 1988), raising the possibility that these sympathetic cotransmitters could contribute to the neural control of renal blood flow (Gabriela A. Eppel *et al*., 2004).

Renal sympathetic nerve activity has been shown to contain oscillations over a range of frequencies from 0.1 to 10 Hz (Malpas SC, 1998). At a higher frequency of renal sympathetic nerve stimulation (0.5 Hz) renin secretion rate is increased without alterations in renal hemodynamic or urinary sodium excretion. At a frequency of renal sympathetic nerve stimulation (1.0 Hz), there is an increase in renal tubular sodium reabsorption (lowers urinary sodium excretion) without change in the renal hemodynamics, but renin secretion rate is further increased. At even higher frequencies of renal sympathetic nerve stimulation (>2.0 Hz) they produce renal vasoconstriction with a decreases in renal blood flow, GFR and urinary sodium excretion (DiBona GF, 1989).

The situation where a renal sympathetic nerve fiber makes sequential contact with multiple effectors is consistent with each effector having a different response threshold (e.g., exhibiting frequency dependence at supramaximal amplitude). However, it is also possible that each effector could respond by virtue of effectors-specific information being encoded in the renal sympathetic nerve discharge pattern (e.g., variations in frequency, amplitude, duration; regular vs. irregular) (DiBona GF, 2000). This suggests that each effector may possess unique response characteristics

in either the time or frequency domain. The situation where unique renal sympathetic nerve fibers specifically and selectively innervate arterioles, tubules, and Juxtaglomerular granular cells suggests that they might be coupled to separate central neuron pools with specific and selective afferent reflex inputs (DiBona GF, 2000). Folkow and colleagues (Folkow B *et al.*, 1958) showed that differences in stimulation threshold of preganglionic fibers were associated with the activation of functionally differentiated effectors (DiBona GF, 2000).

When characterized as to myelination and size of the renal nerves, it appears to be rather homogeneous (DiBona GF *et al.*, 1996). Approximately 96% are unmyelinated fibers with a size range of 0.4–2.5μm. However, when the distribution of fiber diameters was examined, it was found to be bimodal with a primary mode at 1.1 μm and a secondary mode at 1.6 μm (DiBona GF, 2000). This suggests that at least there are two populations of renal sympathetic nerve fibers, possibly subserving different functions (i.e., functional nonuniformity). The conduction velocity is averaged 2.10 ± 0.10 m/s and consistent with unmyelinated C-type fibers (DiBona GF *et al.*, 1996). Strength-duration analysis (at constant frequency) indicated that, for any stimulus duration, lower stimulus strength (volts) was needed to elicit an antidiuretic response rather than a vasoconstrictor response. Thus renal sympathetic nerve fibers producing the effect on the renal tubules (increased sodium and water reabsorption) were not the same as those producing the effect on the intrarenal arterioles (constriction) (DiBona GF, 2000). By analogy with somatic nerves where stimulation threshold is inversely related to fiber diameter, this suggests that the diameters of the nerve fibers involved in the antidiuretic response are greater than the diameters of the nerve fibers involved in the vasoconstrictor response (DiBona GF,

2000). These results confirmed the earlier observations demonstrating that during graded renal nerve stimulation, the response of the renal tubules occurs at a lower level of stimulation than that of the renal arterial vasculature (DiBona GF, 2000).

Renal sympathetic innervations exert its effects on various aspects of renal function that includes the renal hemodynamics, tubular sodium and water reabsorption and renin secretion. In this way, they produce alterations in renal hemodynamic, tubular reabsorption, and renin secretion rate and thus contribute importantly to renal adaption and compensation during normal physiological conditions (DiBona GF, 2000). These effects constitute an important control system in the physiological regulation of arterial pressure and total body fluid and sodium homeostasis. When studies of the neural control of renal function were extended to pathophysiological states such as hypertension, further important observations were proposed (DiBona GF, 2000). In a variety of experimental animal models of hypertension, the rise of arterial blood pressure is completely prevented or reversed by renal denervation; for example, the obesity model of hypertension in the dog is completely prevented by renal denervation in association with a 50% decrease in cumulative sodium retention (Kassab S *et al*., 1995, DiBona GF, 2000). Approximately 30-40% of the renal sodium retention of edema-forming conditions, such as congestive heart failure, cirrhosis (DiBona GF and Sawin LL, 1991) and the nephrotic syndrome (Herman PJ *et al.*, 1989) are dependent on intact renal sympathetic innervations (DiBona GF, 2000). On the afferent side, signals from renal sensory receptors coursing via afferent renal nerves to the neuroaxis are involved in inhibitory renorenal reflexes and excitatory renosystemic reflexes, which, via peripheral sympathoexcitation, contribute to the hypertension of chronic renal

disease (Campese VM and Kogosov E, 1995, Converse R.L *et al*., 1992, DiBona GF, 2000).

## 1.2 Adrenoceptors

Adrenoceptors are membrane bound receptors located throughout the body on neuronal and non-neuronal tissues where they mediate a diverse range of responses to the endogenous catecholamines, i.e. noradrenaline and adrenaline (Robinson and Hudson, 1998). These compounds are responsible for controlling, the cardiovascular, respiratory, neuronal, digestion, pupil dilation and contraction, energetic metabolism and endocrinal function (Beatriz CC and Amaya A, 2001).

### 1.2.1 Classification of adrenoceptors and historical Perspective

Extensive research on adrenoceptors has led to the current knowledge and information regarding adrenoceptors and has facilitated their present classification (Beatriz CC and Amaya A, 2001). The first study on adrenoceptors was started by Dale in 1906. In 1937, Cannon and Rosenblueth put forward a hypothesis, which initiated the idea of classification of adrenoceptors (Beatriz CC and Amaya A, 2001 Cannon and Rosenblueth, 1937, Dale, 1906). Later, Ahlquist in 1948 was the first to establish a pharmacological classification for adrenoceptors into α and β-adrenoceptors (Beatriz CC and Amaya A, 2001).

Ahlquist studied the effects of catecholamine's on various physiological responses of the body that includes contraction and relaxation of the uterus, dilation of the pupil and stimulation of myocardial contraction (Ahlquist, 1948, Magdalena W *et al.*, 2000). He demonstrated that norepinephrine, epinephrine, isoproterenol,