CHAPTER II

BACKGROUND INFORMATION

The kidney is the major route for the elimination of water from the body. Moreover, the renal excretion of water is regulated to maintain the osmolality of the body fluids constant. When water intake is low or when water is lost from the body by other routes (e.g. perspiration, diarrhea), the kidney conserves water by producing a small volume of urine that is hyperosmotic with respect to plasma. When water intake is high, a large volume of hypoosmotic urine is produced. In a normal individual, the urine osmolality can vary from approximately 50 - 1200 mOsm/ kgH_2O and the urine volume can vary from 0.5 - 20 L/ day (Stanton and Koeppen, 1990).

Vasopressin, or antidiuretic hormone (ADH) from the posterior lobe of the pituitary, acts on the kidney to regulate the osmolality and volume of the urine. When plasma ADH levels are low, a large volume of urine is excreted (diuresis)and the urine is dilute. Conversely, when plasma levels of ADH are elevated, a small volume of urine is excreted (antidiuresis) and the urine is concentrated. The secretion of ADH is mainly regulated

by the osmolality of the plasma, but both blood volume and pressure also have an influence (Stanton and Koeppen, 1990 ; West, 1991).

Formation of urine

Formation of urine involves three basic processes : ultrafiltration of plasma by the glomeruli, reabsorption of water and solutes from the ultrafiltrate, and secretion of selected solutes into the tubular fluid (Stanton and Koeppen, 1990).

Ultrafiltration occurs because of Starling forces, hydrostatic pressure in the glomerular capillary (P_{GC}) and the oncotic pressure in Bowman's space (π_{BS}) that drive fluid from the lumen of glomerular capillaries across the filtration barrier into Bowman's space. It is opposed by the hydrostatic pressure in Bowman's space (P_{BS}) and the oncotic pressure in the glomerular capillaries (π_{GC}) (Ganong, 1989 ; Stanton and Koeppen, 1990).

Glomerular filtration rate (GFR) can be altered by changing any of the Starling forces. Physiologically, however, GFR is usually affected in two primary ways :

1. An increase in P_{GC} enhances GFR, and a

decrease in P_{GC} depresses GFR. Changes in arterial pressure are the most frequent cause of variation in P_{GC}.

2. Variations in renal blood flow (RBF). As afferent arteriolar plasma flow increases, GFR rises. As plasma flow in the capillaries increases, π_{GC} rises more slowly. Accordingly, the ultrafiltration pressure increases. A fall in plasma flow decreases GFR.

However, the blood flow remains constant as arterial blood pressure changes between 90 and 180 mmHg, GFR is also regulated over the same range of arterial pressures as renal blood flow. The phenomena whereby RBF and GFR are maintained constant is called "autoregulation".

Tubular function

By the process of reabsorption and secretion, the renal tubules modulate the volume and composition of the urine. Normally, 180 liters of fluid is filtered through the glomeruli each day, while the average daily urine volume is about 1 liters. (Ganong, 1989) because of the reabsorption of renal tubules.

1. Reabsorption by the proximal tubule

Glomerular filtrate is formed by hydrostatic pressure at the approximate rate of 120 milliliters per minutes. Two-thirds of the filtrate is absorbed isoosmotically in the proximal tubule. The filtered water and solutes are reabsorbed by the proximal tubules in two phases : (1) reabsorption of Na^+ with glucose, amino acids and bicarbonate in the first half of the proximal tubule, and (2) reabsorption of Na^+ with chloride in the second half of the proximal tubule (Davies and Wilson, 1975 ; Stanton and Koeppen, 1990). It has been accepted that net sodium reabsorption in the proximal tubule is mediated by an active process which generates a negative potential difference (Barratt et al., 1974 ; Maude, 1974). Sodium diffuses passively across the luminal surface of the cell, and is then actively transported into the lateral spaces between cells, setting up an osmotic gradient which causes the passive removal of water from the tubule (Curran and MacIntosh, 1962; Diamond and Bossert, 1967; Lewy and Windhager, 1968). The oncotic pressure exerted by protein in the peritubular capillaries controls the rate of transfer of reabsorbate from the lateral spaces to the peritubular capillaries. It can be seen that under these circumstances, changes in filtration fraction associated with a rise in glomerular filtration rate would also increase oncotic

pressure and decrease hydrostatic pressure in the efferent capillaries. These changes would favour increased reabsorption (Brenner et al.,1972 ; Horster et al.,1973 ; Green et al., 1974).

Reabsorption in the proximal tubule has been shown to vary with changes in extracellular fluid volume (Brenner and Berliner, 1969). The existence of a natriuretic hormone released in response to expansion of the extracellular fluid volume has been demonstrated to depress sodium reabsorption (De Wardener, 1969).

2. Reabsorption by the Henle's Loop

The remaining fluid passes into the descending limb of the loop of Henle which is permeable to water. Therefore, the fluid in the descending thin limb becomes hypertonic as water moves into the hypertonic interstitium (Jamison, 1970 ; Marsh, 1970 ; Suki and Eknoyan, 1992).

On moving up the ascending limb, sodium is reabsorbed to the extent of about 25% of the filtered load but water is excluded. This mechanism is intimately concerned with the counter - current multiplier system responsible for the medullary osmotic gradient (Suki et al.,1973). It has been suggested that

sodium absorption in the ascending limb is passive both in the thin segment (Imai and Kokko, 1974) and in the thick segment (Burg and Green, 1973 b).

In the thick ascending limb, chloride was once thought to be absorbed by an active electrogenic process. However, it now appears that Na^+, K^+ and Cl^- (in a ratio of 1 : 1 : 2) are cotransported by an electroneutral process, with subsequent back diffusion of K^+ into the lumen along a conductive pathway. Chloride leaves the cell via a conductive pathway, whereas K^+ and Cl^- leave via an electroneutral symport in the basolateral membrance. In addition, there is evidence for a Na^+ / H^+ antiporter on the luminal membrane (Suki and Eknoyan, 1992). Because the thick ascending limb is very impermeable to water, reabsorption of Na^+, Cl^- and other solutes reduces the osmolality of tubular fluid to less than 150 $mOsm/kgH_2O$ (Ganong, 1989).

3. Reabsorption by the Distal tubule

The distal tubule, particularly its first part, is in effect an extension of the thick segment of ascending limb. It is relatively impermeable to water. Sodium is reabsorbed from the tubular fluid in exchange and hydrogen ions. This transport mechanism is largely under the control of aldosterone and accounts for

absorption of up to 5% of the filtered load of sodium, tubular sodium concentration may have fallen to 20 - 40 milliequivalent/liter (Davies and Wilson, 1975 ; Ganong, 1989).

4. Reabsorption by the collecting duct

The cortical collecting tubule, which is also intrinsically impermeable to water, is capable of active sodium and chloride absorption (Hanley and Kokko, 1978). When sodium is the predominant ion species absorbed, the luminal potential is negative, potassium (Grantham et al., 1970) and hydrogen ions (McKinney and Burg, 1978) are secreted passively via a paracellular pathway, but predominantly trans-cellularly across a conductive pathway in the luminal membrane of the principal cell (Stokes, 1981). This process is activated by mineralocorticoids (O'Neil and Helman, 1977). A positive potential may be observed, however, especially in the medullary collecting tubule (Stokes et al., 1978), and this is the result of hydrogen ion secretion (Lombard et al., 1983) mediated via a proton ATPase in the luminal membrane of intercalated cells which is activated by aldosterone (stone et al., 1983). Unlike other nephron segments, the collecting tubule is capable of increasing its intrinsic water permeability in response to anti-diuretic hormone (Grantham and Burg, 1966). Tubular

fluid is lost to the hypertonic medulla because of the osmotic gradient, and a concentrated urine is excreted. In the absence of the hormone, water is still lost to the medulla, but because this amount is small relative to the flow rate, and because the collecting duct is capable of sodium reabsorption, a dilute urine can be excreted (Davies and Wilson, 1975).

Diuretics

Diuretics are substances or drugs which act on the kidney to promote the excretion of water and electrolytes, particularly sodium (Davies and Wilson, 1975), or mean an agent that inhibits tubular absorption of sodium because absorption of sodium in the kidney is responsible for absorption of water, inhibition of sodium reabsorption result in diuresis (Suki and Eknoyan, 1992). Diuretics have been employed in a wide variety of conditions, such as in diabetes insipidus, urinary calculi, renal tubular acidosis, edematous states, hypertension, etc. (Davies and Wilson, 1975 ; Morgan, 1978 ; Dirks and Sutton, 1986 ; Suki and Eknoyan, 1992).

Sites of Diuretic Action and Mechanism of Action

1. Diuretics that act on the distal tubule and collecting system (Potassium - Sparing Diuretics)

1.1. Spironolactone

Spironolactone is a competitive inhibitor of aldosterone at its target site in the distal tubule. It binds to aldosterone receptors and prevents the formation of a protein important in sodium transport (Morgan, 1978). Ochs et al. (1986) described that spironolactone inhibits the formation of the aldosterone complex in the nuclei of kidney epithelial cells of adrenalectomized rats. This occurs at concentration ratios that inhibit the action of aldosterone in the rat in vivo. In vitro studies with kidney tissue slices from rat demonstrate displacement of aldosterone from its specific intracellular receptors by spironolactone (Funder et al., 1974). As a result, less sodium is reabsorbed and less potassium is excreted. However, the amount of sodium absorbed at this site is small in comparison with the overall sodium reabsorption by the nephron. In addition, the aldosterone - induced component is only part of this total amount. Thus, in a normal person the natriuretic effect of spironolactone is relatively small, whereas in patients with hyper-

aldosteronism it may cause a significant natriuresis and diuresis (Morgan, 1978 ; Suki and Eknoyan, 1992).

1.2. Amiloride and Triamterene

Amiloride and triamterene are structurally distinct compounds that have very similar effects on the kidney, although triamterene is primarily effective from the peritubular side of the collecting tubule, whereas amiloride is most effective when placed intraluminally (Gatzy, 1971). Not only do they inhibit the sodium reabsorption induced by aldosterone but also inhibit basal sodium reabsorption. This action is probably due to a change in the permeability of the membrane to sodium (Morgan, 1978). Triamterene inhibits the potential difference in the collecting duct from the peritubular side, and this effect is not completely reversible. Amiloride, from the luminal side, inhibits the potential difference in the distal tubule as well as the collecting duct, and recent studies have suggested that it has an effect in the proximal tubule, although in higher than therapeutic concentration. Furthermore, the effect of amiloride is rapidly and completely reversible when this agent is removed (Gross et al., 1975 ; Kinsella and Aronson, 1980 ; Chan and Giebisch,1981). The inhibition of K^+ transport occurs because no potential difference is developed due to the inhibition of Na^+ reabsorption (Morgan, 1978).

2. Diluting Segment Diuretics

The cortical diluting segment is a functional part of the nephron where sodium transport is the predominant factor. Diuretics that act at this site, of which the thiazides are the principal examples. The thiazides are filtered by the glomerulus and excreted by the proximal tubule, and exert their action from the luminal side of the nephron. The main difference in these drugs relates to their duration of action (Morgan, 1978). The main route of excretion of the thiazides is in the urine. The substituted hydrothiazides are more highly bound to plasma proteins and their renal clearance is lower. Because of their increased lipid solubility, they may undergo some back diffusion in the more distal part of the nephron (Davies and Wilson, 1975). These diuretics produce a greater natriuresis and diuresis than that achieve by distal tubule agents. The increased flow of fluid through the distal nephron means that an increased amount of potassium is initially lost (Morgan, 1976). The Thiazide diuretics also inhibit carbonic anhydrase in the proximal tubule, but with normal doses they are not of great significance. In addition, they also have effect on medullary collecting duct (Wilson et al., 1983).

3. Loop of Henle Diuretics (Loop Diuretics)

Typical examples of drugs that act on the loop of Henle are frusemide, ethacrynic acid and bumetanide. These diuretics inhibit chloride transport that takes place in the loop of Henle. The diuresis able to be achieved is large because 20% of the glomerular filtration of sodium chloride is reabsorbed by this segment. As it acts at this point the increased volume flow through the distal tubule and collecting duct means that potassium loss is accentuated (Morgan, 1978 ; Suki and Eknoyan, 1992).

3.1. Ethacrynic acid

Ethacrynic acid is a powerful diuretic in several animal species and in man but not in rat. Circulating ethacrynic acid is largely protein - bound. A small fraction of unbound drug is filtered by the glomerulus and in addition, there is tubular secretion by the probenecid - sensitive proximal mechanism responsible for transporting other organic acids (Beyer et al., 1965).

Ethacrynic acid and mercurials compete for the same excretory carrier and for the same diuretic receptor (Nigrovic et al., 1973). It acts on the thick ascending limb of Henle's loop probably in the form of

ethacrynic - cysteine complex (Burg and Green, 1973 a). It may be a non - specific inhibitor of Na^+ K^+- ATPase (Martinez - Maldonado et al., 1974) producing a dose related inhibition of Na^+ - K^+ transport in cells. It may also antagonise antidiuretic hormone in the collecting tubules (Abramow, 1974).

3.2. Frusemide (Furosemide)

Furosemide has been very widely used in the relief of edema. It is rapidly absorbed on oral administration and its effect is largely over within 4 hours (Kelly et al., 1974 ; Cutler et al., 1974). Its major effect is on the ascending limb of Henle's loop. Studies on the isolated toad bladder have shown that furosemide displaces cyclic - AMP from specific cyclic AMP binding protein and inhibits the phosphorylation of histones by a cyclic - AMP dependent protein kinase. In the rat, tubular segments dissected out after large doses of furosemide show a marked inhibition of transport ATPase primarily in the loop of Henle and portions of the distal tubule (Davies and Wilson, 1975).

3.3. Bumetanide

Bumetanide is a derivative of metanilamide that displays potent diuretic activity on oral administration in dogs. It is ineffective in rat where it is extensively metabolized (Davies et al., 1974). Bumetanide has an effect similar to that of frusemide

producing a rapid response which is complete within 6 hours. Its major site of action is probably on the ascending limb of Henle's loop and on the proximal tubule (Bourke et al., 1973 ; Davies et al., 1974). Studies with ^{14}C bumetanide have shown that urinary excretion accounts for 65% of an oral dose and that about 18% may be recovered in the feces. After oral administration peak plasma concentrations are attained within 1/2 to 2 hours. The peak urinary excretions of sodium and bumetanide coincide and likewise occur within the first two hours (Davies et al., 1973). This drug is a potent diuretic, being particularly effective in the relief of edema. High oral doses (15 mg) also produce a diuresis in renal failure (Davies et al., 1974).

4. Proximal tubule diuretics

Diuretics, which have action on the proximal tubule, are carbonic anhydrase inhibitors such as acetazolamide and benzolamide. Both drugs are powerful inhibitors of carbonic anhydrase, but they differ in their abilities to penetrate cell membranes. Acetazolamide readily crosses various cellular membranes because of its high degree of lipid solubility, whereas benzolamide is primarily confined to the extracellular fluid because of its limited cellular permeability.

Carbonic anhydrase inhibitors cause acute decrease in GFR without much change in RBF. The reduction in GFR may be due to a decrease in renal plasma flow secondary to increases in afferent and efferent resistances at the single nephron level (Suki and Eknoyan, 1992). Some studies found that the benzolamide-induced reduction in GFR is due to activation of the tubuloglomerular feedback system by the increased distal delivery of solute and that the efferent limb of this system depends on vaso-constriction due to the local action of angiotensin II (Tucker et al., 1978 ; Tucker and Biantz, 1980).

Carbonic anhydrase inhibitors may impair hydrogen - ion secretion into the proximal tubule lumen by one or more of the following mechanisms : (a) inhibiting active hydrogen - ion secretion by the tubular cells, (b) impeding hydrogen - ion secretion by delaying dehydration of carbonic acid formed in the lumen, which raises luminal hydrogen - ion activity, or by (c) inhibiting the efflux of bicarbonate at the basolateral membarane. This inhibition of sodium bicarbonate and water absorption prevent the normal rise in proximal tubule chloride concentration, thereby abolishing the favorable chloride diffusion gradient normally present and resulting in secondary impairment of passive sodium chloride and water absorption. Thus, there are increased deliveries of

sodium, chloride and water to the loop of Henle (Suki and Eknoyan, 1992).

Water diuresis and Osmotic diuresis

The water diuresis produced by drinking large amount of hypotonic fluid begins about 15 minutes after ingestion of a water load and reaches its maximum in about 40 minutes. The act of drinking produces a small decrease in vasopressin secretion before the water is absorbed, but most of the inhibition is produced by the decrease in plasma osmolality after the water is absorbed.

Osmotic diuresis occurs due to the pressure of large quantities of unreabsorbed solute (i.e. mannitol, urea, glucose) in the renal tubules caused an increase in urine volume. Solutes that are not reabsorbed in the proximal tubules exert an appreciable osmotic effect as the volume of tubular fluid decreases and their concentration rises. Therefore, they hold water in the tubules. The result is that the loop of Henle is presented with a greatly increased volume of isotonic fluid. This fluid has a decreased Na^+ concentration, but the total amount of Na^+ reaching the loop per unit time is increased. More fluid passed through the

distal tubule, less water is reabsorbed in the collecting ducts. The result is a marked increase in urine volume and sodium excretion and in excretion of other electrolytes.

In water diuresis, the amount of water that is reabsorbed in the proximal portions of the nephron is normal, but in osmotic diuresis, increased urine flow is due to decreased water reabsorption in the proximal tubules and loops (Ganong, 1989).