# CHAPTER II

# BACKGROUND INFORMATION

## Potassium homeostasis :-

Potassium ion ($K^+$) is the most abundant cation in intracellular fluid (ICF), approximately 98 percent of body potassium ion being located in the cell.There is a difference between the potassium concentration in the two compartment, cell potassium concentration (140 mEq/L) and extracellular potassium concentration (3.5 to 5.5 mEq/L). The chief biological mechanism responsible for maintaining potassium gradient between intracellular and extracellular fluid (ECF) is the active Na-K ATPase pump (Rosa,Williums,and Epstein, 1992 ; Seldin, 1989). The potassium concentration of extracellular fluid is finely regulated because of the role of potassium in the excitability of nerves and muscles. The resting membrane potentials of these tissues are directly related to the ratio of intracellular to extracellular concentrations of potassium. Raising the extracellular potassium concentration lowers the resting membrane potential, thus increasing cell excitability. Conversely, lowering the extracellular potassium concentration hyperpolarizes cell membranes and reduces their excitability (Vander, 1991). Therefore, intracellular potassium concentration are finely regulated by the total amount of potassium in the body (external balance) and by the relative concentration of potassium in the cell versus blood (internal balance) (Willard, 1989).

External balance of potassium is regulated by the kidneys. The average daily dietary intake of potassium is approximately 15 to 100 mEq/L in dogs. Absorption of dietary potassium occurs in the small intestine by passive transport. Approximately 95 percents of dietary potassium is normally excreted in urine daily, and 5 to 10 percent is excreted in faeces (Bovee, 1984). Obligatory urinary potassium losses are proportional to the renal tubular flow in the distal tubules. Aldosterone is the major modifier of renal potassium excretion, it enhances distal tubular secretion of

potassium by increasing renal Na-K ATPase activity and cellular membrane permeability to potassium. This is a powerful means of controlling total body potassium, albeit of slow rate of response (Brobst, 1986 ;Willard, 1989).

Internal potassium balance is principally regulated by insulin and cathecholamines, which have faster response times. Aldosterone is also postulated to affect internal balance, but this is uncertain (Willard, 1989).

**Renal potassium excretion :-**

Potassium is freely filtered at the renal glomerulus. It is reabsorbed at several site along the nephron. Fifty to seventy percent of the filtered potassium is absorbed in proximal tubules. Ten to fifteen percent of the filtered load of potassium reaches the early distal tubule in most physiological conditions. Although a relatively constant fraction of the filtered load of potassium reaches the distal tubule, urinary excretion may vary within a few percent of the filtered load. Thus, transport of potassium between tubular fluid and tubular cells in collecting duct. It is here that final adjustments in potassium reabsorption and secretion are made. The collecting duct are the major sites for renal regulation of potassium excretion (Bovee, 1984 ; Seldin, 1989 ; Wingo, 1993).

**Potassium transport in the collecting ducts :-**

Early micropuncture and microperfusion studies have firmly established that the initial and cortical collecting duct (CCD) are the major sites for final regulation of potassium secretion. Two types of cells, principal cell and intercalated cell, are present in the CCD. The principal cell has Na and K channels at the apical membrane and Na-K ATPase at the basolateral membrane. Na-K ATPase responsible for potassium secretion and sodium reabsorption, whereas the intercalated cell is mainly involved in potassium reabsorption and proton secretion via an apical H-K ATPase. It appears to play an important role in $H^+$ and $HCO_3^-$ handling and in potassium reabsorption during potassium depletion (Rose, 1994; Schlatter, 1993 ; Vander, 1991

; Wang, 1995). At the cortical collecting duct, potassium secretion depends on the flow rate and the rate of sodium reabsorption and thus on the activity of the Na-K ATPase in the basolateral membrane. Inhibition of this pump decreases both sodium reabsorption and potassium secretion (Schalatter, 1993). Certainly, all of reabsorption of Na and the majority of secretion of potassium are located in the principal cell (Stokes, 1990). In addition, a decreased potassium secretion by the action of the principal cells in this nephron segment is likely to be the result of an H-K ATPase in the intercalated cells which increase the reabsorption of potassium the luminal membrane, which would increase the reabsorption of potassium (Gifford, Rome and Galla, 1992 ; Wingo et al., 1990 ; Zhou and Wingo, 1992).

**Hormonal regulation of potassium transport in the collecting duct :-**

Adrenal cortical hormones, specifically aldosterone directly influence the renal excretion of potassium. Glucocorticoids indirectly increase renal potassium excretion by the release of potassium from muscle and other cellular stores. In the absence of adrenal function, the excretion of potassium is decreased resulting in an increase of potassium concentrations in ICF and ECF (Bovee, 1984). Aldosterone plays a major role in potassium homeostasis by augmenting potassium secretion in the principal cells in cortical collecting duct as well as in adjacent cells in the connecting segment and the outer part of the medullary collecting tubule. Aldosterone increases the number of opened sodium and potassium channels in the luminal membrane and enhances the activity of Na-K ATPase in the basolateral membrane (Rose, 1994).

Another hormone, anti-diuretic hormone (ADH, AVP), which increase the water permeability of the luminal membrane of principal cells, also increases sodium reabsorption and potassium secretion in this nephron segment. Insulin and activation of protein kinase C decrease potassium secretion.

The secretion of potassium is also regulated by increased potassium intake or increase in intracellular potassium concentration. The coupling of potassium secretion in this nephron segment and the acid-base status of the organism is well known for human. An increased acid excretion during systemic acidosis is usually

accompanied by a decreased potassium secretion. A coupling between two transport processes occurs via different cell types (Schlatter, 1993).

Renal potassium excretion is regulated by multiple factors acting at several transport sites in response to influences originally outside the kidney. The final rate of excretion of potassium is determined by the net effect of mechanism that may be cooperating or competing. In some situation, the individual regulatory factors may act together, while in other case they may inhibit one another.

**Potassium depletion :-**

Potassium depletion is one of the most common electrolyte disorder. It may result from inadequate dietary potassium, gastrointestinal loss of potassium, or overzealous use of diuretics. The development of potassium depletion may lead to renal changes that worsen the negative balance of cation, thus creating a vicious cycle. The occurrence of potassium depletion activates renalpotassium conserving mechanism(kaliferic), with attenuation of kaliuretic factor. Potassium depletion will reduce aldosterone secretion. The developing hypokalemia may alter basolateral potassium transport in the cortical collecting duct and intracellular potassium depletion will reduce the gradient for this ion across the luminal membrane (Bovee, 1984 ; Mujais and Katz, 1992).

**Renal changes in potassium depletion :-**

It is well known that potassium depletion induces severe alterations of kidney function in mammals. The first renal morphologic changes of potassium depletion occur in the collecting duct which is the main nephron segment involved in the control of potassium excretion (Wright and Giebisch, 1985). The most prominent changes occur in the intercalated cells of the inner stripe of the outer medullary collecting duct (OMCDi) which undergo hypertrophy and ultrastructural changes (Stetson, Wade and Giebisch, 1980). These changes indicate that these cells are involved in potassium absorption. Moreover, dietary potassium restriction enhances

the absorptive potassium rate coefficient in the OMCDi. At physiological flow rates, the changes in potassium absorptive flux would significantly influence the rate of renal potassium excretion (Wingo, 1989).

Recently, the mechanism of potassium absorption has received considerable attention. Doucet and Marsy (1987) described a unique K-dependent ATPase (K-ATPase) activity in the distal nephron of rat and rabbit. Moreover, K-ATPase activity increases significantly with potassium depletion. It was not affect by either the Na-K ATPase inhibitor (ouabain), or the mitochondrial $F_1F_0$ ATPase inhibitor (azide). However, the K-ATPase activity is sensitive to two known inhibitor of gastric H-K ATPase, vanadate and omeprazole, hydrophobic imidazopyridine (Sch28080). (Cheval, et al.,1991; Doucet and Marsy, 1987 ).

It has been studies were reported K-ATPase might be involved in potassium reabsorption and proton secretion in the late distal tubule. Therefore, under conditions such as potassium depletion, there is a net potassium reabsorption instead of secretion along the rat collecting tubule (Linas, et al., 1979).Thus,kidney play an important role in potassium conservation by decreased secretion and by enhanced active reabsorption in the collecting duct (Rose, 1994).

**Functional evidence for a renal H-K ATPase :-**

Characterization of the regulatory nature of H-K ATPase may become accessible via studies in molecular biology and enzymology. However, a role of renal H-K ATPase is supported by biochemical studies on ion transport in isolated perfused OMCDi. The contribution of a functional H-K ATPase in OMCDi was assessed by examining the effect of the gastric H-K ATPase inhibitor (omeprazole) on net K flux and net $CO_2$ flux. Omeprazole profoundly inhibits potassium and proton transport in OMCDi of animals fed a potassium restrict diet absorption tubule derived from animals fed a potassium restricted diet (Wingo, 1989). It has been reported that omeprazole is not only inhibits an H-K ATPase but also inhibits an H-ATPase (Khadouri, et al.1991). Thereby, Sch28080 was used to inhibit potential H-K ATPase activity.(Cheval, Barlet-Bas, and Khadouri, 1991). Furthermore, immunoreactivity

studies in rat and rabbit with mouse monoclonal antibodies raised against hog gastric H-K ATPase revealed diffuse cytoplasmic staining cortical collecting duct, demonstrating the existence of a related antigen (Wingo et al., 1990). These observations are consistent with the renal H-K ATPase which structurally similar to the gastric H-K ATPase.

The H-K ATPase activity is involved in potassium reabsorption against proton secretion which it was stimulated by potassium depletion and was suppressed by potassium loading (Cheval et al., 1989 ; Doucet and Marsy, 1987 ; Garg and Narang, 1989). These results of H-K ATPase activity provide both enzymatic and functional evidence for the potassium reabsorption. However, it has been reported that H-K ATPase is present in the medullary collecting duct of normal rabbits.It indicates that H-K ATPase may play a role in the normal physiological regulation of acid-base and potassium balance(Curran, 1992). A process of potassium reabsorption in medullary collecting duct would provide an important mechanism for precise regulation of potassium excretion in the final urine after secretion of potassium by the cortical collecting duct (CCD). Several studies demonstrated that the H-K ATPase is located in connecting duct, cortical and outer medullary portion of the collecting duct (Khadouri,et al.,1991; Wingo, 1987) . In addition, Helou et al. (1994) reported that H-K ATPase is present in rat inner medullary collecting duct (IMCD) with similar distribution from the proximal to the distal portion of collecting duct. However, H-K ATPase was found in the IMCD of potassium depleted rats but it was not found after two week of potassium depletion.

**Functional evidence for a renal H-K ATPase on distal acidification :-**

The regulation of net renal acid excretion is one of the major functions of the collecting duct. The outer medullary collecting duct (OMCD) and the inner medullary collecting duct are known to possess substantial rates of proton secretion. Urinary acidification in these segments is believed to be in part due to an electrogenic, vacuolar-type H-ATPase. It is sensitive to both N-ethyl maleimide (NEM) and dicyclohexylcarbodiimide (DCCD), known inhibitors of the vacuolar H-ATPase.

Immunocytochemistry studies have localized this H-ATPase in the apical membrane of the intercalated cells of the medullary collecting duct (Brown, Hirsch and Gluck, 1988). These findings clearly support the role of H-ATPase in distal urinary acidification.

In addition, There is another has been proposed that another proton pump, the H-K ATPase, also contribute in collecting duct.This transporter appears identical to the gastric acid pump and can be inhibited by omeprazole, vanadate and Sch28080 (Kurtzman, 1990). An H-K ATPase activity increases in parallel with the surface area of the luminal membrane of intercalated cells during potassium depletion (Stetson, Wade and Giebisch, 1989). It indicates that main role may be conservation of potassium during hypokalemia rather than in regulating acid-base balance (Rose, 1994). However, had evidence demonstrates the presence of H-K ATPase in apical membrane of normokalemic rabbits (Curran, 1992). It indicate that H-K ATPase may play an important role in urinary acidification (Armitage and Wingo, 1993). In addition, Gifford (1992) had been suggested that H-K ATPase is important in the regulation of $HCO_3^-$ excretion under normal and altered acid-base condition.

**Role of aldosterone on distal urinary acidification :-**

It has been recognized that aldosterone exerts major effects on collecting tubule by stimulating the Na-K pump,and H-ATPase activity,increasesing passive potassium secretion,but it does not stimulate the H-K ATPase (Eiam-Ong, Kurtzman,and Sabatini, 1993). Aldosterone would stimulate the H-ATPase directly in both cortical collecting duct (CCD) and medullary collecting duct (MCD) and further increase acid excretion. Thus, excess aldosterone is associated with potassium wastage and excess acid secretion while aldosterone deficiency is associated with potassium and acid retention (Kurtzman,1990). On the other hand,potassium depletion inhibits aldosterone release,which decreases acid excretion and plasma bicarbonate concentration (Eiam-ong, Kurtzman, and Sabatini, 1993). However, it has been established that potassium depletion stimulates the H-K ATPase but the effect of potassium depletion of H- ATPase activity is controlversial (Kurtzman, 1990).

The combination of potassium depletion and aldosterone excess is associated with increased acid excretion and metabolic alkalosis but the combination of potassium depletion and aldosterone deficiency is not yet clear.

**Effects of specific gastric H-K inhibitor on renal H-K ATPase :-**

The presence of an ouabain-insensitive ATPase has been demonstrated in distal nephron segments of mammalian and amphibian tubules. This ATPase is an electroneutral pump sensitive to vanadate and is specifically inhibited by both omeprazole and Sch28080 (Doucet and Marsy, 1987 ; Garg and Narang, 1988). Recently, two classes of specific inhibitors of the gastric H-K ATPase have been developed .Omeprazole,a substituted benzimidazole, is representative of the irreversible class of inhibitors (Fujisaki,et al., 1991).The reversible inhibitors are exemplified by Sch28080, a substituted imidazopyridine compound (Wallmark,et al., 1987). Inhibition of the gastric H-K ATPase by Sch28080 is not reversed by sulfhydryl-reactive reagents, but is competitively inhibited by potassium, suggesting that the drug binds at or near the K-binding site of the pump (Briving, 1988; Wallmark, Briving, and Frylund, 1987).

The previous observations are consistent with a non-electrogenic H for K exchange in the OMCD similar for the gastric H-K ATPase(Wingo,1989). However, omeprazole has been reported to inhibit the vacuolar H-ATPase in vitro, raising the possibility that the effects of this agent on proton transport in the isolated OMCD may be due to inhibition of H-ATPase(Beit,et al.,1987;Mattson,et al.,1991) Further studies of H-K ATPase, however, showed that Sch28080 appeared to be more useful than omeprazole because, it does not need to be preactivated at acid pH in vitro. The renal H-K ATPase is 30 fold more sensitive to Sch28080 than it is to omeprazole and Sch 28080 may be more specific for H-K ATPase than omeprazole which also inhibit NEM sensitive ATPase. Importantly, Sch28080 that is a potent inhibitor of gastric acid secretion. It inhibits the gastric H-K ATPase by reversible interaction with the enzyme that is competitive with potassium (Cheval, et al.,1991; Curran, et al.,1992; Wingo, and Armitage,1993). Subsequently showed that Sch28080 can inhibit

rubidium (Rb) uptake and K-ATPase. The effect of Sch28080 on Rb uptake is not affect by presence of ouabain, suggesting that Rb uptake occur by a mechanism that is unlikely to involve Na-K ATPase. Thus, three structurally distinct inhibitors of the gastric proton pump inhibit K-ATPase activity in the collecting duct and this K-ATPase activity responds appropriately for a K-absorption pump.