CHAPTER V

DISCUSSION

The present investigation was conducted to study the effect of the specific H-K ATPase inhibitor (Sch 28080) on systemic circulation and renal function in hypokalemic dogs. It has been reported in potassium depleted animals that, H-K ATPase activity markedly increase and it appears to play an important role in distal potassium reabsorption as well as urinary acidification (Cheval, Barlet-Bas, and Khadouri, 1990). Two proton ATPase function on urinary acidification has been noted. The first is an electrogenic H-ATPase which is regulated by aldosterone (Sabatini and Kurtzman, 1991). The second is an electroneutral H-K ATPase which is stimulated by potassium depletion. However, aldosterone still plays an important role in potassium regulation (Stokes et al., 1985). Aldosterone excess would stimulate H-ATPase and further increase in acid excretion. Potassium depletion stimulated H-K ATPase and also enhanced acid excretion but would decrease aldosterone (Eiam-Ong, Kurtzman,and Sabatini, 1993 ; Kurtzman, 1991). Regarding to these knowledges, the animals in the present study were treated with furosemide to induce hypokalemia and adrenalectomy was carried out to prevent the effect of aldosterone before administration of Sch28080.

Prolong treated a high dose of furosemide in hypokalemic dogs had a marked reduction on mean arterial pressure (MAP) in group II when compared to the control group (group I) before given Sch28080. This hypotensive effect was primarily due to the loss of water and electrolytes in response to the diuretic action. Moreover, potassium depletion also lead to a reduction in arterial blood pressure in normotensive rats and dogs. The decline in blood pressure was due to decrease in peripheral vascular resistance with a normally maintain cardiac output (Garvez,et al.,1977; Linas, and Dick,1982). However, MAP of either adrenalectomized dogs (group III) or hypokalemic adrenalectomized dogs (group IV) in comparison to the control animals showed nonsignificant decrease, although daily administration of prednisolone acetate

for glucocorticoid replacement were given in the present study. A lack of adrenal steriod hormone in group III and group IV animal especially aldosterone would be a cause of unresponse of the vascular smooth muscle to norepinephrine and epinephrine activity in raising blood pressure (Ganong, 1995). After intrarenal arterial administration of Sch28080, MAP significantly increased in group I and II but HR did not alter; while MAP and HR of group III and IV were not significantly changed. These results indicate that the action of Sch28080 for an increase in MAP in group I and II would be due to vasoconstriction relating to an increase vascular resistance. These results would probably depend on the action of aldosterone which did not appear in animals in group III and IV.

In group I and II, significant reductions of renal hemodynamic (i.e. GFR, and ERBF) coincided with a significant increase in renal vascular resistance (RVR) while no alteration in FF after Sch28080 injection was seen. These findings suggest that renal vasoconstriction was occurred after intrarenal arterial injection of Sch28080. Since an increase in MAP was apparent in group I and II after Sch28080 administration. Therefore, vasoconstrictive effect by the action of Sch28080 would occur at both systemic and intrarenal circulation. However, animals in group III and IV given Sch28080 showed no significant change in GFR, while tendency of decreases in ERPF, ERBF, nonsignificant increases of RVR and FF were noted. Renal vasoconstrictive effect might be apparent predominantly in efferent renal arterioles of animals in groupIII and IV (Vander, 1991). In group III and IV animals, RVR increased less extent than that of group I and II after given Sch 28080. Administration of prednisolone acetate for glucocorticoid replacement in adrenalectomized animal in group III and IV may be responsible for a change of RVR. The action of glucocorticoid on the vasoconstrictor responses to epinephrine, norepinephrine, angiotensin II and vasopressin has been reported in rats (Baylis and Brenner, 1978). The significant increases in FE_{Na} , FE_K and FE_{Cl} after given Sch28080 were seen in hypokalemic animals (group II) and it did not significantly increase in group I,III,and IV animals. These results indicate that the effect of Sch28080 could decrease in renal tubular reabsorption of electrolytes.

The present results showed an increase of FE_K, although very low in plasma K concentration including filterload of K was observed in group II after Sch28080

administration. It is possible that Sch28080 may reduce renal potassium reabsorption by inhibiting H-K ATPase at distal nephron in hypokalemic animals. In addition, furosemide induced hypokalemic animals might enhance electrically neutral H-K ATPase (Kurztman ,1990). This result supports other observations that Sch28080 has been shown to be highly selective inhibitor of H-K ATPase (Cheval, Barlet-Bas,and Khadouri, 1991; Sabatini, and Kurtzman, 1991). However, the cause of increment of FE_{Na} and FE_{Cl} in the present experiment is unclear. It was noted from the present study that Sch28080 injection could increase FE_{Na}, FE_K , FE_{Cl} in group I , II and III. It is possible that, Sch28080 might affect on electrolytes reabsorption at either the proximal tubule or distal tubule.

Adrenalectomized animals in group III showed a greater increase in FE_K when compared to other groups after Sch 28080 administration. This effect would be due to a decrease in H - ATPase which had been shown in adrenalectomized animals with a lack of aldosterone (Khadouri, et al, 1987). Thus H-K ATPase may play a role of acid-base and K balance in this condition. Since, potassium depletion has been shown to enhance H-K ATPase while lack of aldosterone would reduce H-ATPase(Eiam-ong, Kurtzman, and sabatini, 1993). Therefore the major effect of H-K ATPase would be expected to occur in hypokalemic adrenalectomized animals (group IV). However, hypokalemic adrenalectomized dogs showed significant increases of $U_{Na}V$, $U_{Cl}V$, FE_{Na} and FE_{Cl} with no significant changes of U_KV and FE_K after Sch 28080 injection. It seem likely that intrarenal arterial injection of Sch 28080 had no direct effect on renal tubular reabsorption in this group. Significant increases of FE_{Na} and FE_{Cl} may depend on significant increases in FF and V.

The present results show that Sch 28080 markedly decreased urinary acidification in group II and III .The significant reduction of urinary titratable acid excretion ($U_{TA}V$) and urinary acid excretion (UAE) in group II may be explained by two reasons. First, reduction of urinary acid excretion was associated with the reduction in filterload of bicarbonate by a decrease of GFR. Second, a decrease in urinary acid excretion occured with an increase in FE_K. An inhibition of H-K ATPase activity by the action of Sch 28080 would be accounted for a reduction of urinary acid excretion. Potassium depletion enhanced acid secretion by enhancing H-K- ATPase activity which would be inhibited by Sch 28080 and this inhibitor did not affect on

H-ATPase activity (Cheval, Barlet-Bas and Khadouri, 1991:Kurtzman,1990). An experiment in group III demonstrated that significant decreases of $U_{TA}V$, urinary ammonium excretion ($U_{NH4}V$) and urinary acid excretion with an increase in FE_K after given Sch 28080 depended on H-K ATPase activity. These results support the other studies in adrenalectomized animals that H-ATPase activity should be markedly reduced while H-K ATPase should be unaffected (Kurztman, 1990). The present results indicate that H-K ATPase could be stimulated in adrenalectomized animals which the mechanism is unknown.However, the adrenalectomized dogs in the present study were supplemented with prednisolone acetate which might obtained glucocorticoid effect. Thus, stimulation of Na-H exchanger would be suspected (Wilcox, 1982). By this treatment, glucocorticoid may affect either direct or indirect on H-K ATPase activity in adrenalectomized dogs.

In the control animal (group I), $U_{NH4}V$ and urinary acid excretion tended to decrease after Sch 28080 injection while significant reduction of GFR and ERPF were seen. These results indicate that Sch 28080 could decrease urinary acid excretion in the presence of H-K ATPase in normal condition.This finding would be agree with the in vitro study in rabbit that the presence of an H-K ATPase was apparent in apical membrane of normokalemic animal (Wingo, 1990). It indicates that the H-K ATPase might influence on urinary acidification in both potassium depleted animals and normokalemic animals in the present study.

From the observations in hypokalemic adrenalectomized animals (group IV), intrarenal arterial injection of Sch 28080 did not affect on $U_{TA}V$, $U_{NH4}V$ and urinary acid excretion. GFR did not show any alteration in this group. The activity of H-K ATPase would be expected to increase in this group since adrenalectomized animals would lead to reduce H-ATPase activity while severe hypokalemia stimulated H-K ATPase activity. The present results were not be those cases. It is possible that, the dosage of Sch 28080 (10 umol / kg. bw) given in the present study was inadequate to inhibit H-K ATPase in this condition. Eiam-Ong and co-worker (1993) studied in adrenalectomized with glucocorticoid replete rats, replaced with physiologic dose of aldosterone and given furosemide to induce hypokalemia. They found marked increase of H-K ATPase (~ two fold) in cortical collecting duct (CCD) and medullary collecting duct(MCD). These results controverted to the present results. From the

present results, it is possible that H-K ATPase is suppressed by the other unknown mechanism. However, two weeks of potassium depletion has been shown to suppress H-K ATPase activity in the inner strip of in medullary collecting duct (IMCD) (Holou, Aranjo, and Seguro,et al.,1994). Another postulation is that furosemide and glucocorticiod may enhance Na-H exchange which response to maintain urinary acidification in this condition. Thus, the effect of Sch 28080 on urinary acid excretion was not apparent in this group.

The present study demonstrates that Sch 28080 did not affect on Posm in all groups. In group I, II and III showed a reduction of Cosm and free water reabsorption but slight elevation of FE_{H2O} was apparent after Sch 28080 administration. A decrease of water reabsorption coincided with decreases in tubular reabsorption of Na, K and Cl in group I, II and III. Therefore, the action of Sch 28080 was more likely natriuretic effect, which would be accounted for increasing in $U_{Na}V$, $U_{Cl}V$, FE_{Na} , FE_{Cl} and FE_{H2O}, although the reduction of GFR. RPF and filterload were decreased. These findings are similar to the observation in vanadate studies that it would inhibit water reabsorption in the proximal tubule (Higashi and Bello-Reuss, 1980) and would decrease sodium reabsorption along the assending limb of Henle's loop (Westenfelder, Hamburger and Gracie , 1981). Furthermore, vanadate has also been reported to inhibit the initiation of the AVP-induced increase in hyperosmotic permeability at distal tubule to cAMP formation (Edwards and Granthan, 1983 b). Hoch's study (1992) gave a controversial result that Sch 28080 augmented ADH induced water transport in toad urinary bladder. In contrast to the experiment in group IV which demonstrated that there were no changes of Posm and Uosm through experimental period. Cosm and free water reabsorption significantly increased while C_{H2O} showed nonsignificant decrease. It was noted that GFR, FF, V, FE_{Na} and FE_{Cl} increased in this group. It seem likely that the action of Sch 28080 for natriuresis in hypokalemic adrenalectomized dog depends on an increase of renal hemodynamic. However, the direct effect of Sch 28080 on renal tubular function in hypokalemic adrenalectomized dogs is unclear.

In conclusion, the present study demonstrates that the effect of Sch28080 on renal function in control, hypokalemic group and adrenalectomized group showed a significant decreases in renal hemodynamics while RVR significantly increased when

compared to the pretreated period. Urinary acidification markedly decreased in hypokalemic group and adrenalectomized group after Sch28080 injection. The intrarenal arterial injection of Sch28080 (10 mol/kg.bw) showed a maximum of inhibitory effect on renal function within 40 minutes. Sch28080 injection failed to inhibit urinary acidification in hypokalemic adrenalectomized group. However, in vivo studies of the effect of Sch28080 on H-K ATPase showed that it is dependent on either plasma potassium concentration or adrenal hormone levels.