CHAPTER V

DISCUSSION

The present results demonstrate that intravenous infusion of sodium metavanadate in anesthetized dogs induced a striking but reversible increase in mean arterial blood pressure and decrease in heart rate (Figure 1). An increment in mean arterial blood pressure could theoretically be attributed to either increased cardiac output or increased peripheral resistance. However, in the previous studies in dogs and rats, vanadate would decrease cardiac output which would be due to both decreased stroke volume and heart rate and myocardial contractile force fell (Inciarte et al., 1980; López-Novoa and Garrido, 1986). The decrease in heart rate was apparently produced by no direct effect on the chronotropic mechanism of the heart, but some indirect mechanism, perhaps arterial baroreceptor reflex. It has been reported that bilateral vagotomized dogs, both arterial pressure as well as heart rate were signifcantly elevated during sodium orthovanadate infusion (Hom, Chelly, and Jandhyala, 1982). From the present finding showed that intravenous infusion of sodium metavanadate combination with either atenolol (group III, selective beta-1 adrenoceptor blocker) or acetylcholine (group V, parasympathomimetic drug) produced a marked decrease in heart rate that was elicited by vanadate. When intravenous infusion of sodium metavanadate in dogs combination with verapamil (group VI, a calcium channel blocker), an attenuated decrease in heart rate was seen. These results demonstrate that the reflex bradycardia might initiate by a rise in systemic arterial pressure subsequent to the effect of vanadate increasing baroreceptor afferent discharge in aortic arch and carotid sinus. The stimulation of the cardioinhibitory area increasing efferent cholinergic vagal tone and decreasing efferent discharge in the sympathetic cardiac nerves might be expected

to occur. Increasing impulses in the cholinergic vagal cardiac fibers will decrease heart rate whereas decreasing impulses in the noradrenergic sympathetic nerves to the heart will decrease either the cardiac rate (chronotropic effect) or the force of cardiac contraction (inotropic effect). However, most previous studies suggested that the decreased stroke volume seemed unlikely entirely from a rise in afterload, in part from a fall in left ventricular preload subsequent to pulmonary vasoconstriction by vanadate. The other possibility was that contractility decreased, perhaped subsequent to the increment of coronary resistance, decreased coronary flow and myocardial tissue P_{O_2} and pH (Inciarte et al., 1980; Sundet et al., 1984). According to Borchard and co-worker (1979) demonstrated that a striking difference in the inotropic action of vanadate on isolated heart preparations strongly produced negative intropic effects in the atrium though it produced positive inotropic effects in the ventricular myocardium. On the other hand, vanadate reduced the force of contraction of stimulated left atrium and spontaneously beating right atrium. However, it increased the force of contraction of papillary muscles of ventricular myocardium. The rise in total peripheral resistance appeared to result largely from vasoconstriction. Vanadate has been shown to produce a potent vasoconstriction with increment in peripheral vascular resistance which apparently included all components of the peripheral vascular bed in dogs (Inciarte et al., 1980; Hom, Chelly, and Jandhyala, 1982) as well as in cats (Sánchez-Ferrer et al., 1988). The rise in the packed cell volume during intravenous infusion of vanadate might be reflect splenic contraction and transcappillary fluid efflux (Inciarte et al., 1980). In the rats the hemodynamic alterations in response to vanadate were qualitatively similar (Jadhav and Jandhyala, 1983; López-Novoa and Garrido, 1986). It seems likely, therefore, that the hypertension produced by vanadate is related to an increase in peripheral vascular resistance.

In the present experiments, intravenous infusion of sodium metavanadate caused decreases in glomerular filtration rate less than effective renal plasma flow and effective renal blood flow (Figure 2,3). Hence, the ratio of the glomerular filtration rate and effective renal plasma flow, the filtration fraction insignificantly increased (Figure 4), indicating a predominant in efferent arteriolar vasoconstriction than afferent arteriole. These changes would be accounted for a marked increase in renal vascular resistance (Figure 4). There were other supported reports in dogs which indicated that an intravenous or intrarenal arterial infusion of vanadate induced a rise in renal vascular resistance causing reduction in renal blood flow and glomerular filtration rate (Higashi and Bello-Reuss, 1980; Inciarte et al., 1980; López-Novoa, Mayol, and Martínez-Maldonado, 1982; López-Novoa et al., 1982; Benabe, Cruz-Soto, and Martínez-Maldonado, 1984) and as similar as in rats (Hatfield and Churchill, 1981; López-Novoa and Garrido, 1986).

Many possibilities for renal vasoconstriction occurred during vanadate administration. One possible mechanism for vasoconstriction is that these effects results from inhibition of sarcolemmal Na^+-K^+ATPase. Since vanadate has been shown to inhibit Na^+-K^+ATPase, but the amount of inhibition apparently varies in the different tissues (Nechay et al., 1986). The rise in plasma potassium concentration during intravenous infusion in this studies might be compatible with an inhibition of Na^+-K^+ATPase. An inhibition of Na^+-K^+ATPase would reduce the transmembrane gradient of sodium which would lead to cell depolarization in vascular smooth muscle. This could result in an increase in the influx of extracellular calcium (Benabe, Cruz-Soto, and Martínez-Maldonado, 1984). On the other hand, Na^+-K^+ATPase inhibition may also increase intracellular calcium by decreasing the efflux of calcium through the sodium-calcium exchange mechanism in the plasma membrane (Nechay et al., 1986). Other differences might be related to actions of vanadate that are

independent of Na^+-K^+ATPase. Vanadate also inhibited the Ca^{2+}ATPase of sarcoplasmic reticulum and plasm membrane which is considered to be responsible for the high affinity ATP-dependent Ca^{2+} uptake or storage or efflux leading to reduced cytoplasmic calcium concentration (Rossi, Garrahan, and Rega, 1981; Sánchez-Ferrer et al., 1988). Cytosolic calcium could also rise that would be associated with a decrease in the calcium release from the sarcoplasmic reticulum. This would then result in an accumulation of calcium for the interaction with the troponin-tropomyosin complex which activated interaction of cross-bridge between actin and myosin causing contracture of vascular smooth muscle. The increment in cytoplasmic calcium ion from any of these mechanisms may serve as a stimulus for vasoconstriction. In present studies, combination of verapamil with vanadate (group VI, inhibition of calcium entry to cell through the voltage-operated channels) caused significantly attenuated the increment in systemic arterial pressure and renal vascular resistance. An improved the renal hemodynamics by the effect of vanadate was seen in this group. These results might be suggested that cardiovascular and renal hemodynamics effects caused by vanadate were mediate in part via an increase intracellular calcium by increasing calcium influx. According to the experiment in dogs by López-Novoa, Mayol, and Martínez-Maldonado (1982) showed that pretreatment with acetylcholine (incresing sodium influx most likely enhances calcium efflux through a sodium-calcium exchange) could be ameliorated the effects by intrarenal sodium orthovanadate (0.5 μmole/min) and suggested that an increment of intracellular calcium was due to the reduction of the calcium efflux from the cell. In contrast to the present experiment, animals in group V infused with acetylcholine could blunt the effects of intravenous sodium metavanadate on cardiovascular and renal hemodynamics. These results might be suggested that an increment of intracellular calcium was not due to decreasing the calcium efflux from the cell. This finding supports the previous suggestions that voltage-dependent calcium channels are required for calcium entry in to the cytosol in vanadate-induced

vasoconstriction and the extracellular calcium concentration is the critical determinant of vanadate-induced vasoconstriction (Benabe, Cruz-Soto,and Martínez-Maldonado,1984; Sundete al., 19 84).

It seems liely that the observed hemodynamic changes produced by vanadate in the present study were partially due to an inhibition of Na^+-K^+ATPase rather than Ca^{2+}ATPase. It has been shown that Ca^{2+}ATPase activity has not been shown to be altered by diminished extracellular calcium or by a slow calcium-channel blocker (Benabe, Cruz-Soto, and Marti 'nez-Maldonado, 1984). Vanadium was also reported to stimulate monoamine oxidase and inhibited norepinephrine uptake by autonomic nerve terminal (Schroeder, Balassa, and Tipton, 1963). On the other hand, it might released norepinephrin from adrenergic sympathetic nerve ending or increased norepinephrin concentration in the cleft between the terminal and smooth muscle cell (Sánchez-Ferrer et al., 1988). Hypercatecholamia in this setting could induced the vasoconstriction which are indirectly effects of vanadate. The present study indicates that prazosin (group II, selective alpha-1 adrenoreceptor blocker, and atenolol (group III, selective beta-1 adrenoreceptor blocker) significantly prevented the increment in systemic arterial pressure but they did not prevented renal hemodynamics response that were elicited by vanadate. This finding might support the previous suggestion that vanadate produced cardiovascular alterations via postsynaptic alpha-1 adrenoreceptor and beta-1 adrenoreceptor (Hom, Chelly, and Jandhyalo, 1982). However, stimulation of the renal nerves has been shown to cause renal vasoconstriction in both cortex and medulla and a marked decrease in renal blood flow. This effect is medÛ¥-□+@ by alpha-1 adrenoreceptors which respond preferentially to catecholamine released from sympathetic nerve endings and to a lesser extent by postsynaptic alpha-2 adrenoreceptors which respond primarity to circulating and actions of catecholamine (Kopp, Bradley, and Hjemdahl, 1983). The beta-1 receptor mediate the cardiac

responses to beta adrenergic stimulation, whereas the beta-2 receptor is known to mediate the peripheral vascular responses to beta adrenergic stimulation (Lands et al., 1967). These hypothesis may be applied to explain in the present results of renal hemodynamics and renal vascular resistance in prazosin and atenolol effects which were not prevented the effect of vanadate. On the other hand, a mechanisms for vanadate effects on renal vasculature may be mediated a part by interfering the catecholamine effects via postsynaptic alpha-2 adrenoreceptor and beta-2 adrenoreceptor.

In addition to hemodynamic changes was accompanied with a decrease in renin secretion during given vanadate which could be dependent on extracellular calcium concentration. Increment in intracellular calcium by decreasing its efflux by vanadate or increasing its influx with high extracellular potassium or increasing angiotensin II caused reduction of renin secretion (Churchill and Churchill, 1980, López-Novoa et al., 1982; Jadhav and Jandhyalo, 1983). The present study showed that enalapril maleate (group IV) (angiotensin-converting enzyme inhibitor) significantly prevented the systemic arterial pressure but they did not prevented renal hemodynamics response that were elicited by vanadate. These findings may support the previous suggestion that vanadate produced cardiovascular alterations by a mechanisms mediated a part by increasing angiotensin II concentration and related to resulted in reducing of renin secretion (López-Novoa et al., 1982). Since the action of vanadate on renal hemodynamic was not mediated via the renin-angiotensin system.

The effects of sodium metavanadate which can be attributed to renal tubular sites of action include : the present data indicates that after intravenous infusion of sodium metavanadate markedly declined but reversible in urine flow rate, sodium excretion, potassium excretion. and chloride excretion (Figure 2; Table3,4,5), but it markedly rose in bicarbonate excretion (Table 6). Fractional water excretion and

fractional free water clearance (Figure 21,22) significantly decresed after intravenous infusion of sodium metavanadate. Sodium metavanadate significantly exhibited a marked depression of the renal threshold for glucose and bicarbonate, whereas the marked depression in maximal tubular reabsorption for glucose (Table 11, Figure 23) and bicarbonate (Table 12, Figure 24). Moreover, it significantly reduced tubular secretion and excretion of PAH (Table 13, Figure 25).

Comparison of these findings with those reported previously by other reveals little disagreement. These results are different from those obtained in rats. Although renal vasoconstriction also occurs when rat kidney are exposed to vanadate, urine flow rate and salt excretion increse, whether vanadate is administered to intact rats as a bolus as continous intravenously or intrarenally infusion (Day et al., 1980; Higashi and Bello-Reuss, 1980; Hatfield and Churchill, 1981; Westenfelder, Hamburger, and Garcia, 1981). Since profound diuresis is seen even when the GFR is unchanged, vanadate must inhibit sodium and water reabsorption in renal tubules rather than cause diuresis by processes tied only to hemodynamic alterations. A study by micropuncture technique in both whole kidney and single nephron suggested that vanadate decreased in water reabsorption in the proximal tubule (Higashi and Bello-Reuss, 1980). Furthermore, vanadate has been shown to depress both free water formation, sodium reabsorption along the assending limb of Henle's loop (Westenfelder, Hamburger, and Garcia, 1981) The resulting inhibition in outer medullary Na^+-K^+ATPase activity correlated well with the simultaneous impairment in diluting capacity. Vanadate has also been reported to inhibit the initiation of the AVP-induced increase in hyperosmotic permeability at a site distal to c-AMP formation and caused a rapid decrease in lumen-negative transepithelial voltage which would decreased free water and sodium reabsorption in the cortical collecting tubule (Edwards and Grantham, 1983b) and in toad bladder (Arruda and Westenfelder, 1983). These results must interfere with

urinary concentration. This present results produced neither diuresis nor natriuresis. These results are similar to the other experiments in dogs that the renal vascular bed effects of vanadate appear to predominate over the tubular effects. Therefore, there is a sequent decrease in salt and water excretion which is probably secondary to the decrease in glomerular filtration rate and volume depletion (López-Novoa, Mayol, and Martínez-Maldonado, 1982; López-Novoa et al., 1982). The natriuresis and water effect of vanadate is reversible and is secondary to inhibition of the basolateral Na^+-K^+ATPase which occurs throughout the entire nephron, renal cortex likely binds more vanadate. The major mechanism of action of vanadate has been shown by inhibition of the Na^+-K^+ATPase, and lead to change in cytosolic calcium levels by either an Na^+-Ca^{++}exchange mechanism or Ca^{++}-ATPase which located on the basolateral tubular side. According to the present results, prazosin (group II), enalapril maleate (group IV) and acetylcholine (group V) did not change in peritubular physical factors, excepted for verapamil (group VI) could attenuted the response of renal functions which were elicited by vanadate. Unpropotionate changes in filtered load, urinary excretion and tubular reabsorption of electrolytes in the present results might be indicated that sodium metavanadate not only interfered to renal hemodynamics but also directly interfered to renal tubular function (Figure 3-14).

Primary reports in rats indicate that vanadate infusion results in a decrease in proximal tubules maximal bicarbonate and glucose reabsorption and p-aminohippurate (PAH) secretion (Higashi and Bello-Reuss, 1980; Westenfelder, Hamburger, and Garcia, 1981; Edwards and Grantham, 1983a). All of these effects correlate with the simultaneous increase in sodium excretion suggesting a relationship of Na^+-K^+ATPase inhibition. Some have speculated that vanadate, by inhibiting a greater fraction of the proximal tubule Na^+-K^+ATPase activity, would lead to glycosuria, phosphaturia and bicarbonaturia, a syndrome identical to proximal renal tubular acidosis (RTA).

These present results might not show only decrease in maximal transtubule glucose reabsorption and transtubule bicarbonate reabsorbtion but also decrease in renal threshold. These results suggest that the main site of diffuse tubular cell demage was the proximal convolution which might result in decreased luminal membrane permeability. Moreover, vanadate depressed renal blood flow that resulted in decrease secretion and excretion of PAH. The present results support that sodium, chloride and water reabsorption decreased in this tubular site.

The mechanism for the lack of consistent effect of vanadate on renal sodium and water reabsorption cannot be adequately explained by the present results. Nevertheless some major possibilities can be considered. First, This response is dependent on the state of hydration of the animal and is seen only at relatively high concentrations of vanadate (Day et al., 1980; Higashi and Bello-Reuss, 1980). This indicates a dissociation between vascular and tubular actions of vanadate. The next possibility is that trace amounts of vanadium can be detected in most mammalian tissue; the highest concentrations are often found in the kidney, especially in the renal cortex (Bogden et al., 1982). On the other hand, the major role of renal Na^+-K^+ATPase in sodium reabsorption is in the medulla (ascending limb of Henle 's loop) and that it has a relatively small role in proximal sodium reabsorption, so that natriuresis may not occur by vanadate infusion. This possible effect may be similar to the effect of cardiac glycoside on sodium and water reabsorption in both renal cortex and medullar (Martinez-Maldonado et al., 1972). Finally, fractional reabsorption of sodium and water could be decreased in the proximal tubule, but the excess sodium and water reabsorbed in more distal sites of the nephron (Higashi and Bello-Reuss, 1980).

The present experiments demonstrate that an intravenous infusion of sodium metavanadate caused hyperkalemic hyperchloremic distal renal tubular acidosis.

Plasma anion gap was normal but urine anion gap significantly increased after vanadate infusion (Table 7). Blood pH significantly reduced, while urine pH was unable to less than 5.5 (Table 8). Utilizing the urine-to-blood gradient of P_{CO_2} (U-B P_{CO_2}) during maximal urinary alkalinization significantly reduced during acute sodium metavanadate infusion (Table 12). The urinary excretion of titratable acidity and ammonium significantly decreased, although the urinary excretion of bicarbonate did not alter, so that fractional acid excretion significantly reduced after sodium metavanadate infusion (Table 9). These data demonstrate that animals with sodium metavanadate infusion had a preserved capacity defect for distal acidification. The excretion of potassium underthese experiment condition was reduced which was due to impaired potassium secretion.

Theoretically, however, vanadate could cause distal acidification defect. It could do so by an indirect effect abolishing cortical collecting duct transepithelial voltage, or it could do so by a direct effect inhibiting of the collecting duct proton pumps. Distal urinary acidification has traditionally been thought to occur via an electrogenic H^+-ATPase. This enzyme is found in the intercalated cells of the collecting duct: it is stimulated during chronic metabolic acidosis, it inhibited by vanadate (Arruda, Sabatini, and Westenfelder, 1981). Recently, it has become clear that the collecting duct also contains an electroneutral H^+-K^+ATPase . While the existing cell type principle versus intercalated is currently under debate, the enzyme is found in greatest quantity in the cortical collecting duct; it is stimulated by potassium and inhibited in vitro by vanadate (Garg and Narang,1988; Dafnis et al., 1992). The present experiment was different in rats which developed hypokalemic distal RTA (classical distal RTA) in association with inhibition of collecting tubule H^+-K^+ATPase activity. These present results suggest that inhibition of renal Na-K^+ATPase alone should result in hyperkalemia, and if one of the collecting tubule H^+- ATPase is also inhibited, hyperkalemic distal RTA will result.

The change of plasma catecholamine and renin level and the enzymes activity of vascular and proximal and distal tubular site entire of the nephron should be further examined.

In conclusion, the effects of vanadate on cardiovascular system and renal function are likely to be complex. The present findings demonstrate that an increase in peripheral vascular resistance is the major factor underlying the hypertension produced by vanadate. The cardiovascular system alterations are mediated directly via an increase intracellular calcium by increasing calcium influx through voltage-operating channels, a part by indirect effects of vanadate are mediated by an increase in catecholamines release particulary interfering the central autonomic neurogenic effects via postsynaptic alpha-1 adrenoreceptor and beta-1 adrenoreceptor, and also indirectly mediated by increasing angiotensin II concentration. Sodium metavanadate has both renal vascular and renal tubular effects. The defects in renal vascular, proximal and distal tubular function have been shown to relate to direct actions of both vascular and renal tubular cells ATPase system, primarily in Na-K^+ATPase.