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Cardiovascular and Renin Responses to Vanadate in the Conscious Dog: Attenuation after Calcium Channel Blockade (41786)

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Abstract. The effects of vanadate on cardiovascular function and on the secretion of renin and vasopressin were investigated by infusing sodium orthovanadate (0.32 μmole/kg · min) intravenously into five conscious dogs. Vanadate caused significant increases in mean arterial pressure, total peripheral resistance, pulmonary arterial pressure, and cardiac output. These data illustrate that the hemodynamic effects of vanadate in the conscious dog are similar to those of the anesthetized dog but that minor differences do exist. Vanadate significantly suppressed plasma renin activity, but plasma vasopressin was unchanged. The effects of vanadate also were investigated in the same dogs on another day after administration of the calcium channel blocker, verapamil (0.3 mg/kg bolus + 0.01 mg/kg · min). After calcium channel blockade, the increases in arterial pressure and pulmonary arterial pressure induced by vanadate were attenuated, and cardiac output did not increase. Calcium channel blockade also prevented the vanadate-induced decrease in plasma renin activity. These data suggest that the cardiovascular and humoral alterations produced by vanadate in the conscious dog are at least partially mediated by changes in intracellular calcium.

Since the discovery of vanadate as an inhibitor of Na-K-ATPase (1), many of its effects on hemodynamics and renal function have been investigated. In conscious rats (2), vanadate produces a marked diuresis and natriuresis. In anesthetized cats (3) and anesthetized dogs (4), the major effects of vanadate administration are increases in arterial pressure and renal vascular resistance and decreases in urine flow and renal blood flow. The sympathetic nervous system does not appear to be responsible for these effects; α and β adrenergic antagonists do not modify the cardiovascular effects of vanadate in anesthetized cats (3). It has been suggested that the vascular effects of vanadate may be related to the inhibition of vascular smooth muscle ATPase and changes in cytosolic calcium content (4); however, in anesthetized cats (3) the hemodynamic and renal responses produced by vanadate are unchanged by calcium channel blockers.

The purpose of this study was to investigate the hemodynamic and renal effects of intravenous sodium orthovanadate. In order to evaluate whether changes in intracellular calcium concentration were responsible for the observed responses, we also assessed the effects of vanadate after administration of the calcium channel blocker, verapamil. All of these experiments were performed in conscious dogs to avoid possible complicating influences from anesthesia (5) and acute surgery.

Materials and Methods. *Surgical preparation.* Each dog was prepared surgically for hemodynamic monitoring 2 weeks before the experiments started. Anesthesia was induced with sodium pentobarbital (25 mg/kg, iv) and supplemented as necessary. A left thoracotomy was performed aseptically through the fourth intercostal space. The pericardium was opened, and an electromagnetic flow probe was placed around the aorta at its origin. Catheters were inserted into the descending aorta, main pulmonary artery, and the right and left atria. The pericardium was loosely approximated, and the free ends of the catheters and flow probe were passed subcutaneously to the interscapular area and passed through the skin. A polyethylene drainage catheter was inserted into the pleural cavity through the seventh intercostal space. After the chest was closed, the pneumothorax was reduced by connecting the drainage catheter to a controlled vacuum source. After several hours the catheter was sealed and left in place

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for 2 days so that any accumulated intrathoracic fluid could be withdrawn. One week after the initial surgery, the dogs again were anesthetized with sodium pentobarbital, and catheters were placed in the thoracic aorta and inferior vena cava via a branch of the femoral artery and a tributary of the femoral vein, respectively. Penicillin (500,000 U/day) was administered for 3 days following each operation. Catheters were kept patent by daily flushing with a dilute solution of sodium heparin.

Experimental protocol. Experiments were performed on the conscious dog (19.0 ± 0.7 kg body wt) as it rested quietly in a Pavlov stand. The hemodynamic and renal effects of vanadate were evaluated under two conditions: (i) while the dogs received a continuous infusion of isotonic saline (0.25 ml/min), and (ii) on a separate day while they received an infusion of the calcium channel blocker, verapamil, in isotonic saline (0.3 mg/kg bolus + 0.01 mg/kg · min). Each infusion was started 10 min before the experiment began. Each experiment was divided into three consecutive 30-min periods: control, experimental, and recovery. Sodium orthovanadate in isotonic saline (0.32 μmole/kg · min) was infused during the experimental period of both control and calcium-blocked experiments. Urine samples were collected at 10-min intervals through a Foley catheter, and an equal amount of isotonic saline was given intravenously to replace the volume lost. Blood pressures were recorded continuously with Statham P23Db pressure transducers that were balanced every 10 min to eliminate possible errors caused by baseline drift. Cardiac output was measured with an electromagnetic flowmeter (Biotronex Laboratory, Inc.); an autozeroing circuit (6) was used to minimize baseline shifts in the aortic flow tracing. Heart rate was determined from the aortic flow signal. Blood pressures and aortic flow were monitored on an oscillographic recorder interfaced with a PDP 11/34 computer (Digital Equipment Corp.) Each hemodynamic variable was sampled 100 times per second and averaged each minute by the computer. Stroke volume and total peripheral resistance were calculated from the average values of cardiac output, arterial pressure, and heart rate. One-minute averages of all hemodynamic data were displayed on a video screen and stored on a flexible diskette for later analysis.

Blood samples (25 ml) were withdrawn from the aorta 5 min before the end of the control, experimental, and recovery periods. Part of the sample (3 ml) was collected in a chilled tube containing EDTA for determination of plasma renin activity. The remainder was collected in a chilled tube containing lithium heparin for determination of hematocrit and plasma values of vasopressin, osmolality, sodium, and potassium. The volume of each blood sample was replaced immediately with an equal amount of 6% dextran in isotonic saline. Urine and plasma sodium and potassium concentrations were measured with a flame photometer (Instrumentation Laboratory, Model 143) and osmolality by freezing-point depression with a high-precision osmometer (Advanced Instruments, Model 3R) immediately following the experiment. Plasma renin activity was determined by radioimmunoassay of angiotensin I with reagents supplied by a commercially available kit (Clinical Assays, Inc.). Plasma vasopressin was determined by radioimmunoassay as described previously (7).

Statistical analysis. The 1-min averages for the hemodynamic data were averaged over 10-min periods corresponding to each urine collection period. All data within each group of experiments were analyzed using an analysis of variance for repeated measurements (8). When statistical significance ($P < 0.05$) was indicated, Dunnett's test was used to identify the significant differences between the means of each interval and the mean of the three control periods. All data are expressed as the mean ± standard error of the mean unless otherwise noted. Comparisons between treatments were made using an unpaired t test.

Results. The effect of intravenous sodium orthovanadate on systemic hemodynamics is shown in Fig. 1. Aortic pressure increased from a control value of 109.8 ± 1.0 to 137.0 ± 4.7 mm Hg ($P < 0.01$) during the experimental period while total peripheral resistance increased from 3.17 ± 0.03 to 3.77 ± 0.42 PRU ($P < 0.01$). Pulmonary arterial pressure increased from 18.4 ± 0.1 to 22.7 ± 1.3 mm Hg ($P < 0.01$) and left atrial pressure increased from 5.9 ± 0.1 to 9.5 ± 2.1 mm Hg ($P < 0.01$). Cardiac output increased modestly, but sig-

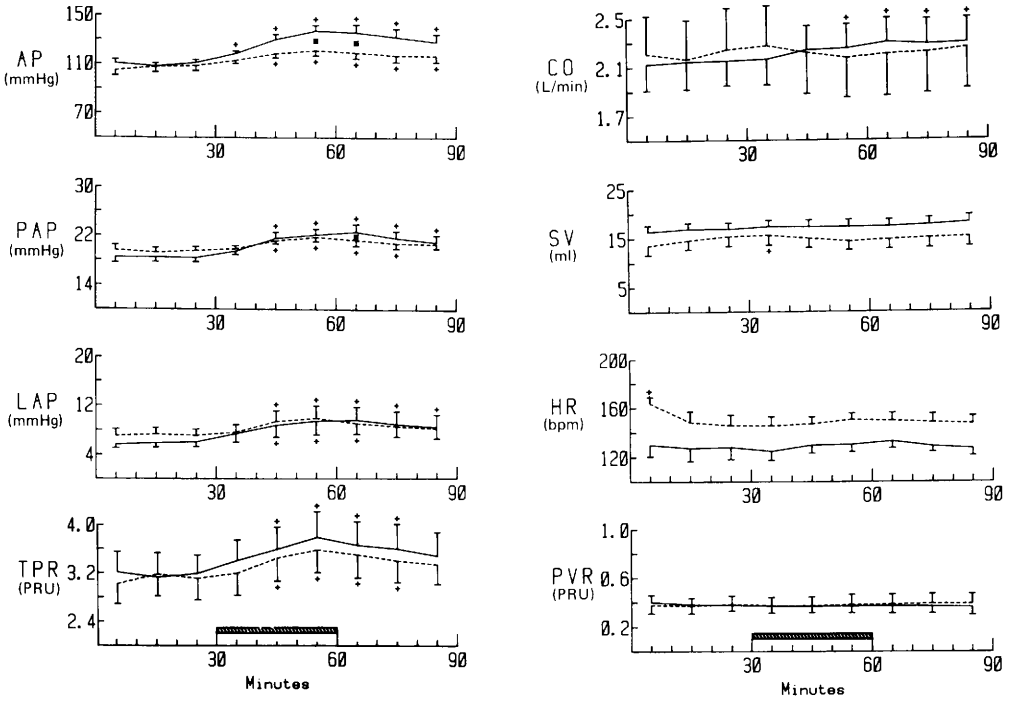

FIG. 1. Hemodynamic responses to vanadate infusion alone (solid line) and after calcium channel blockade with verapamil (dashed line). The period of vanadate infusion is denoted by the cross-hatched bar. Abbreviations: arterial pressure (AP), pulmonary arterial pressure (PAP), left atrial pressure (LAP), total peripheral resistance (TPR), cardiac output (CO), stroke volume (SV), heart rate (HR), and pulmonary vascular resistance (PVR). Crosses denote significant differences from control ($P < 0.05$) and asterisks denote differences between groups ($P < 0.05$).

nificantly from 2.14 ± 0.01 to 2.30 ± 0.20 liter/min ($P < 0.01$). Heart rate, pulmonary vascular resistance, and stroke volume were not changed significantly by vanadate.

Infusion of vanadate caused a significant decrease in plasma renin activity from 1.95 ± 0.57 to 1.21 ± 0.41 ng AI/ml/hr ($P < 0.01$) during the experimental period, and this persisted during the recovery period (Fig. 2). Vanadate also caused a significant increase in hematocrit from 35 ± 3 to 38 ± 2% ($P < 0.05$) as illustrated in Fig. 2, but plasma vasopressin and plasma osmolality were unchanged during the entire experiment.

Figure 3 depicts the effects of vanadate on renal function. Vanadate caused no significant changes in urine flow or sodium excretion during the experiment.

The hemodynamic effects of vanadate differed somewhat in the presence of verapamil (Fig. 1). Verapamil initially caused a significant increase in heart rate as can be seen during the first 10 min of the control period, but heart rate then decreased significantly and stabilized during the remainder of the control period; infusion of vanadate had no significant effect on heart rate. Aortic pressure increased from 106.9 ± 1.6 to 120.9 ± 4.2 mm Hg ($P < 0.01$) and pulmonary arterial pressure increased from 19.5 ± 0.2 to 21.8 ± 0.7 mm Hg ($P < 0.01$) in response to vanadate in the presence of verapamil. These increases were attenuated ($P < 0.05$) when compared to the responses elicited by vanadate alone. Vanadate also caused an increase in total peripheral resistance from 3.1 ± 0.1 to 3.6 ± 0.4 PRU ($P < 0.01$) and in left atrial pressure from 7.2 ± 1.0 to 9.9 ± 2.0 mm Hg ($P < 0.01$) after the administration of verapamil, but cardiac output and pulmonary vascular resistance did not change significantly even though stroke volume increased from 14.5 ± 0.8 to 15.6

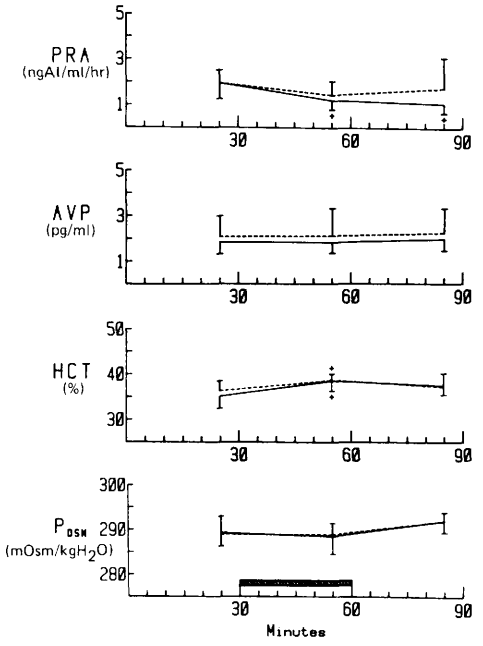

FIG. 2. Changes in plasma renin activity (PRA), arginine vasopressin (AVP), hematocrit (HCT), and plasma osmolality (Posm) in response to vanadate under normal conditions (solid line) and after calcium channel blockade with verapamil (dashed line). Notation as in Fig. 1.

± 2.0 ml ($P < 0.01$). Plasma renin activity did not change, indicating that verapamil effectively prevented the decrease elicited by vanadate under control conditions. Hematocrit increased significantly from 36 ± 2% during the control period to 38 ± 1% ($P < 0.05$) during the experimental period (Fig. 2), but plasma vasopressin and plasma osmolality were unchanged.

The effects of vanadate on renal function after the administration of verapamil are shown in Fig. 3. Urine flow showed an initial increase during the control period and then returned to control values during the rest of the experiment. Sodium excretion showed an initial increase which appeared to parallel the increase in urine flow but did not change significantly from control values during the experiment. The initial increase in both urine flow and sodium excretion was not statistically significant.

To test the efficacy of the calcium block, verapamil was infused into two dogs at twice the dose; the dogs then were given the usual dose of vanadate. The arterial pressure response to vanadate was not diminished further after the larger dose of verapamil.

Discussion. These experiments demonstrate that intravenously administered sodium orthovanadate causes increases in aortic blood pressure, pulmonary arterial pressure, and total peripheral resistance in the conscious dog. The increase in blood pressure is largely the result of an increase in total peripheral resistance although the small increase in cardiac output also contributes. Left atrial pressure showed a consistent increase in response to vanadate; consequently, pulmonary vascular resistance did not change even though pulmonary arterial pressure increased significantly.

The responses of arterial pressure, pulmonary arterial pressure, and total peripheral resistance to vanadate in the conscious dog were similar to those observed in the anesthetized dog. Anesthesia, however, may alter some of the cardiovascular effects of vanadate, since cardiac output, heart rate and left atrial pressure decrease and pulmonary vascular resistance increases after vanadate administration to the anesthetized dog (4). We gave vanadate at a dose (0.32 μmole/kg • min) comparable to one dose (estimated to be 0.34 μmole/kg • min) used in a previous study in anesthetized dogs (4), so it is doubtful that the different results are attributable to differences in the amount of vanadate given. It is more likely that anesthesia alters the cardiovascular response (5) elicited by vanadate.

Verapamil, a calcium channel blocker, sig-

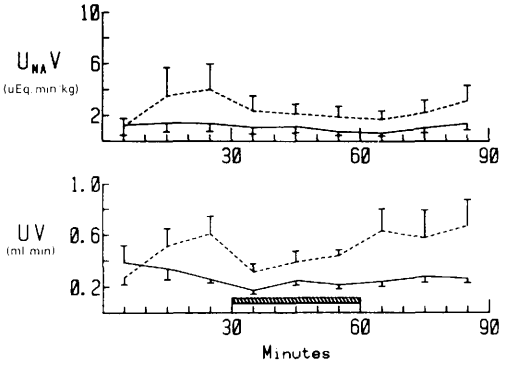

FIG. 3. Changes in sodium excretion ($U_{Na}V$), and urine flow (UV), in response to vanadate under normal conditions (solid line) and after calcium channel blockade with verapamil (dashed line). Notation as in Fig. 1.

nificantly attenuated the increases in aortic pressure and pulmonary arterial pressure that were elicited by vanadate. This suggests that the increase in blood pressure caused by vanadate is mediated in part by an increase in intracellular calcium. These results differ from those reported to occur in the anesthetized cat where verapamil caused no change in the aortic pressure response to vanadate (3). We have no explanation for this discrepancy, but it may be related to species differences or to some unknown effect of anesthesia.

Vanadate has the ability to inhibit two enzymes, Na-K-ATPase and Ca-ATPase, but the amount of inhibition apparently varies in different tissues. It seems likely that the observed hemodynamic changes produced by vanadate in this study were partially due to an inhibition of Na-K-ATPase rather than Ca-ATPase; it has been shown that sarcoplasmic reticulum Ca-ATPase has a low affinity for vanadate and that the binding is antagonized by both Ca^{++} and ATP at physiological concentrations (9). The hemodynamic responses to vanadate also may be due to a central neurogenic effect (10).

Studies using intact rats (2, 11) or perfused rat kidneys (12) have shown that vanadate produces potent diuretic and natriuretic effects which have been attributed to a decrease in water reabsorption in the proximal tubule secondary to an inhibition of Na-K-ATPase (2). This response is dependent on the state of hydration of the animal and is seen only at relatively high concentrations of vanadate in the conscious rat (2). The concentration needed to produce this diuretic and natriuretic effect also is relatively high in perfused rat kidneys (12). In contrast, in anesthetized cats (3) and anesthetized dogs (4) in a normal state of hydration, vanadate produces neither diuresis or natriuresis; rather there is an intense vasoconstriction, a concomitant decrease in renal blood flow, and a marked decrease in urine flow. Papaverine, acetylcholine, and intravascular volume expansion attenuate the renal effects of vanadate infused into the renal artery of anesthetized dogs (13) implying that the decreased urine flow may be secondary to a decrease in renal blood flow. We observed no significant change in urine flow during the entire experiment; therefore, renal function appears to be less affected by vanadate in the conscious dog than in the anesthetized dog.

Vanadate has been shown to produce a calcium-dependent inhibition of renin secretion in rat kidney slices (14) and in the anesthetized dog (15) when infused into the renal artery. Our data reveal that vanadate also causes a calcium-dependent inhibition of renin secretion in the conscious dog. The inhibition of renin by vanadate (14, 15), as well as the inhibition of renin secretion by vasopressin (16), has been linked to an increase in intracellular calcium. A recently published study (17) demonstrated that renin secretion is dependent on Na-K-ATPase activity and that by blocking with ouabain, a furosamide-induced increase in renin secretion may be prevented. This concept is compatible with our demonstration that vanadate does not lower plasma renin activity in dogs treated with the calcium channel blocker, verapamil. Vanadate had no detectable effect on the regulation of vasopressin in our experiments; plasma vasopressin did not change during the course of either experiment.

In conclusion, we have shown that intravenous vanadate given to the conscious dog causes changes in systemic hemodynamics which are similar to the responses elicited in anesthetized dogs, although the responses do differ somewhat. The increases in total peripheral resistance, aortic pressure, pulmonary arterial pressure, and the decrease in plasma renin activity after vanadate administration all appear to be mediated in part by an increase in intracellular calcium.

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