PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 169, 401–405 (1982)

Evidence for Centrally Mediated Effects of Vanadate on the Blood Pressure and Heart Rate in Anesthetized Dogs (41365)

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Abstract. Cerebroventricular administration of sodium orthovanadate (0.2 μmole/kg/min for 20 min ivt) produced significant increases in the arterial blood pressure of chloralose-anesthetized dogs. Following bilateral vagotomy, both arterial pressure as well as heart rate were significantly elevated during ivt infusion. These effects were completely abolished when the animals were pretreated with an autonomic ganglionic blocker, hexamethonium. In contrast, intravenous administration (iv) of the same dose of vanadate significantly increased the arterial pressure only in the vagotomized dogs and this effect was accompanied by a reduction in the heart rate. Pressor effects of iv vanadate were potentiated after hexamethonium while the bradycardic effects were virtually eliminated. This study demonstrates that vanadate can produce cardiovascular alterations via neurogenic mechanisms. It is suggested that these centrally mediated effects and their relationship to sodium pump inhibition should be given further consideration in evaluating the role of vanadium in the etiology of hypertension.

Vanadium, a trace element, occurs widely in various animal tissues (1). Ever since Cantley *et al.* reported that vanadium is a potent inhibitor of Na^+-K^+-ATPase (2), several investigators have been concerned with the possible physiological and pathological significance of this finding. Cardiovascular effects of this agent could be particularly important since sodium pump inhibition has been postulated to play a role in the etiology of volume-expanded, low-renin type(s) of hypertension (3). It has been demonstrated that intravenous administration of sodium orthovanadate produces potent vasoconstrictor effects and increases the arterial blood pressure in the anesthetized animals (4, 5). Since vanadium-induced alterations in Na^+ pump activity may also occur in the neuronal tissues and affect their function, the present investigation is undertaken to evaluate whether vanadate can alter arterial blood pressure and heart rate by mechanisms mediated via the central nervous system.

Materials and Methods. Mongrel dogs of either sex weighing between 12 and 14 kg were anesthetized with α-chloralose (Pointet Girard, Paris) 100 mg/kg iv. Skeletal muscle relaxation was enhanced by intramuscular injection of *d*-tubocurarine, 0.2 mg/kg. After endotracheal intubation, animals were placed on artificial ventilation throughout the experimental period. Arterial blood pressure was recorded from a catheterized femoral artery via Statham (P-23 dc) pressure transducer. A Grass cardiotachograph was utilized to monitor heart rate from the pressure pulse. Both the parameters were recorded on a Grass polygraph. A femoral vein was catheterized for intravenous administration of the drugs.

Perfusion of the cerebrolateral ventricles. Procedure for cerebroventricular perfusion in the experimental animals was described in detail previously (6, 7). Briefly, the head of the animal was secured firmly in a stereotaxic apparatus (David Kopf). A stainless steel cannula (18 gauge) was introduced into a cerebrolateral ventricle and secured firmly to the skull with dental cement. Modified artificial cerebrospinal fluid (CSF) was continuously perfused into the ventricle and allowed to flow out freely via a polyethylene tube placed in the cisterna magna. Rate of perfusion was maintained constant at 0.1 ml/min with a Sage infusion pump. Composition of the CSF used in this study is as follows: NaCl, 8.1; KCl, 0.331; $CaCl_2$, 0.156; $MgCl_2$, 0.061; NaH_2PO_4, 0.049; $NaHCO_3$, 1.5; urea, 0.110; sucrose, 0.037; ascorbic acid, 0.066 (represented as g/liter). The pH was

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adjusted to 7.3 to 7.4; CSF was maintained at 37°.

Drugs. Sodium orthovanadate (Na_3VO_4 from Fisher) was dissolved in CSF and buffered with sodium dihydrogen phosphate so that the pH of the final solution would be 7.35. Concentration of NaCl in this solution was modified so that the sodium content in this vanadate solution was not different from that in normal CSF. Hexamethonium bromide was dissolved in normal saline.

During the stabilization period of 60 min, artificial CSF was continuously infused into the lateral ventricles. Then the CSF is replaced with that containing vanadate. In a few preliminary experiments sodium orthovanadate was infused at a rate of 0.1 μmole/kg/min for 15 to 20 min. Since this dose did not produce any significant changes in the blood pressure and heart rate, in all the subsequent studies, a dose of 0.2 μmole/kg/min was infused intraventricularly (ivt) for 20 min and changes in the blood pressure and heart rate were recorded. This dose was calculated on the basis of formula weight of Na_3VO_4. It is considered that the effects of centrally administered vanadate could be due to leakage of the substance into the peripheral circulation; thus, the same dose of vanadate (in CSF) was also infused via a femoral vein at a rate of 0.1 ml/min for 20 min and the effects on heart rate and blood pressure were compared with those observed during ivt infusion. In several groups of dogs the effects of ivt and iv infusion of vanadate were studied before and after bilateral vagotomy and after vagotomy plus autonomic ganglionic blockade with hexamethonium (5 mg/kg, iv).

All the data are presented as means ± SEM. Statistical significance of the changes produced by vanadate in the same group were determined by Dunnet's test (20). Analysis of variance was used when the data from different groups were compared (Digital minicomputer system, MINC-11).

Results. Resting blood pressure and heart rate values of control dogs and those after bilateral vagotomy and vagotomy plus hexamethonium before vanadate administration are shown in Table I.

During the control period of 60 min, infusion of CSF did not produce any changes in the blood pressure or heart rate. In the dogs with intact vagi, ivt infusion of vanadate (0.2 μmole/kg/min) produced significant increases in the blood pressure accompanied by slight reductions in the heart rate; after these changes reached a plateau, which occurred between 15 and 20 min, infusion was stopped (at 20 min; Fig. 1). Both the parameters returned approximately to prevanadate levels within 60 to 90 min after the infusion was stopped. After recovery, these actions of vanadate could be reproduced in the same animals. In the vagotomized dogs, ivt infusion of vanadate produced significant increases in the blood pressure as well as heart rate and these pressor effects were slightly greater than those observed in the dogs with vagi intact. Pretreatment of the animals with hexamethonium completely abolished the ivt effects of vanadate in the vagotomized dogs.

In contrast, intravenous infusion of the same dose of vanadate to the dogs with intact vagi, caused significant reductions in the heart rate; however, increases in the arterial pressure (<10 mm Hg) were not statistically significant. Only after bilateral

TABLE I. RESTING BLOOD PRESSURE AND HEART RATE VALUES OF GROUPS OF CHLORALOSE-ANESTHETIZED DOGS PRIOR TO THE ADMINISTRATION OF VANADATE

Group[a]	Mean blood pressure (mm Hg) (mean ± SEM)	Heart rate (beats/min) (mean ± SEM)
Control	103 ± 7.2	97 ± 8.4
After vagotomy	140 ± 4.7	191 ± 6.0
After vagotomy + hexamethonium, 5 mg/kg, iv	117 ± 9.5	147 ± 5.6

[a] $N = 9$ in each group.

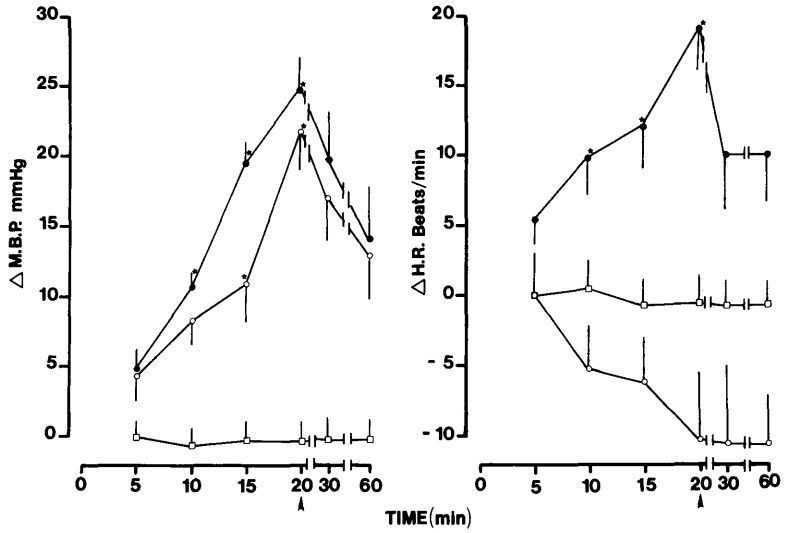

FIG. 1. Effects of intraventricular administration of sodium orthovanadate (0.2 μmole/kg/min) on the blood pressure and heart rate of chloralose-anesthetized dogs. Absolute changes in the mean blood pressure and heart rate (mean ± SEM) are shown on the ordinate. Open circles (○), before vagotomy; closed circles (●), after vagotomy; open squares (□), after vagotomy plus hexamethonium. $N = 9$ in each group. A star indicates statistical significance of the change from the resting values ($P < 0.05$). Infusion was stopped after 20 min as indicated by an arrow.

vagotomy, did significant increases in blood pressure occur during iv infusion of vanadate; these pressor effects were still accompanied by significant decreases in the heart rate (Fig. 2). After pretreatment of the vagotomized dogs with hexamethonium, pressor effects of iv vanadate were potentiated while the bradycardic effects were essentially eliminated (Fig. 2).

Discussion. Intraventricular administration (ivt) of vanadate to chloralose anesthetized dogs produced significant increase in the blood pressure which was accompanied by a slight and insignificant decrease in the heart rate. But in the vagotomized animals, heart rate was also significantly enhanced during ivt infusion of vanadate. Obviously in the dogs with vagi intact, activation of vagi, either centrally induced by vanadate or reflexly mediated, was masking the tachycardic effects of this agent. These pressor and tachycardiac effects of ivt vanadate were apparently mediated via neurogenic mechanisms since autonomic ganglionic blockade with hexamethonium completely abolished these actions. It is important to note that while ivt vanadate significantly elevated arterial pressure in the dogs with vagi intact, intravenous administration of the same dose failed to produce similar increases in the blood pressure. Significant pressor effects to iv vanadate were evident only after bilateral vagotomy, even though these effects were still accompanied by reductions in the heart rate. Thus it appears that reflex bradycardia in the chloralose anesthetized dogs is mediated via both components of the autonomic nervous system. This observation is consistent with the data obtained after ganglionic blockade which abolishes baroreceptor compensations. Pressor responses to iv vanadate were potentiated after pretreatment of the animals with hexamethonium, while the accompanying reductions in heart rate were virtually eliminated. Thus, the data further suggest that pressor effects of iv vanadate are due to a direct action on the vasculature and in the doses studied it has no direct effect on the chronotropic mechanisms of the heart. In addition, the results clearly demonstrate that the effects noted during ivt administration of vanadate are not due to any leakage

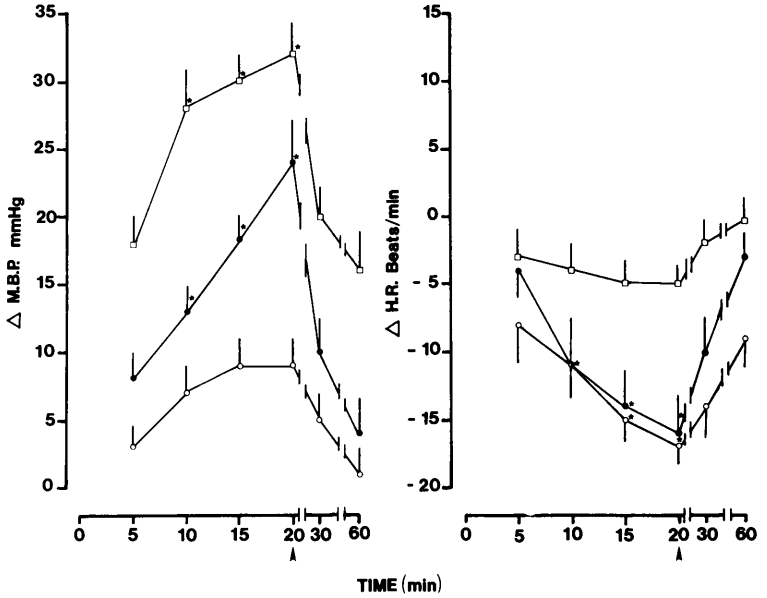

FIG. 2. Effects of intravenous administration of sodium orthovanadate (0.2 μmole/kg/min) on the blood pressure and heart rate of chloralose-anesthetized dogs. Absolute changes in the mean blood pressure and heart rate (mean ± SEM) are shown on the ordinate. Open circles (○), before vagotomy; closed circles (●), after vagotomy; open squares (□), after vagotomy plus hexamethonium. $N = 9$ in each group. A star indicates statistical significance of the change from the resting values ($P < 0.05$). Infusion was stopped after 20 min as indicated by an arrow.

of the compound into peripheral circulation.

Peripheral hemodynamic effects of vanadate were studied by other investigators in anesthetized cats and dogs (4, 5, 8). Inciarte *et al.* reported effects of several intravenous doses of vanadate in the dogs anesthetized with sodium pentobarbital (5). The lowest dose used in their study is comparable to that employed in the present investigation and at that dose vanadate was not effective in producing any significant changes in the arterial pressure until after 30 to 40 min of infusion (5). In the studies of Larsen *et al.*, iv infusion of 0.5 μmole/kg/min of sodium orthovanadate also failed to produce any essential changes in the arterial blood pressure even after 70 min of infusion to chloralose anesthetized cats (4). Borchard *et al.* showed that in cats anesthetized with urethane plus chloralose, a bolus injection of 500 μg/kg, iv, produced approximately 20 mm Hg increase in the blood pressure accompanied by a reduction in the heart rate; in the same study, higher doses reduced arterial pressure due to cardiac depression (8). In all these studies, vagi were not sectioned. Thus the dose used in the present study (0.2 μmole or 36.8 μg/kg/min) is lower than, or comparable to, those employed by the other investigators.

The relationship between the cardiovascular effects of vanadate and its ability to inhibit Na^+-K^+-ATPase, has yet to be established. Ozaki and Urakawa, based on their *in vitro* studies on guinea pig aorta concluded that the vasoconstrictor effects of vanadate were not due to inhibition of the Na^+ pump (9). Rapp also suggested that vascular actions of vanadate are independent of its actions on Na^+-K^+-ATPase (10). Further, vanadium has been shown to affect several other enzyme systems such as cardiac adenylcyclase and cyclic AMP (11, 12) and Ca^{2+}-Mg^{2+}-ATPase in the brain (13). Thus it is difficult to establish at the present time, the exact mechanisms involved in the effects of vanadate. However, it should be noted that cardiac glycosides which are established inhibitors of the sodium pump, produced increases in the blood pressure and heart rate following in-

traventricular administration to anesthetized, vagotomized cats and dogs (14). Thus it is possible that central effects of vanadate reported in this study may be related to sodium pump inhibition. Witkowska and Brezezinski showed that subacute or chronic oral administration of sodium metavanadate produced significant reduction in the norepinephrine and increases in the dopamine and serotonin levels of rat brains (15). Whether these effects are related to sodium pump inhibition or not, these studies do demonstrate that peripherally administered vanadate can alter brain metabolism and perhaps function.

Vanadium is present in human tissues and serum concentrations may vary from 0.6 nmole to 8.0 μmole per liter and thus it can be considered as an endogenous substance (16). Content of vanadium in human subjects can reach higher levels due to certain environmental situations and/or certain pathological conditions such as renal disease (17, 18). Steffen *et al.* have recently shown that prolonged dietary administration of vanadate produces significant increases in the arterial pressure in the Sprague–Dawley rats (19). The present study demonstrates that small doses of vanadate, when administered centrally, produced significant increases in the arterial pressure even in the presence of vagal compensation. The neurogenically mediated actions of vanadate, whether they are related to sodium pump inhibition or not, should be given serious consideration in evaluating the relationship between vanadium and hypertension.

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Received July 30, 1981. P.S.E.B.M. 1982, Vol. 169.