Effect of Prolonged Dietary Administration of Vanadate on Blood Pressure in the Rat

ROBERT P. STEFFEN, PH.D., MOTILAL B. PAMNANI, M.D., PH.D.
DAVID L. CLOUGH, PH.D., STEPHEN J. HUOT, B.A., SHEILA M. MULDOON, M.D.
AND FRANCIS J. HADDY, M.D., PH.D.

SUMMARY Vanadate, a potent naturally occurring Na⁺,K⁺-ATPase inhibitor thought to have a role in regulating Na⁺-K⁺ pump activity, was fed to uninephrectomized rats drinking tap water or a 1% solution of sodium chloride for as long as 56 weeks. Feeding was achieved by adding sodium orthovanadate to normal rat chow equivalent to 100 or 200 ppm vanadium by weight. In the rats drinking tap water, systolic pressure gradually increased over a period of several weeks and then was sustained in a dose-related manner for the duration of the treatment. The increased pressure was not associated with changes in water intake, urine output, or urinary sodium excretion but correlated positively with plasma vanadium levels ranging from 0.04 to 0.27 mg/ml. Increased pressure was associated with increased heart-to-body-weight ratio but did not appear to occur in a small group of animals drinking the 1% solution of sodium chloride. These findings, considered in the light of others, indicate that vanadate deserves continued study in relation to hypertension.


KEY WORDS  • dietary vanadate in rat  • systolic blood pressure  • water intake  • urine output  • sodium excretion  • vanadium blood levels  • heart and body weight

Vanadium is found in a variety of plants and animals, many of which are ingested by human beings, and has long been thought to be essential for normal growth and development of animals.¹ In solution, it forms oxanions that resemble the more familiar phosphate compounds. At pH values and concentrations likely to be found in the body, oxyvandium exists principally in two states, vanadium V (vanadate, HVO₄⁻) and vanadium IV (vanadyl, VO²⁺).²

It was only recently discovered that vanadate is a potent inhibitor of Na⁺,K⁺-ATPase. This occurred quite by accident. In 1975, Charney et al.³ reported that a certain commercial brand of adenosine triphosphate (ATP) obtained from horse skeletal muscle contained an inhibitor of Na⁺,K⁺-ATPase. In 1977, Josephson and Cantley⁴ isolated a potent Na⁺,K⁺-ATPase inhibitor from skeletal muscle and two other groups⁵,⁶ noted the contaminant in the commercial brand of ATP. Cantley et al.⁷ then reported that the contaminant was vanadate. Interest has intensified because vanadate is a naturally occurring powerful inhibitor of Na⁺,K⁺-ATPase which co-purifies with ATP derived from muscle. This suggests that vanadate may have a physiological role in controlling the rate of the sodium pump.⁸,⁹

Vanadate inhibits Na⁺,K⁺-ATPase from a variety of tissues and species, and the intensity of inhibition increases when the external potassium concentration is raised over physiological ranges or when external sodium concentration is reduced below physiological ranges.⁴ In intact tissues, vanadate must gain entrance to the cell to act, i.e., it inhibits Na⁺,K⁺-ATPase and the sodium pump from the cytoplasmic side. Consequently, the extent of inhibition in intact cells will depend on a major extent on the permeability of the cell membrane to vanadate. Other ATPases are also inhibited by vanadate, particularly in high concentrations.¹⁰ Thus, while the cellular action of vanadate is similar to that of ouabain in some respects, it is different in others; vanadate acts from the inside rather than the outside of the cell, inhibition increases rather than decreases as a function of the external potassium concentration, and vanadate is less specific than ouabain for Na⁺,K⁺-ATPase.

It has long been known that ouabain stimulates the contractile activity of the heart and blood vessels. Jackson¹¹ reported many years ago that vanadate reduces the blood volume of various organs by a direct action, and Schroeder¹² reported in 1957 that the urinary excretion of vanadium is increased in some
patients with essential hypertension. These observations stimulated us to conduct a detailed study of the cardiovascular actions of vanadate in anesthetized dogs. We found that vanadate is a potent direct constrictor of blood vessels and that intravenous infusion produces a modest rise in blood pressure (BP) due entirely to a rise in total peripheral resistance. This is associated with antidiuresis due to renal vasoconstriction (rather than diuresis as seen in rats. Certain of the cardiovascular effects occur with calculated increments in plasma vanadium concentration as low as 0.4 μg/ml (levels as high as 2.0 have been reported in humans with renal insufficiency).

These findings, along with the knowledge that human foods vary greatly in vanadium concentration, encouraged us to examine the effect of a long-term dietary supplement of vanadate on the systolic BP of the rat.

Methods

Two experiments were conducted in adult male Sprague-Dawley rats weighing approximately 250 g.

Experiment 1

In the first experiment, 20 rats with documented normotension had the right kidney removed under ether anesthesia. Following recovery, they were housed separately in metabolic cages. Oral intake was normal rat chow and tap water. Water intake, urine output, and urinary sodium output were observed daily for 1 week. The 20 animals were then randomly divided into four equal groups. One group continued to receive normal rat chow, which contains 0.3 parts per million (ppm) vanadium by weight, and tap water. The second group received normal chow and drank a 1% solution of sodium chloride. The third group ate normal rat chow supplemented with sodium orthovanadate equivalent to 100 ppm vanadium by weight and drank tap water. The fourth group ate the same vanadate-supplemented chow and drank the salt solution. Fluid intake, urine output, and sodium output were measured daily and systolic BP and weight weekly for 9 weeks. The animals were then sacrificed and their hearts weighed.

The BP was measured by the tail cuff plethysmographic method and urine sodium concentration by flame photometry. Pressures in the vanadium-supplemented groups were compared to appropriate drinking fluid control groups using Student’s t test.

Experiment 2

The second experiment differed from the first in that the groups were larger, all rats drank tap water, two levels of dietary vanadium were employed, and fluid intake and output were not measured. Twenty-seven adult male Sprague-Dawley rats with documented normotension were uninephrectomized and then observed for 2 weeks while eating normal rat chow and drinking tap water. The animals were then divided into three equal groups. All groups drank tap water.

One group continued to eat normal rat chow and the other two groups ate normal rat chow supplemented with sodium orthovanadate, equivalent to 100 ppm vanadium in the one group and 200 ppm vanadium in the other group. Body weight and tail systolic pressure were measured weekly for 56 weeks. The animals were then sacrificed and heart weight measured. Plasma vanadium concentration was measured by atomic absorption spectroscopy. Tail artery norepinephrine content was measured by high performance liquid chromatography with electrochemical detection and expressed as μg/g tissue wet weight. Pressures, weights, and heart-to-body weight ratios were compared statistically among groups with Duncan’s Multiple Range Test. The relation of blood pressure to plasma vanadium concentration in all three groups was evaluated by linear regression analysis.

Results

Experiment 1

In the first experiment, one animal in the vanadate-saline group died 3 weeks following initiation of the experimental diet. Figure 1 shows body weight as a function of time in the remaining animals. The vanadate-supplemented groups gained weight more slowly than their respective control
DIETARY VANADATE AND HYPERTENSION/STEFFEN ET AL.

FIGURE 2. Effect of dietary vanadate on urinary sodium excretion in uninephrectomized Sprague-Dawley rats drinking tap water or a 1% solution of sodium chloride. Vanadate treatment and numbers of animals in each group are the same as in figure 1. Each symbol represents the mean daily output. Sodium chloride drinking increased sodium output, but vanadate was without effect either while the rats drank the solution of sodium chloride or tap water.

FIGURE 3. Effect of dietary vanadate on systolic blood pressure (BP) of uninephrectomized Sprague-Dawley rats drinking tap water or a 1% solution of sodium chloride. Vanadate treatment and numbers of animals in each group are as in figure 1. The BP was measured with the tail cuff plethysmographic method. From the 3rd to the 9th week, the BP in the vanadate-tap water group was significantly greater than in the tap water group (p < 0.05 to 0.005). Pressure in the vanadate-saline group was not significantly greater than in the saline group at any time.

FIGURE 4. Effect of two levels of dietary vanadate on the weight of uninephrectomized Sprague-Dawley rats drinking tap water. After 6 weeks, each symbol represents the mean of 4 weekly weights.

These data led us to the second experiment in which the long-term effect of a vanadate-supplemented diet was examined in animals drinking only tap water. Two animals in the group receiving the higher vanadate concentration died early in the experiment. The effects on body weight in the remaining animals are shown in Figure 4. The animals receiving the higher concentration of vanadate gained weight more slowly than the control group but, in contrast to our experience in the first experiment, those receiving the lower concentration gained weight at the same rate as the control group. Figure 5 shows that the systolic BP in both vanadate groups exceeded that in the control groups. Water consumption, urine output, and urinary sodium output per 100 g/24 hrs promptly increased in the groups drinking saline relative to those groups drinking tap water, but there was no difference in these variables between the groups eating vanadate supplemented chow relative to those eating normal chow, i.e., vanadate did not produce diuresis or natriuresis. Figure 2 shows the changes in sodium excretion.

Figure 3 shows that the systolic BP was significantly elevated in the vanadate-tap water group relative to the tap water group by the third week and remained elevated until the experiment was terminated. Vanadate did not cause a significant increase in pressure in the animals drinking saline. Heart-to-body-weight ratios were not significantly influenced by vanadate.

Experiment 2

These data led us to the second experiment in which the long-term effect of a vanadate-supplemented diet was examined in animals drinking only tap water. Two animals in the group receiving the higher vanadate concentration died early in the experiment. The effects on body weight in the remaining animals are shown in Figure 4. The animals receiving the higher concentration of vanadate gained weight more slowly than the control group but, in contrast to our experience in the first experiment, those receiving the lower concentration gained weight at the same rate as the control group. Figure 5 shows that the systolic BP in both vanadate groups exceeded that in the control
group by the third to the eighth week of treatment. It also shows that the BP in the group receiving the higher concentration exceeded the BP in the group receiving the lower concentration by the 20th week, and that the BPs in both groups remained elevated for the duration of the experiment. The transient decrease in BP beginning at the 40th week in the group receiving the higher concentration is the result of an inadvertent but serendipitous withdrawal of vanadate treatment for 2 weeks.

At the end of the 56th week, the animals were sacrificed and plasma vanadium measured in most of the animals in each group. Figure 6 shows that vanadium was undetectable in the plasma of the control animals but that it was elevated in those receiving vanadate, more so in those receiving the higher concentration. The figure also shows that there was a significant positive correlation between plasma vanadium concentration and the animals' last recorded BP. Relative to the control group, the heart-to-body-weight ratio was significantly elevated only in the group receiving the higher concentration of vanadate. This increase, however, was due to the lesser body weight. Absolute heart weight was not significantly different. The heart-to-body-weight ratios in the control, 100 ppm, and 200 ppm groups were 2.56 ± 0.16 (SE) × 10⁻², 2.79 ± 0.75 × 10⁻², and 2.75 ± 0.29 × 10⁻² (p = 0.05) respectively.

Endogenous norepinephrine in the tail artery was not significantly different among the three groups.

Discussion

These studies show that dietary ingestion of small amounts of vanadate, a potent naturally occurring Na⁺,K⁺-ATPase inhibitor, slowly increases BP in the uninephrectomized rat drinking tap water. The response is dose-related and sustained for the duration of treatment. The increased pressure is not associated with changes in water intake, urine output or urinary sodium excretion, but correlates positively with plasma vanadium levels, ranging from 0.04 to 0.27 μg/ml. The increased BP was associated with increased heart-to-body-weight ratio but did not appear to occur in a small group of animals drinking a 1% solution of sodium chloride.

Elevated BP in rats has also been observed following intravenous infusion of sodium orthovanadate, which produces much higher serum levels of vanadium (1.2-2.2 μg/ml) and, in preliminary experiments, following subcutaneous implantation of sodium orthovanadate in Silastic, which produces a similar plasma level of vanadium (0.17 μg/ml) (Pamnani M, Huot S, Dalton D: unpublished observation). Thus, vanadate can raise BP in the rat by several routes of administration.

The hemodynamic mechanism of the elevated pressure was not studied. In the anesthetized dog, however, intravenous infusion of vanadate increases pressure entirely by raising total peripheral resistance; cardiac output actually decreases. Resistance to
blood flow through the renal and coronary vascular beds increase. Intrabrachial infusion raises resistance to blood flow through the skin and skeletal muscle vascular beds of the dog forelimb, due mainly to constriction of small vessels but also in part to constriction of large arteries and veins. On intravenous infusion, the decrease in cardiac output is associated with a decrease in left ventricular fiber shortening. Intracoronary infusion with coronary flow held constant constricts the coronary vascular bed but has little effect on left ventricular fiber shortening and dP/dt max. Intravenous administration also increases arterial pressure in the cat, and this is associated with increases in the resistance to flow through the beds supplied by the renal artery, hepatic artery, coeliac artery, and portal vein, but not those supplied by the femoral and carotid arteries. These responses are not influenced by α-blockade, β-blockade, or Ca++ antagonists. Thus, in the dog and cat, intravenous administration of vanadate appears to raise pressure by a direct vasoconstrictor action.

Studies in isolated preparations point in the same direction. The isolated canine saphenous vein responds to vanadate with increased tension and this is not blocked by phentolamine or verapamil, and is only partially blocked by removing calcium from the bathing fluid and adding EGTA (Muldoon S: unpublished observation). Some isolated preparations of cardiac muscle respond to vanadate with a positive inotropic effect, while others do not and, at least in cat papillary muscles, the positive inotropic effect is not blocked by propranolol, phenolamine, or reserpine. The isolated rat heart is among those preparations that respond to vanadate with a positive inotropic effect.

The cellular mechanism of the increased contractile activity in vascular and some cardiac muscles is not clear. Vanadate inhibits Na+,K+-ATPase from a variety of tissues and species, including the rat heart. In our hands, 10⁴ M sodium vanadate inhibits the Na+,K+-ATPase activity of rat heart microsomes by 50% (Clough D: unpublished observation). This suggests that the increased contractile activity is related to Na+,K+-ATPase and Na+–K+ pump inhibition, as in the case of the cardiac glycosides. However, the concentration range of vanadate required to increase cardiac contractility is far less than the concentrations needed to inhibit purified Na+,K+-ATPase. If these lower concentrations are added to the isolated guinea pig heart, neither a positive inotropic effect nor an inhibition of 86Rb uptake (a measure of Na+–K+ pump activity) is seen. They also fail to contract the isolated saphenous vein.

In the isolated dog femoral artery, pretreatment with even higher concentrations of vanadate (10⁴ M) fails to block potassium-induced relaxation (a response thought to be due to stimulation of Na+,K+-ATPase and the Na+–K+ pump), whereas this response is blocked by ouabain (Muldoon S: unpublished observation). These discrepancies may be related to failure of penetration of vanadate to the cytoplasmic surface of the cell membrane where it acts on Na+,K+-ATPase, but some mechanism other than Na+,K+-ATPase inhibition must be involved in the response of the isolated saphenous vein to vanadate. Here 10⁴ M vanadate increases tension but does not reduce ouabain-sensitive 86Rb uptake. These findings do not rule out the possibility that on long-term treatment, as in our studies, the vanadate enters the vascular smooth muscle cell and produces vasoconstriction via inhibition of Na+,K+-ATPase. In fact, 7 weeks after implanting sodium orthovanadate in Silastic subcutaneously in the rat, ouabain-sensitive 86Rb uptake by the tail artery is significantly reduced (Pamnani M, Huot S: unpublished observation).

The elevated pressure with dietary administration of vanadate was associated with an increased heart-to-body weight ratio but not with an increase in absolute heart weight. Vanadium is considered to be essential for the growth of animals. We found that vanadate excess also inhibits growth. Little is known of the possible influence of a prolonged excess of vanadate, as in our study, on cell hyperplasia and cell hypertrophy. Perhaps the absence of an increase in absolute heart weight and the slowed growth rate are both the result of an effect on cell division and/or cell growth.

We also failed to observe diuresis or natriuresis, as has been reported during intravenous administration of vanadate in rats. However, the blood levels of vanadium achieved during intravenous infusion far exceeded those achieved during dietary administration of vanadate in our study (1.2–2.2 vs 0.04–0.27 µg/ml respectively).

We also failed to observe elevation of pressure when the animals drank a solution of 1% sodium chloride. This bears further study, however, since the number of animals in the group was small. A high salt intake tends to raise extracellular sodium concentration and decrease extracellular potassium concentration. These changes minimize the inhibitory effect of vanadate on Na-K pump activity. Perhaps these two observations are related. It is also possible that drinking the salt solution causes a more rapid elimination of vanadium; fluid intake and output clearly increase.

We do not know whether uninephrectomy is a requirement for the BP response in the rat to dietary vanadate. Uninephrectomy was included in the protocol simply because it sensitizes the animals to other hypertensinogenic procedures. Findings reported since the initiation of our study indicate that the normal anesthetized rat responds to vanadate administered intravenously with an increase in mean arterial pressure. Thus, uninephrectomy may not be a requirement in the case of dietary vanadate in the unanesthetized rat.

Acknowledgments
We acknowledge the excellent assistance of Josephine Johnston, Robert Whitmore, Martha McShane, Patricia Prather, John Schwartz, Jr., and James Otto.
References

6. Hudgins PM, Bond GH: (Mg2+ + K+) dependent inhibition of NaK-ATPase due to a contaminant in equine muscle ATP. Biochem Biophys Res Commun 77: 1024, 1977
Effect of prolonged dietary administration of vanadate on blood pressure in the rat.
R P Steffen, M B Pamnani, D L Clough, S J Huot, S M Muldoon and F J Haddy

_Hypertension_. 1981;3:I173
doi: 10.1161/01.HYP.3.3_Pt_2.I173

_Hypertension_ is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1981 American Heart Association, Inc. All rights reserved.
Print ISSN: 0194-911X. Online ISSN: 1524-4563

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://hyper.ahajournals.org/content/3/3_Pt_2/I173

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in _Hypertension_ can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to _Hypertension_ is online at:
http://hyper.ahajournals.org//subscriptions/