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Effect of Intracerebroventricular Vanadate Administration on Salt and Water Intake and Excretion in the Rat[1,2,3]

EMMA CHIARAVIGLIO AND CONSTANZA LOZADA

[4]*Instituto de Investigación Médica, Mercedes y Martin Ferreyra*
Casilla de Correo 389, 5000 Córdoba, Argentina

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CHIARAVIGLIO, E. AND C. LOZADA. *Effect of intracerebroventricular vanadate administration on salt and water intake and excretion in the rat.* PHARMACOL BIOCHEM BEHAV 24(6) 1503–1508, 1986.—The effect of vanadate (VO^{-}_{3}), an "in vitro" inhibitor of Na,K-ATPase activity, on sodium and water intake and excretion of Na-depleted and water deprived rats, was investigated. Injection of sodium orthovanadate (Na3VO4, H_2O 14) 1 μl, 1.0 mM solution, 51 ng/μl free base vanadium (V) into the 3rd brain ventricle (3BV) inhibited by 34% the sodium intake induced by peritoneal dialysis (PD). Urinary water and sodium excretion increased and potassium excretion decreased. The same concentration of vanadate administered by continuous infusion into the 3BV (1 μl/hr, 24 hr, 51 ng/μl, 1.2 μg/24 hr) during 24 hours after PD, decreased sodium intake by 69%. The same rate of infusion through the jugular vein failed to inhibit sodium intake or to increase urinary water and sodium excretion. Injections into lateral hypothalamus were also ineffective. Vanadyl (VO^{+}_{2}), the reduced form of vanadate, did not affect sodium intake. Similar or larger doses of vanadate injected into the 3BV of water deprived rats, did not modify water intake significantly. The present results suggest that the Na-K, active transport system is involved in salt and water balance regulation at the central nervous system level.

Na,K-ATPase Vanadate Diuresis Natriuresis Sodium appetite Water intake

VERNEY [22] showed that hypothalamic sensors sensitive to changes in the osmolality of extracellular fluid (osmoreceptors) were involved in the regulation of antidiuretic hormone (ADH) release. Later on, Andersson [1] observed that hypertonic NaCl, but not hypertonic saccharide solution, injected into the hypothalamus induced drinking, suggesting that juxtaventricular sodium sensors, influenced directly by Na concentration of the cerebrospinal-fluid (CSF), should mediate thrist and ADH release.

The important role of Na concentration of the CSF in the initiation and satiation of Na appetite has been extensively studied by Weisinger *et al.* [24] in the sheep. The authors suggested that the neural system subserving Na appetite is more responsive to changes in Na concentration than changes in osmolality of the CSF. Acute Na depletion by PD in the rat produced dramatic changes in CSF sodium concentration, while infusion of hypertonic CSF into the 3BV decreased Na intake induced by Na depletion [12]. These reports provide evidence for the involvement of sodium sensors mediating Na appetite. Furthermore, the location of the sensors seems to be close to brain ventricular system or at least accessible by intraventricular infusion [12,24].

The hypothesis that cerebral sodium sensors participate in the regulation of fluid and salt balance has stimulated experiments to find out in what manner NaCl ions might initiate sensor activity. This possibility is also supported by effects of Na,K-ATPase inhibitors reported in several species. In the Na deficient sheep intracarotid infusion of ouabain inhibited salt appetite [15] and virtually abolished water intake in response to intracarotid infusion of hypertonic NaCl, angiotensin II or water deprivation [23]. In the rat hypothalamic implant of ouabain suppressed water intake [8,9]. In the goat, intracerebroventricular infusion of ethacrynic acid (another inhibitor of enzymatic Na transport) prevented drinking and increased ADH release [21], suggesting that an active Na transport may be essential for the excitation of cerebral sensors involved in salt and water balance.

Vanadium (V), a trace element, occurs widely in various

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TABLE 1

EFFECT OF A SINGLE INJECTION INTO THE 3RD BRAIN VENTRICLE (3BV) OF VANADATE (1 μl/51 ng V) ON URINE VOLUME AND ON URINARY Na AND K EXCRETION DURING THE 2 HOUR INTAKE TEST GIVEN TO SODIUM DEPLETED RATS AFTER PERITONEAL DIALYSIS

		Urine		Na excretion		K excretion	
	N	ml/100 g	Δ%	μEq/100 g	Δ%	μEq/100 g	Δ%
Control artCSF	10	0.62 ± 0.11		8.4 ± 1.26		437 ± 111.6	
Vanadate mM							
0.5	5	0.98 ± 0.3	58	37.0 ± 9.0*	340	173 ± 41.1	60
1.0	6	0.84 ± 0.2	35	41.6 ± 13.0*	395	331 ± 42.4	24
1.5	6	0.83 ± 0.2	34	88.7 ± 17.2*	956	302 ± 43.0	31

Mean ± SEM.

One way ANOVA, *F=19.4, 11.1 and 8.5 $p<0.01$ for vanadate 0.5, 1.0 and 1.5 mM respectively, when compared with artCSF.

animal tissues including the brain. It is an essential nutrient; its lack impairs growth in rats and chickens. In the body fluids vanadium will be in the form of vanadate, VO^{-}_{3} (+V oxidation state). Vanadate can be reduced in the tissues to +IV oxidation state: vanadyl (VO^{+}_{2}) (see [20] for review). Since it was shown recently that certain vanadium compounds are among the most potent known inhibitors of Na,K-ATPase activity [7,11] several investigators have been concerned with the physiological significance of this finding. As the activity of the Na pump has been postulated to play a role in the etiology of thirst [8, 9, 21, 23] and sodium appetite [15] it was interesting to use vanadate as a "tool" to study water and sodium balance. The purpose of the present experiment was to study whether intracerebral administration of vanadate would affect sodium and water intake and excretion in the rat.

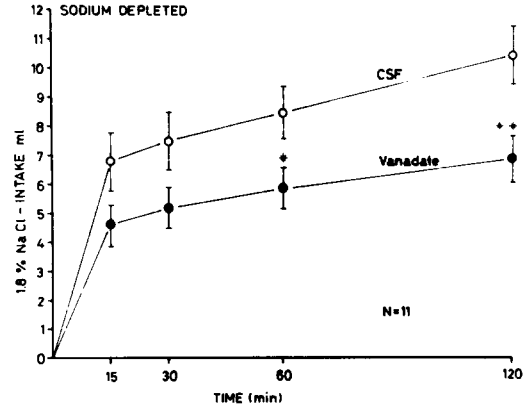

FIG. 1. Effect of the administration of vanadate 1.0 mM solution (1 μl/51 ng/μl vanadium) into the 3rd brain ventricle, on the cummulative intake of 1.8% NaCl solution in rats depleted of sodium. Mean±SEM. *F(1,20)=5.4, $p<0.05$; **F(1,20)=9.2, $p<0.01$.

METHOD

Fifty one male albino rats, weighing 250–350 g at the beginning of the experiment were used. Animals were on a 14 hr light/10 hr dark cycle with a room temperature near 23°C, fed ad lib with a balanced diet (NaCl content: 11 g/kg) and free access to water. Experiments were conducted between 9:00 and 13:00 hr. Before each experiment, the animals were pre-exposed to the testing cage, handled and trained in the assigned experimental procedure.

Sodium orthovanadate (Na_3VO_4, H_2O 14 Fisher Sc. Co., MW 436) stock solution 100 mM was prepared in distilled water, adjusting the solution to pH 7.0. At this pH the vanadium salt will be stable in the form of vanadate (VO^{-}_{3}). Working solutions (0.5, 1.0, 1.5 and 1.75 mM) were freshly prepared every day, dissolving the appropriate amount of stock solution in artificial cerebro spinal fluid CSF (artCSF) pH 7.4 used as vehicle. The pH of the final solution would be 7.2–7.3. The composition of artCSF was similar to endogenous CSF. The concentrations of the various components in mM/liter were as follows: NaCl 130; KCl 2.8; $CaCl_2$ 1.2; $NaCO_3H$ 20; $MgCl_2$ 1.0; $NaPO_4H_2$ 0.5. Isotonic artCSF had an Na concentration of 150 mM, 300 mosm/kg H_2O. A large volume of artCSF was made in advance and stored frozen in 5 ml vials. In order to convert vanadate (VO^{-}_{3}) into the less active vanadyl (VO^{+}_{2}) ascorbic acid was used as reducing agent. Ascorbic acid (Sigma, MW 176) stock solution 100 mM was prepared in distilled water. The working solution 1.0 mM was freshly prepared in CSF. An equimolar solution of vanadate (1.0 mM) plus ascorbic acid (1.0 mM) was made into 1 ml CSF 10 μl of vanadate stock solution plus 10 μl ascorbic acid stock solution.

Surgery

Under tribromoethanol anesthesia (200 mg/kg) the rats were implanted with a stainless steel cannula (15 mm in length and 0.6 mm o.d.) into the 3BV according to the König and Klippel stereotaxic coordinates [18]. The cannula was anchored with a screw and fixed to the skull with dental cement. In a group of five animals the cannula was aimed at the lateral hypothalamic nucleus. A recovery period of 1 week was given before starting the experimental procedure. In another group of animals a jugular catheter (Vinyl tube o.d. 0.96 mm) was inserted under ether anesthesia, 1 day prior to experimentation. After surgery, the animals were caged individually with food and water ad lib.

Sodium depletion by peritoneal dialysis (PD). Animals were treated by an intraperitoneal injection of 5% glucose solution warmed to 37°C, with a volume equivalent to 10% of the rat body weight (b.wt.). One hour later the ascitic fluid

TABLE 2

EFFECT OF CONTINUOUS INFUSION OF 1.0 mM VANADATE SOLUTION (1 μl/HR, 51 ng VANADIUM/HR), ON URINE VOLUME AND ON URINARY Na AND K EXCRETION DURING THE 24 HR FOLLOWING PERITONEAL DIALYSIS IN RATS

		Urine		Na^+ excretion		K^+ excretion	
	N	ml/100 g	Δ%	μEq/100 g	Δ%	μEq/100 g	Δ%
				A. 3BV infusion			
Control Vehicle	4	3.8 ± 0.6		9.0 ± 1.4		2687 ± 298	
Vanadate 1.0 mM	4	10.3 ± 2.8*	171	31.0 ± 9.8*	244	960 ± 299†	64
				B. Intravenous infusion			
Control Vehicle	6	5.4 ± 0.7		4.2 ± 1.8		1920 ± 580	
Vanadate 1.0 mM	6	7.1 ± 1.4	32	6.1 ± 3.9	45	1801 ± 315	6

Mean ± SEM.

One way ANOVA: *F=5.9 and 5.3, $p<0.05$ for urine and Na, respectively, †F=11.3, $p<0.01$ for K, as compared with control group.

A. Infusion into the 3rd brain ventricle (3BV). B. Intravenous infusion.

was recovered by inserting a needle into the peritoneal cavity (Volume injected: 34.0±1.0 ml; recovered: 33.7±1.2 ml) (99.1% N=12). The Na concentration of the removed dialyzate was 94.1±2.1 mM and the amount of Na withdrawn was: 0.843±0.02 mM/100 g rat (mean±SE, N=10). Following PD rats were caged individually without food and with distilled water as the only drink. During the 24 hr post-dialysis period rats drank 4.4±0.6 ml/100 g b.wt., excreted 8.7±1.0 ml/100 g b.wt. of urine and lost 9.3% of b.wt. (from 342.7±10.6 g to 310.8±9.7 g, N=11). Sham dialyzed rats drank 3.7±0.5 ml/100 g b.wt. of water, excreted 3.5±0.8 ml/100 g b.wt. of urine and lost 7.6% of b.wt. (from 235±3 g to 217±3.6 g, N=10).

Infusion procedure. All 3BV pulse injections were made in a volume of 1 μl over a 20 sec period. The injector, 1 mm longer than the guide cannula was left into the cannula for another 20 sec before being pulled out. Each rat was its own control and received a drug injection between two CSF injections. Mean data from the two CSF injections were used as control, unless the mean group values between the first and second test differed by $p<0.05$. Continuous central (3BV) or systemic (jugular vein) infusion were carried out by an osmotic minipump (MP Alzet), implanted under light ether anesthesia under the loose skin of the back delivering 1 μl/hr. The MP was connected through a vinyl catheter to the injector into the guide cannula or into the jugular catheter. After recovery, the same rats were implanted with an MP filled with the vehicle, serving as control. The urine excreted during the test was collected, measured and stored frozen for Na and K determination. The analysis was carried out with a Perkin-Elmer Model 380 atomic absorption spectrophotometer. At the end of the experiments rats were killed with an overdose of ether and brains fixed for verification of cannula placement. Data were analyzed by one way analysis of variance and Student's *t*-test. A *p*-value of 0.05 was accepted as significant.

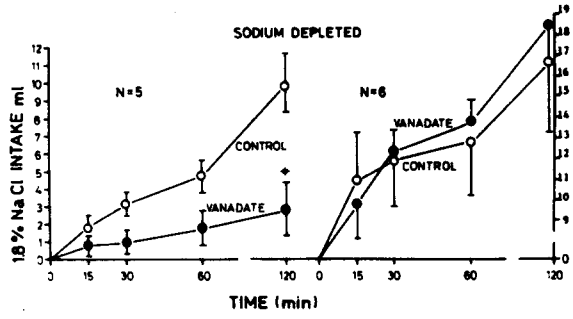

FIG. 2. Effect of continuous infusion of 1.0 mM vanadate solution (1 μl/hr, 51 ng/μl vanadium (V)) on the cummulative intake of 1.8% NaCl solution induced by sodium depletion. Left: infusion into the 3rd brain ventricle. Right: intravenous infusion. Mean±SEM. *F(1,8)=14.7, $p<0.01$.

RESULTS

Effect of Vanadate in Sodium Depleted Rats

Twenty-four hours after PD a group of cannulated rats received a 3BV pulse injection of 1 μl of CSF. Ten minutes later two graduated tubes containing 1.8% NaCl and water respectively, were offered. Cumulative intake volume was recorded. During 2 hours, not injected rats drank 11.0±0.8 ml of 1.8% NaCl solution and 2.8±0.6 ml of water (N=28). Injection of 1 μl of vanadate (1.0 mM 51 ng vanadium free base V) into the 3BV decreased significantly the intake of NaCl solution: 6.9±0.8 ml as compared with the control value of rats injected with CSF: 10.5±1.0 ml, $p<0.01$, N=11 (Fig. 1). Water intake did not change significantly between experimental and control values: 2.0±0.9 ml and 1.6±0.8 ml, respectively. The sodium depletion was repeated in the same animals separated by 2–3 days. Each time, the animals re-

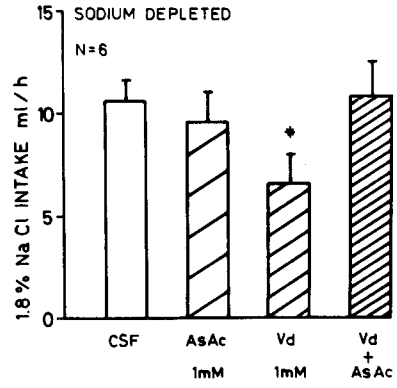

FIG. 3. Sodium chloride intake induced by sodium depletion in rats injected into the 3rd brain ventricle with 1 μl of CSF (vehicle), ascorbic acid (As Ac 1 mM), vanadate (Vd 1 mM) and vanadate plus As Ac. Mean±SEM. *$p<0.01$ as compared with CSF. Student's t-test.

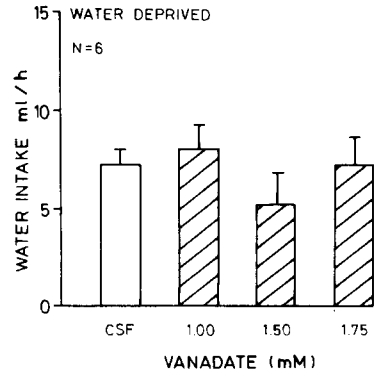

FIG. 4. Effect of the administration of increasing doses of vanadate into the 3rd brain ventricle, on the water intake induced by water deprivation. The effective doses were: 51.0, 76.5 and 89.2 ng/μl of vanadium free base (V). Means±SEM.

ceived 1 μl vanadate solution, 10 min before access to drinking fluids (0.5 mM vanadate (VO^{-}_{3}), 25 ng/μl vanadium (V) N=7, or 1.5 mM vanadate, 76.5 ng/μl N=10) in a random sequence. Intake volume and urine excreted were recorded as described. The same procedure was repeated, this time the animals received a second control injection of CSF. Injection of 0.5 or 1.5 mM vanadate did not affect significantly the 1 hour intake of 1.8% NaCl or water as compared with values following injection of CSF. After vanadate 0.5 mM the intakes were: NaCl 8.0±1.3 ml, water 1.6±0.75 ml. CSF NaCl 7.0±0.1 ml, water 0.3±0.2 ml (N=7). Following vanadate 1.5 mM, NaCl intake was 9.6±1.0 ml, water 1.1±0.5 ml, (N=10). artCSF NaCl 8.5±0.7 ml, water 1.1±0.6 ml (N=10). Vanadate, 1 μl, 1.0 mM injected into the lateral hypothalamic nucleus did not change the 2 hours NaCl intake compared with the control value: 10.1±0.9 ml, 11.5±1.2 ml, N=5, respectively.

Urine and urinary Na excreted during the intake test increased with the three doses of vanadate while K excretion decreased (Table 1).

Effect of Continuous Vanadate Infusion

A group of 5 rats with cannula in the 3BV were infused with distilled water (vehicle) by means of an MP, during 1 hr prior to 24 hr post-PD. During infusion, rats were caged individually without food and with distilled water to drink; urine volume, Na and K excreted during the infusion period were measured. After 24 hr the infusion was stopped by pulling out the injector and the intake of 1.8% NaCl and water was recorded during 2 hr. The same procedure was repeated 2–3 days later in the same rats, this time holding an MP filled with 1.0 mM vanadate solution delivering at a rate of 1 μl/hr 51 ng/μl, 1.2 μg/24 hr free base vanadium (V). Distilled water was used as vehicle in the continuous central infusions, because it was seen in a previous experiment that 24 hr infusion of CSF alone decreased sodium intake. In 6 rats with catheter in the jugular vein an MP was implanted as described above but this time the MP was filled with isotonic saline (0.15 M NaCl) as vehicle, and connected to the intravenous catheter 1 hr prior to PD. Vanadate 1.0 mM was infused at the same rate (1 μl/hr during 24 hr). Infusion stopped and the two fluids' intake was recorded. The central vanadate infused rats drank 2.9±1.2 ml. Compared with the intake of the same rats infused with CSF (9.5±1.3 ml) N=5, the difference was significant $p<0.001$ (Fig. 2). Urinary excretion during the infusion period (10.3±2.8 ml/100 g b.wt.) increased compared with control value (3.8±0.6 ml/100 g b.wt.). When the same amount of vanadate was given by intravenous route, the difference in NaCl ingested by rats infused with vanadate or CSF was not statistically significant: (18.2±2.0 ml and 16.3±3.6 ml respectively, N=6 (Fig. 2). These animals excreted 7.1±1.4 and 5.4±0.7 ml of urine/100 g b.wt. when they received vanadate or CSF, respectively. At difference with the centrally vanadate injected rats, urinary Na excretion was not increased when rats were injected by intravenous route (Table 2).

Effect of Vanadyl Injection

Six rats with cannula in the 3BV were depleted of Na by PD and kept on distilled water with no food for 24 hr. Ten min before giving access to the drinking tubes, they were centrally injected with 1 μl CSF. The NaCl and water ingested for 1 hr were taken as the control intake. The same rats, under the same experimental conditions, received in a random sequence central injections separated from each other by 2–3 days recovery: 1 μl ascorbic acid (1 mM); 1 μl vanadate (VO^{-}_{3}) (1 mM); 1 μl of an equimolar solution containing 1 mM ascorbic acid plus 1 mM vanadate. Ascorbic acid reduce vanadate VO^{-}_{3} to vanadyl (VO^{+}_{2}), a compound which "in vitro" prevents the inhibition of Na-K,ATPase [17].

As can be seen in Fig. 3 sodium intake was not affected by the 3BV injections of vanadate combined with ascorbic acid. The volume of NaCl drunk (10.8±1.6 ml) did not differ from the value observed after injection of ascorbic acid alone (9.6±1.4 ml) or CSF (10.5±0.9 ml). However Na intake was significantly reduced (6.6±1.5 ml) N=6, $p<0.01$ after vanadate (Fig. 3).

Effect of Prior Water Deprivation

Six cannulated rats deprived of water during 24 hr, food available, received a control injection of CSF. Ten minutes

after injection water was given to drink during one hour. The intake was recorded as control. After 2–3 days recovery, the same rats treated as before received one of the following vanadate injections in 1 μl volume, in a random sequence: 1.0 mM, 1.5 mM and 1.75 mM vanadate (51.0, 76.5 and 89.2 ng/μl free base vanadium V). Water intake was recorded. A second control value was obtained injecting 1 μl CSF.

Water intake did not change significantly in rats receiving 1 μl injection of vanadate, compared with the intake observed after injection of CSF (Fig. 4).

DISCUSSION

The present studies show that injection of vanadate into the 3BV caused a decrease in sodium intake and increase in diuresis and natriuresis in rats depleted of sodium by PD. The injection made into the lateral hypothalamic nucleus was ineffective, strengthening the idea that the cerebral receptors involved in the regulation of salt and water homeostasis are in close contact with the CSF, near to the walls of the 3rd ventricle [13–24]. Cantley [11] working with purified enzyme from dog kidneys reported that vanadium is a powerful "in vitro" inhibitor of the Na, K-ATPase; if the "in vivo" action reported here is exerted by inhibition of the enzyme, the results would support the hypothesis that the enzymatic active transport of Na may be essential in the process of excitation and/or transduction of the putative receptor. The evidence that vanadate inhibits Na,K-ATPase activity in the neural tissue was shown in squid axons [6] even though the axon membrane is structurally different from the nerve cell membrane.

The concept that salt appetite may depend on the rate of active sodium transport was first reported by Denton [15] in sheep. Several authors working on rats, goats and sheep added further information, suggesting that the activity of receptors mediating thirst and ADH release would depend on enzymatic cation transport [2–4, 8, 9, 21, 23]. In the present report, Na depleted rats responded to vanadate by increasing diuresis and natriuresis, despite the fact that they were not in salt and water balance [16]. The same renal effect was reported previously in rats with expanded volume, after vanadate administration [3,4].

Central injection of vanadate in dosages that did not affect drinking behavior were able to induce diuresis and natriuresis, suggesting the existence of two separate neural systems subserving behavior and renal function. This is in line with the observation that it might be sodium sensitive neurons responsive to the changes in sodium concentration of the CSF which evoke Na appetite [24]. Besides, there are osmoreceptors located in the supraoptic nucleus and surrounding tissue, subserving vasopressin release [19]. Whether vanadate activates these two different groups of cells has to be demonstrated. The present experiments did not allow us to explain the mechanism by which vanadate exerts its action. Observations on the humoral natriuretic factor suggest that the natriuretic hormone causes natriuresis by inhibiting distal sodium transport, due in part, to suppression of the renal Na,K-ATPase activity (see [10] for review). Research on the atrial natriuretic factor leads the authors [14] to conclude that this factor contains an extremely powerful inhibitor of renal tubular NaCl reabsorption, unrelated to Na,K-ATPase inhibition. In our experiments the same amount of vanadate which produces natriuresis by central injection was ineffective by intravenous route, giving rise to the idea of the existence of a central natriuretic mechanism that remains to be elucidated. Vanadate is involved in an active transport other than Na,K-ATPase like Ca, Mg-ATPases [17]. On the other hand, ventricular injection of vanadate, together with a reducing agent ascorbic acid did not affect sodium appetite (see Fig. 3). This experiment is relevant to favor the idea that the Na-transport enzyme could be linked to receptor activation. It was reported that while vanadate inhibits "in vitro" Na,K-ATPase [7], vanadyl is inactive or a mild inhibitor [17].

Vanadate did not affect water intake induced by water deprivation. Ouabain and ethacrynic acid, both inhibitors of active Na transport, have been reported to inhibit thirst in goats and sheep [21–23]. We cannot explain the lack of effect of vanadate on water intake as compared with ouabain. Ouabain is a highly specific inhibitor of the Na,K-pump, acting at sites accessible from the extracellular medium. Vanadate, on the contrary, acts at sites accessible from the intracellular medium [5]. Whether this difference accounts for the lack of effect on water consumption is unknown. The action of vanadate is potentiated by concentration of K ions at the extracellular surface [7], a fact that should be relevant in animals with high serum and CSF potassium concentration as those treated with PD [12].

The centrally mediated effects of vanadate described here, whether they are related to the cellular sodium pump or not, should be taken into consideration; vanadium is a natural substance present in body tissues and its relationship with water-sodium homeostasis regulation deserves further studies. The nature of the enzymatic mechanism involved in the excitation of putative sensors controlling sodium intake and excretion remains an open question.

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