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EFFECTS OF DIETARY VANADIUM EXPOSURE ON LEVELS OF REGIONAL BRAIN NEUROTRANSMITTERS AND THEIR METABOLITES

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Abstract—Adult male CD-1 mice were treated with various levels of vanadate in drinking water for 30 days. The levels of catecholamine and indoleamine neurotransmitters and their major metabolites were measured in six different brain regions. Vanadium caused a dose-related decrease in norepinephrine (NE) levels in hypothalamus, the region rich in this biogenic amine. Levels of the NE metabolite, vanillylmandelic acid (VMA), correspondingly decreased in the same region. Although hypothalamic dopamine (DA) also showed a significant decline, vanadium had little effect on DA metabolites. Levels of 5-hydroxytryptamine (5-HT) and its metabolite, 5-hydroxyindoleacetic acid (5-HIAA), were not influenced. Levels of DA were not affected in the corpus striatum, where the highest levels of this amine are observed. Effects of vanadium on various biogenic amines and their metabolites were only marginal in other brain regions. Results suggest that vanadium has a selective effect on adrenergic pathways, and effects on other hypothalamic amines appear to be secondary. These observations support the pro-oxidant potential of vanadate ion on catecholamines suggested earlier.

There has been an increased amount of interest in the biochemical and toxicological effects of vanadium [1]. Vanadium has been suggested as an essential trace element; however, no definite role for this ubiquitous material has yet been identified. Its several biological properties include: the inhibition of Na^+, K^+-stimulated ATPase [2] and glucose-6-phosphatase [3], stimulation of adenylcyclase [4], and an insulinomimetic effect [5]. *In vivo*, vanadate is reduced rapidly to a vanadyl form by an NADH-dependent reductase [6]. Specific vanadate-dependent, NADH- and NADPH-stimulated oxygenase systems have been suggested in the microsomal fractions [7, 8].

It is presumed that vanadate has oxidizing properties in mammalian cells and rapidly causes oxidation of glutathione in erythrocytes [9]. A dose-dependent increase in glutathione concentration, however, was noticed in cultured epithelial cells incubated in the presence of orthovanadate [10]. Although neurological disorders have been attributed to vanadium poisoning, relatively little attention has been paid to specific biochemical changes produced by this element. Witkowska and Brzezinski [11] reported that both subacute and chronic administration of the toxic doses of vanadium caused alterations in whole brain levels of norepinephrine (NE), dopamine (DA) and 5-hydroxytryptamine (5-HT). This effect was suggested to be induced by the pro-oxidant potential of vanadate on catecholamines and lipid membranes [12]. Lipid peroxidation was increased in several tissues of rat and mouse, particularly in brain [12]. The *in vitro* uptake and release of NE were both decreased by the addition of metavanadate to cortical and hypothalamic slices [13]; the inhibition was dependent on both time and vanadium concentration.

Vanadium is effectively absorbed after oral administration [14, 15]. The present study investigated the dose-related effect of oral vanadate administration on the regional levels of a variety of neurotransmitters and their metabolites. Levels of the biogenic amines are intimately related to behavioral and neurochemical alterations, and neurological effects are often associated with the toxicologic properties of vanadium [16].

MATERIALS AND METHODS

Adult male CD-1 mice (Charles River, Wilmington, MA), 27 ± 3 g initial weights, were acclimated for 1 week with the surroundings and housed in groups. They were fed commercial rodent chow and drinking water *ad lib.* throughout the duration of study. Feed and water consumption were measured continuously, and animals were weighed once a week. Treatment of the animals (five per group) were started by changing the drinking water with water containing various concentrations of sodium orthovanadate.

Reagent grade sodium orthovanadate (Fisher Scientific, Fairlawn, NJ) was dissolved in deionized water, and the pH was adjusted to 7 to prevent polymerization of vanadate ions. This stock solution was diluted with normal drinking water to provide vanadium levels of 0, 8, 25 and 75 ppm. The levels indicated in this report are those of vanadium, used as orthovanadate. The treated water was changed frequently, and water intake was recorded to estimate the vanadium intake. Based on average water consumption, the estimated vanadium intake corresponded to 0, 1.88, 7.07 and 17.24 mg vanadium

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kg/day. An additional 0.14 mg/kg of vanadium per day was provided by the commercial food (containing 1 ppm vanadium), and this level was added to the dose. The total vanadium intake (food plus water) was rounded to the nearest tenth of a mg.

Following a 30-day treatment, the animals were killed by decapitation and their brains were quickly excised. Each brain was sampled at 0° in the following parts: cerberal hemispheres, cerebellum, hypothalamus, corpus striatum, medulla oblongata and the midbrain [17]. The midbrain comprised all anatomical structures not included in other parts. The groups were sampled in a random order, and all sampling was completed between 10:00 and 11:00 a.m. to avoid any possible diurnal variation in the neurochemicals.

The tissues were immediately placed in preweighted vials containing several volumes (in relation to tissue weight) of ice-cold 0.05 M $HClO_4$ and reweighed. Brain regions were homogenized and centrifuged at 10,000 *g* for 60 min, and the supernatant fraction was passed through a 0.2 μm pore filter by gentle centrifugation. These filtrates were analyzed for major catecholamines and indoleamines, namely dopamine (DA), norepinephrine (NE), serotonin (5-HT), and their principal metabolites, i.e. vanillylmandelic acid (VMA), 3,4-dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA) and 5-hydroxyinodoleacetic acid (5-HIAA), using high-performance liquid chromatography with a multiple electrode electrochemical detector [18]. The analytical system consisted of Bioanalytical Systems (West Lafayette, IN) model LC 150 electrochemical analyzer, model LC 3A amphoteric detector, LC 22A temperature controller and a Biophase ODS column. The column temperature was maintained at 30°. The mobile phase and other conditions were similar to those described earlier [19]. Levels of brain biogenic amines or their metabolites were calculated from the response of analytical standards (Sigma Chemical Co., St. Louis, MO). The detector responses were linear up to 500 ng/ml ($r^2 > 0.99$).

Values in various regions are indicated as mean ± S.E. of five animals per group. The statistical differences were computed by comparing the treatment group to the control group using a *t*-test.

RESULTS

The levels of vanadate used in this study were apparently nontoxic as indicated by the growth rate of animals in different treatment groups. There were no treatment-related changes in food or water intake of any of the groups (data not shown). The food intake provided 0.1 mg/kg/day of vanadium, a quantity much smaller than the lowest dose level for vanadium from water.

The effect of vanadate on selected neurotransmitters was limited to the hypothalamic region. Figure 1 indicates the influence of vanadium on NE and its principal metabolite, VMA, in this region. Both neurochemicals showed a dose-dependent decrease. The level of NE was decreased significantly in all treatment groups. The three vanadate doses caused a decrease of 45, 46 and 48%, respectively,

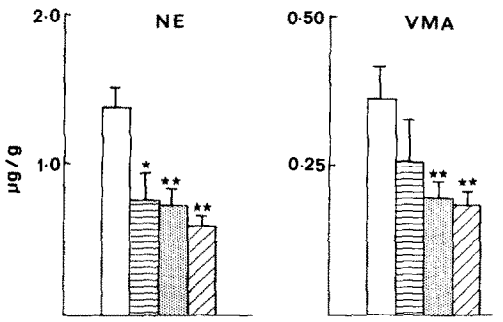

Fig. 1. Concentrations of norepinephrine (NE) and its metabolite, vanillylmandelic acid (VMA), in the hypothalamus of mice exposed to various levels of vanadate in drinking water. The bars (left to right) indicate control group (open bar), vanadium at the low dose (2.0 mg/kg/day, horizontal hatch), vanadium at the medium dose, 7.2 mg/kg/day (shaded), and the highest dose of vanadium (17.4 mg/kg/day, cross-hatched). Each point indicates mean ± S.E.M. of five samples. The statistically significantly differences are indicated: (*) $P < 0.05$, and (**) $P < 0.005$.

for this important neurotransmitter. The levels of the metabolite VMA were correspondingly decreased by 29, 45 and 50% in the three treatment groups respectively.

Figure 2 illustrates the levels of two other neurotransmitters in hypothalamus, DA and 5-HT. The levels of DA were decreased significantly in all treatment groups. The decreases were 30, 39, and 48%, respectively, in the three groups given vanadate in drinking water. The effects on 5-HT levels were not remarkable; the highest treatment level caused a 28% decline, which was not statistically significant at the $P = 0.05$ level.

The levels of other catecholamine and indoleamine metabolites in hypothalamus are shown in Table 1. The dopamine metabolites HVA and DOPAC were decreased nonsignificantly by the vanadate treatment. Treatment by vanadate caused a 31% decrease in the hypothalamic 5-HIAA but the decrease was not statistically significant.

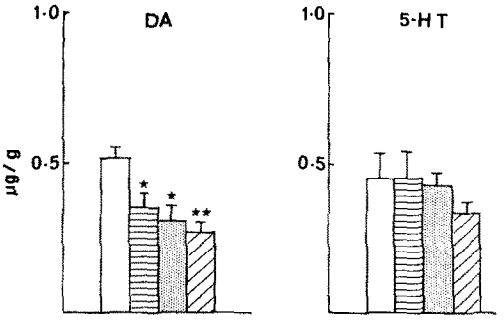

Fig. 2. Levels of dopamine (DA) and serotonin (5-hydroxytryptamine, 5-HT) in the hypothalamic region of mice treated with various amounts of vanadate in drinking water (control, open bar; low, horizontal hatch; medium, shaded; and high dose, cross hatch). Each bar indicates mean ± S.E.M. of five animals, with statistical significance as (*) $P < 0.05$ and (**) $P < 0.005$.

Table 1. Levels of biogenic amine metabolites in hypothalamic region of mice treated with vanadate in drinking water for 30 days

Vanadium (mg/kg/day)	Brain amine or metabolite (μg/g wet tissue)		
	HVA	DOPAC	5-HIAA
0.1	0.079 ± 0.023	0.031 ± 0.020	0.278 ± 0.068
2.0	0.078 ± 0.024	0.038 ± 0.013	0.237 ± 0.055
7.2	0.059 ± 0.005	0.046 ± 0.012	0.244 ± 0.016
17.4	0.052 ± 0.013	0.023 ± 0.010	0.192 ± 0.039

Values are mean ± S.E.M. of five samples in each case.

The levels of NE and its metabolite, VMA, in non-hypothalamic regions are indicated in Table 2. In general, vanadate appeared to decrease the levels of these chemicals in other regions but the effects were only occasionally significant. The concentration of NE decreased in the midbrain, while that of VMA showed a significant decrease in the corpus striatum.

The levels of DA and its various metabolites in the regions other than hypothalamus are given in Table 3. No effect of DA level was apparent in striatum, the region with the highest concentration of this chemical. The dose-dependent decline in DA levels in the cerebral cortex was not statistically significant. Similarly, the levels of 5-HT and its metabolite, 5-HIAA, shown in Table 4, indicated no influence of vanadate treatment in any region of the brain.

DISCUSSION

The results reported here indicate that oral long-term intake of vanadate has potential neurotoxic effects. The neurochemical alterations were observed at vanadium levels which otherwise caused no observable toxic effects. The food and water intake, as well as the growth rate, were not influenced at the doses employed. Similarly, there were no apparent behavioral effects of vanadate treatment.

There is little information available on the regional distribution of various amine transmitters in mouse central nervous system. The values obtained in this study were comparable to those reported earlier in selected brain regions for this species [20]. The data presented here should serve as a baseline for future investigations involving neurochemical evaluations in various brain regions. The relative consistency of values for various amines other than NE and its metabolite, VMA, in non-hypothalamic regions, and their relatively small variability within a group, suggest that these estimates are valid for various brain regions.

It appears that vanadium selectively alters the adrenergic pathway in mammalian brain. The concentrations of both NE and is metabolic product, VMA, were reduced in the hypothalamus, the region with the highest concentrations of these chemicals. There was a general trend of depletion of NE in several other regions, although a statistically significant reduction was seen only in two other regions, i.e. midbrain and striatum. Vanillylmandelic acid levels were altered in the striatum but not in the other non-hypothalamic regions. Although vanadate treatment decreased hypothalamic DA levels, it appears that this was a secondary effect since the levels of DA were not affected in striatum, the brain region containing the highest concentration of this amine. No effect was apparent on the indoleamine (5-HT) or its metabolite in any brain region.

The results on NE levels in this study are in agreement with those reported earlier during subacute or chronic vanadium poisoning [11]. An increase in DA and 5-HT levels in whole-brain homogenates of vanadium-treated rats has been reported [11]. The presence of vanadate *in vitro* prevents the uptake of NE by rat brain cerebral cortex slices [13]. The release of cerebral NE was accelerated at micromolar concentrations of vanadate, whereas an inhibition of such release was reported by the authors at relatively higher levels of metavanadate. It was proposed that vanadate promotes the oxidation of catecholamines, an effect also demonstrated by enhanced formation of adrenochrome and aminochrome *in vitro* [12]. However, the lack of DA depletion in striatum in this study, and also reported for whole brain earlier [11], suggests that the *in vivo* effects of vanadium are selective on NE only. It is not known if the selectivity is dependent on the heterogeneous distribution of vanadium in various brain regions.

Lipid peroxidation in mouse and rat brain is increased by vanadium [12]. Similar effects are produced by other transition metals such as manganese [21]. Manganese has been shown to produce free radicals and cause subsequent oxidation of catecholamines [22], although in our preliminary studies, the toxic doses of this metal increased various neurotransmitter levels in several brain regions [22]. Vanadate is an active oxidant under physiological conditions [8, 23] which may oxidize susceptible biogenic amines, or components of the enzymes involved in synthesis of these amines.

Consideration should be given to the bioavailability of dietary vanadate. Although vanadate is absorbed by the gastrointestinal tract, the estimates of the extent of its absorption vary widely, ranging from 1% in human [24] to 31% in rats [15]. The levels of vanadate in brain equilibrate slowly with those in blood and concentrations in brain up to only half that of blood have been reported 5 days after parenteral vanadate administration [25].

The selectivity of vanadate on the adrenergic sys-

Table 2. Effect of various dietary levels of vanadium on norepinephrine and its metabolite, vanillylmandelic acid, in various brain regions (other than hypothalamus) of mice

Chemical	Vanadium (mg/kg/day)	NE and VMA levels (μg/g wet tissue)				
		Cerebral hemispheres	Cerebellum	Corpus striatum	Midbrain	Medulla oblongata
Norepinephrine	0.1	0.330 ± 0.031	0.186 ± 0.034	0.376 ± 0.032	0.601 ± 0.054	0.578 ± 0.073
	2.0	0.248 ± 0.033	0.159 ± 0.018	0.376 ± 0.073	0.460 ± 0.062	0.588 ± 0.024
	7.2	0.324 ± 0.029	0.194 ± 0.034	0.302 ± 0.031	0.411 ± 0.037*	0.484 ± 0.013
	17.4	0.319 ± 0.022	0.174 ± 0.009	0.260 ± 0.034*	0.416 ± 0.055*	0.470 ± 0.012
Vanillylmandelic acid	0.1	0.235 ± 0.015	0.229 ± 0.023	0.279 ± 0.011	0.239 ± 0.015	0.235 ± 0.019
	2.0	0.185 ± 0.010	0.187 ± 0.011	0.173 ± 0.021†	0.192 ± 0.028	0.227 ± 0.006
	7.2	0.209 ± 0.006	0.193 ± 0.019	0.197 ± 0.022‡	0.184 ± 0.021	0.181 ± 0.009
	17.4	0.201 ± 0.007	0.210 ± 0.007	0.196 ± 0.023‡	0.234 ± 0.019	0.227 ± 0.008

Values are mean ± S.E.M. of five samples each.
* Significantly different from the control group at $P < 0.05$.
† Significantly different from the control group at $P < 0.01$.
‡ Significantly different from the control group at $P < 0.025$.

Table 3. Influence of vanadium intake on the regional brain levels of dopamine and its major metabolites, homovanillic acid and 3,4-dihyroxyphenylacetic acid, after 30-day treatment in mice

Chemical	Vanadium (mg/kg/day)	Levels of DA and metabolites (μg/g wet tissue)				
		Cerebral hemispheres	Cerebellum	Corpus striatum	Midbrain	Medulla oblongata
Dopamine	0.1	1.223 ± 0.181	0.011 ± 0.001	4.969 ± 0.736	0.288 ± 0.029	0.055 ± 0.009
	2.0	1.125 ± 0.193	0.014 ± 0.002	6.113 ± 0.387	0.273 ± 0.025	0.079 ± 0.010
	7.2	0.927 ± 0.168	0.021 ± 0.005	5.623 ± 0.456	0.244 ± 0.037	0.058 ± 0.006
	17.4	0.899 ± 0.067	0.020 ± 0.004	5.795 ± 0.436	0.341 ± 0.044	0.070 ± 0.005
Homovanillic acid	0.1	0.178 ± 0.030	0.007 ± 0.002	0.539 ± 0.074	0.131 ± 0.020	0.035 ± 0.009
	2.0	0.207 ± 0.040	0.004 ± 0.002	0.647 ± 0.031	0.132 ± 0.012	0.051 ± 0.004
	7.2	0.128 ± 0.009	0.007 ± 0.003	0.494 ± 0.032	0.101 ± 0.015	0.032 ± 0.002
	17.4	0.128 ± 0.010	0.019 ± 0.007	0.531 ± 0.056	0.122 ± 0.006	0.044 ± 0.007
3,4-Dihydroxyphenyl acetic acid	0.1	0.149 ± 0.019	0.013 ± 0.004	0.491 ± 0.071	0.092 ± 0.021	0.026 ± 0.008
	2.0	0.197 ± 0.038	0.020 ± 0.007	0.606 ± 0.042	0.119 ± 0.011	0.063 ± 0.008
	7.2	0.125 ± 0.016	0.025 ± 0.009	0.503 ± 0.049	0.105 ± 0.022	0.050 ± 0.011
	17.4	0.145 ± 0.006	0.026 ± 0.006	0.527 ± 0.040	0.113 ± 0.009	0.060 ± 0.004

Values are mean ± S.E.M. of five samples each.

Table 4. Levels of serotonin (5-hydroxytryptamine) and its principal metabolite, 5-hydroxyindoleacetic acid, in various brain regions of mice after a 30-day dietary exposure

Chemical	Vanadium (mg/kg/day)	Levels of 5-HT and 5-HIAA (μg/g wet tissue)			
		Cerebral hemispheres	Cerebellum	Corpus striatum	Midbrain	Medulla oblongata
5-Hydroxytryptamine	0.1	0.608 ± 0.026	0.108 ± 0.024	0.581 ± 0.059	0.886 ± 0.109	0.598 ± 0.083
	2.0	0.561 ± 0.051	0.111 ± 0.011	0.610 ± 0.084	0.964 ± 0.116	0.794 ± 0.043
	7.2	0.587 ± 0.037	0.156 ± 0.043	0.479 ± 0.069	0.791 ± 0.069	0.643 ± 0.045
	17.4	0.628 ± 0.020	0.156 ± 0.024	0.563 ± 0.063	0.899 ± 0.065	0.685 ± 0.036
5-Hydroxyindoleacetic acid	0.1	0.206 ± 0.013	0.062 ± 0.007	0.293 ± 0.027	0.484 ± 0.061	0.309 ± 0.014
	2.0	0.217 ± 0.030	0.066 ± 0.008	0.329 ± 0.039	0.516 ± 0.063	0.369 ± 0.014
	7.2	0.166 ± 0.012	0.074 ± 0.017	0.262 ± 0.017	0.399 ± 0.038	0.282 ± 0.017
	17.4	0.168 ± 0.010	0.070 ± 0.004	0.241 ± 0.031	0.392 ± 0.031	0.262 ± 0.018

Values are mean ± S.E.M. of five samples each.

tem needs further investigation. We are currently studying the regional distribution of vanadium in brain regions, and the turnover rates of various neurotransmitters. The effects of low levels of vanadate exposure on adrenergic receptor will also be evaluated.

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