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Effects of Transition Metals and of Metal-Binding Antihypertensive Agents on Tryptamine Oxidase and Dopa Decarboxylase* (33536)

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Several antihypertensive agents, thought to lower blood pressure by a direct effect on vascular smooth muscle, were also noted to have the capacity to bind transition and related metal ions. Included among the clinically useful antihypertensive agents were the substituted hydrazines, 1-hydrazinophthalazine (Apresoline) and 1,-4-dihydrazinophthalazine (Nepresol) (1), and nitroprusside (2). Clinically less effective agents included the simple inorganic ions, thiocyanate (3) and azide (4). Because of the association of antihypertensive and metal-binding effects, the antihypertensive potency of other compounds known to bind metals tightly was evaluated. This evaluation revealed that experimental hypertension in animals could be corrected by ethylenediaminetetraacetate (5) and certain mercaptans (6). The mechanism by which metal-binding agents act on vascular muscle, if that is actually the basis of their antihypertensive effect, is speculative. Many of the metals which they bind, however, are found in human tissues (7). The possibility that their activity may be related to the binding of a specific metal led us to examine the effect of these substances and of transition and the related subgroup II metal ions on the activity of two crude enzyme systems: tryptamine oxidase and dihydroxyphenylalanine (dopa) decarboxylase, both of which involve the metabolism of vasoactive amines.

Methods. A homogenate containing monoamine oxidase was prepared from fresh livers of guinea pigs, using an equal volume of ice cold 0.067 *M* sodium phosphate buffer (pH 7.4). The homogenate was centrifuged in the cold, the resulting supernatant was dialyzed twice in the cold with stirring for 5 hr against 10 vol of the same phosphate

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buffer, and the dialysate thus obtained was frozen until needed (8). To assay enzymatic activity, oxygen consumption was measured by the Warburg procedure. The reaction flask contained: 1.8 ml of the enzyme preparation, 0.2 ml of the compound to be tested, 0.1 ml of 0.02 *M* potassium cyanide to inhibit side reactions (10), and 0.2 ml of 0.25 *M* tryptamine as substrate introduced from the sidearm to start the reaction.

A dialysate containing dopa decarboxylase was similarly prepared from a homogenate of fresh kidneys of guinea pigs (9). To assay enzymatic activity, carbon dioxide production was measured by the direct method of Dixon and Warburg (10). The reaction flask contained: 1.6 ml of the enzyme preparation, 0.1 ml of the compound to be tested, 0.3 ml of 1 *M* sulfuric acid, initially placed into the center well, to stop the reaction at the termination of the experiment, and 1.0 ml of 0.05 *M* dopa as substrate introduced from the sidearm to start the reaction.

The effects of di-, tri-, and tetravalent ions of the first transition series of metals from titanium through copper (Ti, V, Cr, Mn, Fe, Co, Ni, and Cu) and the effects of divalent ions of the metals of subgroup II (Zn, Cd, and Hg) were tested on both enzyme systems. Except for vanadyl sulfate, chlorides of the metals were used throughout. The metal ions were tested at concentrations (in the reaction mixture) of 10^{-2}, 10^{-3}, and 10^{-4} *M*. Assays were performed in quadruplicate, and two controls were run simultaneously, one without the metal and the other without the enyzme preparation. The following antihypertensive compounds were assayed in the same manner as the metals: 1-hydrazinophthalazine (Apresoline), 1,-4-dihydrazinophthalazine (Nepresol), sodium nitroprusside, sodium thiocyanate, sodium azide, sodium hydrogen ethylenediaminetetraacetate (pH 7.0), and β-mercaptopropionic acid.

Results. Of the metal ions and metal-binding agents tested, those which produced at least 15% change in the rate of tryptamine oxidation by hepatic homogenates are indicated in Fig. 1. Although most metals were without significant effect, large amounts of

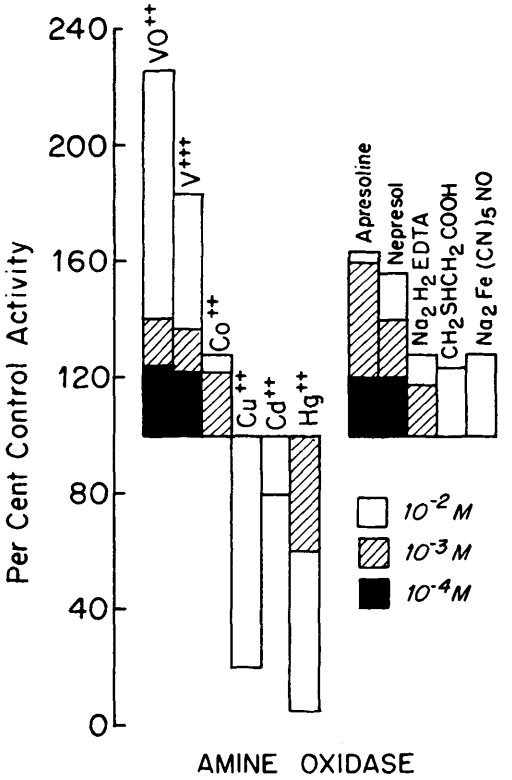

FIG. 1. Activation or inhibition of tryptamine oxidation by dialyzed hepatic homogenate in the presence of three different concentrations of metal ions and metal-binding agents: The open bars indicate changes produced by 10^{-2} *M*, the cross-hatched bars by 10^{-3} *M*, and the solid bars by 10^{-4} *M* concentrations of the test substances in the reaction flasks. All of the antihypertensive drugs tested appear in the figure except thiocyanate and azide. Changes in enzymatic activity or less than 15% are not indicated.

copper and somewhat smaller amounts of mercury were markedly inhibitory, while vanadium and cobalt enhanced tryptamine oxidation. Tetravalent vanadium ion, at a concentration of 10^{-2} *M*, more than doubled enzymatic antivity. Even at concentrations of 10^{-5} and 10^{-6} *M*, this ion consistently increased oxygen uptake, the average amounts being 16 and 10%, respectively. Cobalt was the only other metal which enhanced enzymatic activity; however, at a concentration of 10^{-2} *M*, the increase was only about 30%. Most of the antihypertensive agents tested enchanced tryptamine oxidation and

TABLE I. The Effects of Vanadyl Sulfate and Sodium Cyanide on Oxygen Consumption by Dialyzed Hepatic Homogenate with and without Added Tryptamine.[a]

	0	$VOSO_4$ (10^{-4} *M*)	$VOSO_4$ (10^{-3} *M*)	$VOSO_4$ (10^{-2} *M*)
0	174 (24)	251 (33)	276 (48)	288 (107)
NaCN (10^{-4} *M*)	173 (22)	249 (40)	275 (44)	327 (95)
(10^{-3} *M*)	150 (0)	229 (8)	256 (0)	333 (61)
(10^{-2} *M*)	68 (0)	122 (0)	146 (0)	314 (41)

[a] Note: The tabulated numbers indicate oxygen consumption in μl/hr. Numbers without parentheses are values in the presence of 0.02 *M* tryptamine and parenthetical numbers are values without any added tryptamine.

none inhibited it; moreover, the pair of substituted hydrazines had effects at concentrations of 10^{-4} *M*.

To examine the striking increases in oxygen uptake induced by vanadium, enzymatic activity was studied as a function of the concentration of vanadyl ion in the presence of several concentrations of cyanide ion, added to inhibit other oxidative reactions, and in the presence and absence of the substrate, tryptamine. In the absence of either vanadium or cyanide, the oxygen uptake by hepatic homogenate without substrate was small. Addition of 10^{-3} *M* vanadyl ion doubled this small uptake. Addition of 10^{-3} *M* cyanide abolished it, either without or with vanadium. Tryptamine increased oxygen uptake by the homogenate sevenfold in the vanadium and cyanide free system. Additions of 10^{-3} *M* vanadyl ion further increased uptake by more than 50%. Addition of 10^{-3} *M* cyanide as well had relatively little effect (Table I).

Metal ions and metal-binding agents which produced at least 15% change in the rate of dopa decarboxylation by renal homogenates are indicated in Fig. 2. In this system, unlike the amine oxidase system, all of the metals tested had an effect; all were inhibitory at concentrations of 10^{-2}*M*, but only mercury and cadmium were inhibitory at concentrations of 10^{-4} *M*. The pair of substituted hydrazines were also potent inhibitors, with 10^{-5} *M* Apresoline producing 13% inhibition and 10^{-6} *M* Nepresol 17% inhibition.

Discussion. The survey reported here of the effects of transition and related metal ions on a pair of relatively crude enzyme systems involved in the metabolism of two vasoactive amines is comparable to one recently reported by Rifkin who, while studying the effects of a similar series of metals on ATPases from rat kidney, observed inhibition of monovalent cation stimulated ATPase by vanadium, copper, silver, lead, and all three subgroup II metals (11). We found that vanadium, and to a much lesser extent, cobalt were able to increase tryptamine oxidation by dialyzed homogenate from pig liver and that relatively low concentrations of mercury and cadmium inhibited dopa decarboxylation by dialyzed homogenate from guinea pig kidney. Low concentrations of substituted hydrazines, the only antihypertensive compounds tested that have significant continuing use in treating human hypertension, were also able to stimulate tryptamine oxidation and to depress dopa decarboxylation. This combination of effects would tend to prevent the formation of amines and to increase the destruction of those that were formed. Although catecholamines are not known to be increased in most human hypertension, at least some therapeutic effects of antihypertensive agents, e.g., methyl dopa, have been associated with interference with catecholamine and possibly serotonin metabolism (12, 13).

The two enzyme preparations used here were relatively crude; however, even apparently pure preparations of monoamine oxidase may be complicated by the presence of isoenzymes (14). Despite the heterogeneity of our preparation, the increased oxygen uptake induced by vanadium appeared to involve tryptamine oxidation, since it was observed in the presence of 10^{-3} *M* cyanide which effectively inhibited oxidation in the absence of tryptamine (Table I).

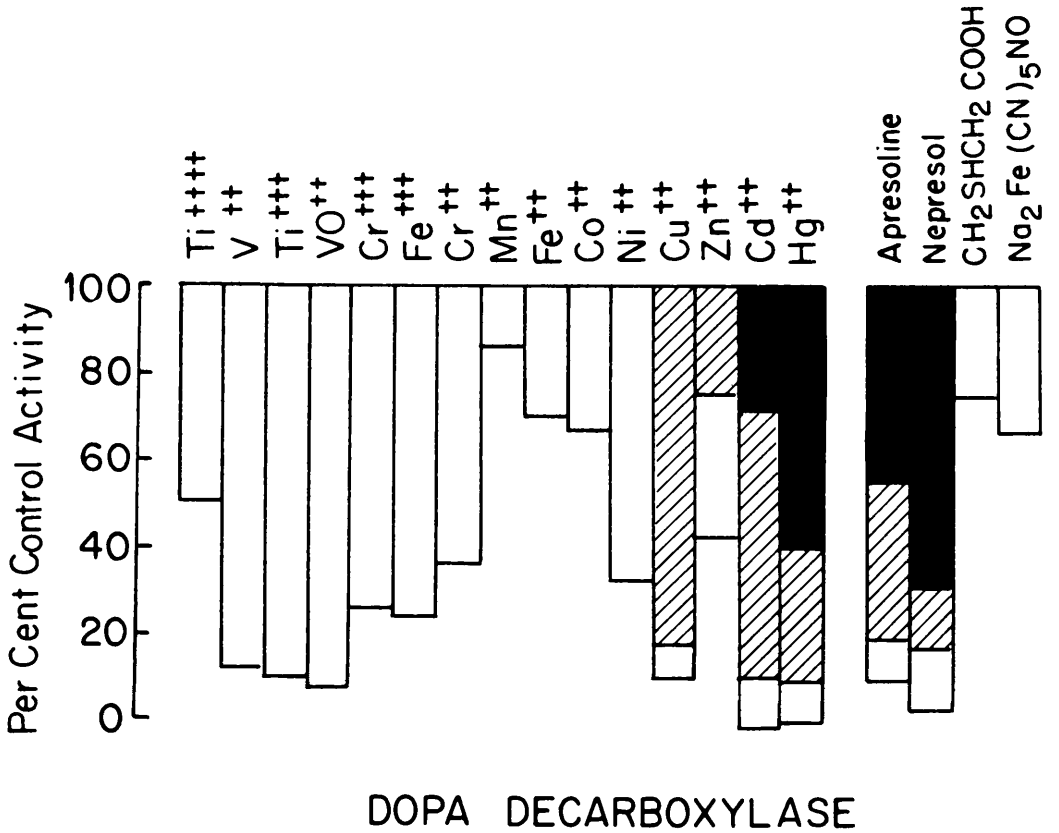

FIG. 2. Inhibition of dopa decarboxylation by dialyzed renal homogenate in the presence of three different concentrations of metal ions and metal-binding agents. The conventions are those of Fig. 1.

There is very little work by others which can be related to the stimulation of amine oxidation by vanadium reported here, although the *in vivo* effect of vanadium on the metabolism of 5-hydroxytryptamine has been studied in a few dogs. One animal received an intravenous injection of 2.5 mg of $NaVO_3$/kg of body weight; during the next hour it excreted 17% more 5-hydroxyindoleacetic acid than during the control period (15). This isolated result is consistent with vanadium-induced stimulation of tryptamine oxidation.

At concentrations of 10^{-4} *M*, only mercury and cadmium inhibited dopa decarboxylase activity significantly. Whereas there is normally little mercury in human tissues, there is a considerable amount of cadmium, and that metal is largely concentrated in the kidneys (16). The very marked predilection of cadmium for a particular tissue sets it apart from almost all other trace elements, and it is truly unique in being about 10 times as concentrated in human kidney as in human liver and in being several times more concentrated in liver than in other organs (16). It accumulates in the human kidney with age up to the fourth decade of life (17), the infant having less than one-hundredth the renal concentration of the adult (16). In addition, renal cadmium varies with the geographic origin of the subject; Negroids from Burundi were observed to have significantly less and Mongoloids from Asia significantly more renal cadmium than Caucasoids from Switzerland, India, and the United States (16). For American adults, the average concentration of cadmium in the kidney approximates 2×10^{-4} *M* (17), twice the minimum concentration which inhibited dopa de-

carboxylation *in vitro*. It has been suggested that cadmium may be related to human hypertension (18). Data consistent with such a relationship include: First, human beings dying of hypertension are reported to have abnormally high concentrations of renal cadmium (18); second, an intra-arterial injection of 20 μg of cadmium is acutely hypertensive for rats (19) and third, 5 parts of cadmium per million parts of food induce chronic hypertension in rats (20).

Summary. The effects of transition and subgroup II metal ions on the activities of crude preparations of tryptamine oxidase and dopa decarboxylase, obtained from fresh guinea pig livers and kidneys, were assayed with the Warburg method. Tryptamine oxidation was accelerated by 10^{-6} *M* vanadyl ion. Cobalt had a similar but less potent effect. Dopa decarboxylation was inhibited by 10^{-4} *M* mercuric or cadminum ions. Moreover, 10^{-4}*M* or lower concentrations of two clinically effective antihypertensive hydrazines also caused acceleration of tryptamine oxidation and inhibition of dopa decarboxylation.

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