Acute Pressor Effects of Intra-Arterial Cadmium and Mercuric Ions In Anesthetized Rats.* (30659)

H. Mitchell Perry, Jr. and Aniece Yunice
Hypertension Division, Department of Internal Medicine, Washington University School of Medicine, and the Medical Service Veterans Administration Hospital, St. Louis, Mo.

Vasoactivity of cadmium and mercury has recently been demonstrated in three ways. Intra-arterial injections of less than 50 μg of either element into anesthetized rats have produced acute but transient increases in diastolic pressure, as described here. Micromolar concentrations of mercuric ion have induced single slow contractions in spirally-cut strips of rabbit aorta, as will be described. Finally, after drinking water containing 5 parts per million of cadmium for several months, rats have become hypertensive(1).

Methods. Normal adult female Sprague-Dawley rats weighing from 200 to 250 g, maintained on tap water and Purina laboratory chow *ad libitum*, were anesthetized with 3.0 mg of intraperitoneal pentobarbital sodium per 100 g of body weight. The femoral artery was exposed and a #22 needle was inserted, with the orifice directed centrally. The needle was tied in place, blocking flow to the leg. Arterial pressure was recorded with a Sanborn electromanometer; 0.9% NaCl with 100 units of heparin per ml was used in the extracorporeal part of the system. A recorded diastolic pressure which remained within a 10 mm range between 70 and 100 mm Hg for 4 minutes was required before an animal was tested. Test materials were dissolved in 0.15 ml of 0.9% NaCl and injected *via* the intra-arterial needle. Immediately after injection, the needle was flushed with 0.15 ml of 0.9% NaCl. The total time of injection and subsequent flushing was 30 seconds, during which arterial pressure was not recorded. Except where explicitly stated, each animal received only one injection of one test material.

Results. Small quantities of cadmium were pressor, but larger amounts were briefly depressor. The typical pressor response induced by a small quantity of intra-arterially injected ion was prompt and considerable but transient; it was not associated with a significant change in pulse rate (Fig. 1). From 40 μg to 10 μg of cadmium ion, as either the chloride or the sulfate, per 100 g of body weight raised the diastolic pressure approximately 25 mm Hg within one minute of the end of the injection. Still smaller amounts of cadmium were usually inert, although occasionally 5 μg was markedly pressor. In contrast from 320 μg (half of the average acutely lethal dose) to 80 μg of the ion, lowered the diastolic pressure approximately 25 mm Hg by the end of the injection (Fig. 2). Eight minutes after injection of any quantity from 320 μg to 10 μg of cadmium ion, the diastolic pressure was from 5 to 15 mm Hg above the control level.

Zinc, which is also a subgroup II element, had no similar pressor effect. Quantities ranging up to 320 μg (a quarter of the average acutely lethal dose) of zinc ion, as the chloride, per 100 g of body weight were inert (Fig. 2). On the other hand, mercuric ion produced greater increases in diastolic pressure than cadmium ion and, unlike the latter, was not depressor in large concentrations. Quantities ranging from 280 μg (a quarter of the average acutely lethal dose) to 70 μg of mercuric ion, as either the chloride or the sulfate, per 100 g of body weight, raised

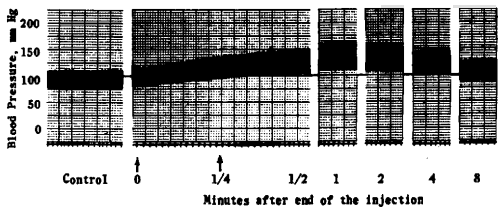

FIG. 1. Typical pressor response to cadmium: Arterial pressure of an anesthetized 202 g rat after intra-arterial injection of 40 μg of Cd^{++} as the chloride. The pressure rose from a control level of 112/76 mm Hg to a maximum of 163/110 mm Hg within 1 minute of the end of the injection.

* This investigation was supported by USPHS Research Grants OH 106 and HE 02577.

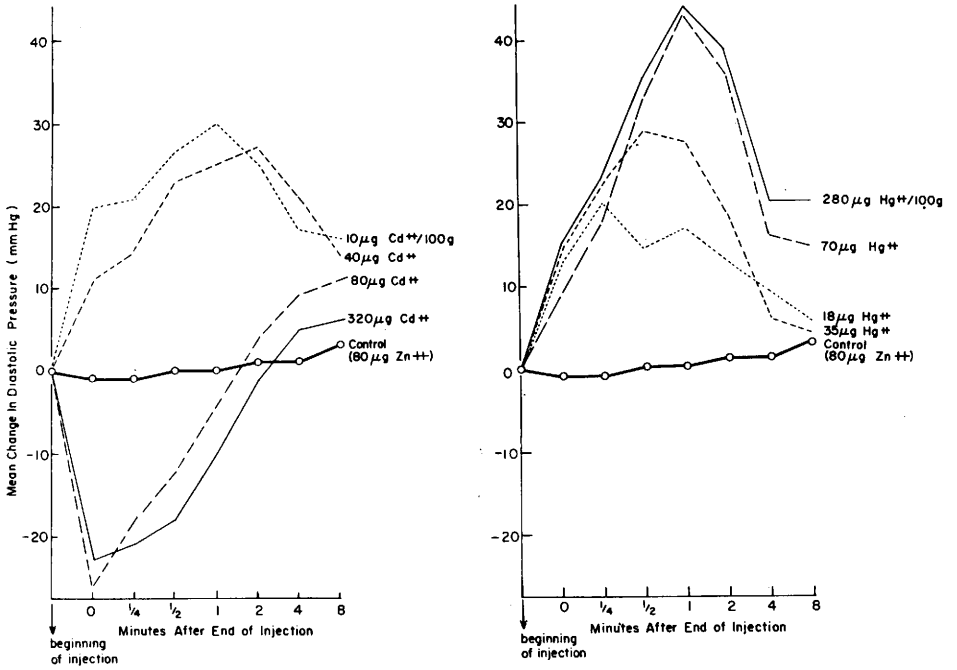

FIG. 2. Mean changes in diastolic pressure for 4 groups of 6 rats each which received $CdCl_2$, for 4 groups of 6 rats which received $HgCl_2$, and for a control group of 6 rats which received $ZnCl_2$. Standard deviations of the mean changes were always less than 15 mm Hg and mean changes from the initial pressure were always significant at the 1% level when they exceeded 10 mm Hg.

the diastolic pressure more than 40 mm Hg (Fig. 2).

No pressor effect was observed for organic cadmium and mercury compounds. Quantities of dimethyl cadmium, dimethyl mercury, and meralluride containing 100 μg of cadmium or mercury per 100 g of body weight had no effect on diastolic pressure. Pressor responses similar to those induced by inorganic cadmium and mercuric ions were induced by other metallic ions, although none was effective in such small quantities as cadmium. Vanadyl, barium, and silver ions were markedly pressor, while lead was inert even in very large quantities (Table I).

The pressor effect of cadmium was reversed both by a metal-binding agent, which presumably bound the cadmium and rendered it inert, and by an antihypertensive agent thought to act directly on vascular musculature. A minute after the end of an injection of 20 μg of cadmium ion per 100 g of body weight, when the pressor effect was near maximal, 5000 μg of $Na_2CaEDTA$ (ethylenediamine tetraacetate) per 100 g of body weight produced an immediate decrease in diastolic pressure to below the control level, with a rapid rebound to a level somewhat above the control level (Fig. 3). $Na_2CaEDTA$ was chosen to permit binding of cadmium ions but prevent binding of sodium, potassium, magnesium, and calcium ions. Under similar conditions 250 μg of hydralazine per 100 g of body weight produced a slower, but

TABLE I. Pressor Effects of Intra-Arterial Injections of Various Metallic Ions.

Metal	10 μg/100 g	100 μg/100 g	1000 μg/100 g
VO^{++}	2	36*	died
Ba^{++}	2	24*	56*
Ag^{+}	3	15*	38*
Cu^{++}	0	5	32*
Pb^{++}	2	6	6

Tabulated values are mean increases in diastolic pressure for 5 groups of 6 rats each, one group for each metal. Each group received 10 μg of metallic ion per 100 g body weight by intra-arterial injection. After 10 min., 100 μg was injected, and 10 min. later an additional 1000 μg was injected. In no case did the diastolic pressure differ significantly ($p < 0.05$) from the baseline when the second injection of metal was given.

* Increase significant at 1% level.

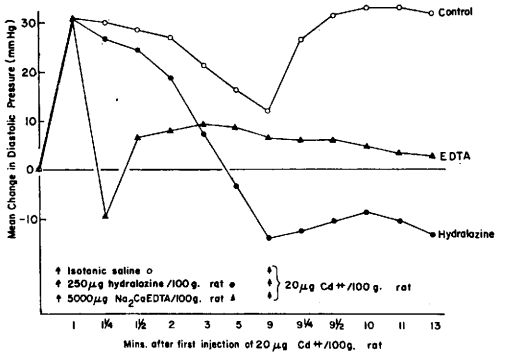

FIG. 3. Mean changes in diastolic pressure for 3 groups of 6 rats each. The 13-minute period considered here began at termination of an injection of 20 μg of Cd^{++}, as the chloride, per 100 g of body weight. One minute later, 0.9% NaCl alone or with either $Na_2CaEDTA$ or hydralazine was injected. After 8 more minutes, all animals received a second injection of $CdCl_2$. Differences in diastolic pressure which exceeded 12 mm Hg were significant at the 1% level. The persistent slight increase in diastolic pressure *above the "zero" baseline* following $Na_2CaEDTA$ and the persistent somewhat greater decrease *below it* following hydralazine are similar to the effects of these two agents when they were given without antecedent cadmium ion.

eventually more profound, fall in diastolic pressure (Fig. 3). Eight minutes after the injection of cadmium ion when partial recovery from the pressor effect had occurred, a second similar dose typically raised the diastolic pressure to its previous maximum level; both $Na_2CaEDTA$ and hydralazine blocked such a pressor response to a second dose of cadmium ion (Fig. 3).

For 3 rats bilateral nephrectomy and adrenalectomy did not interfere, for several hours at least, with the pressor response to 20 μg of cadmium ion per 100 g of body weight. For another 3 rats the pressor response to similar amounts of cadmium ion was not influenced by the production of complete autonomic blockade with 0.5 mg of hexamethonium chloride per 100 g of body weight.

Discussion. Although the pressor effect of cadmium has not previously been stressed, we have reported a pressor response in rats. It followed the intravenous injection of 50 μg of cadmium ion per 100 g of body weight and was more delayed and less marked than the response described here(2). Others have cited *depressor* effects: progressive hypotension in dogs followed weekly intravenous injections of 100 μg of cadmium ion per 100 g of body weight(3), while in cats and rabbits the intravenous administration of half that amount of cadmium(4) produced immediate but transient hypotension. Without citing actual data, Vargas and Cafruny, who worked primarily with organomercurials but used mercuric ions as well, allude to the "prolonged constrictor action of mercury"(5).

The significance of the metal-induced pressor effect described is unknown. The route and intensity of exposure used here are artificial; nonetheless man is exposed to cadmium. As evidence of this, the average American adult accumulates about 4500 μg of cadmium in his kidneys during the first 20 years of his life, and some Japanese accumulate more than 5 times that much(6). The unique concentration of cadmium in the kidney and to a lesser extent in the liver is well documented(6) without being understood. The biologic half-life of cadmium is very long (7); however, normal human beings do excrete some cadmium(8). The chemical form in which cadmium occurs in man has not been defined, although a possible model is suggested by the cadmium-containing metallothionine in equine kidney(9) and the similar cadmium-containing protein in rabbit liver (10). The biologic effects of cadmium in mammalian tissues are unknown, but its ability to uncouple mitochondrial phosphorylation and oxidation is intriguing(11).

Summary. From 10 to 40 μg of intra-arterial cadmium ion per 100 g body weight reproducibly raised the diastolic pressure of anesthetized rats an average of 25 mm Hg. The effect was nearly maximal within one minute and had decreased by half within 8 minutes. Larger doses of cadmium were transiently depressor. The pressor effect was not immediately inhibited by adrenalectomy, nephrectomy, or autonomic blockade. Zinc produced no similar pressor response, but mercury did. The minimum effective dose of mercuric ion was larger, but moderate doses were more pressor, and large doses were not depressor. Vanadyl, silver, barium, and cupric ions also had some pressor activity. The pressor effect of cadmium was immediately reversed by a chelating agent and more slowly

reversed by an anti-hypertensive agent thought to act locally on vascular smooth muscle.

1. Schroeder, H. A., Vinton, W. H., Jr., Am. J. Physiol., 1962, v202, 515.
2. Schroeder, H. A., Perry, H. M., Jr., J. Lab. & Clin. Med., 1955, v46, 416.
3. Fatkhullaev, I., Izv. Akad. Nauk UzSSR, 1959, v6, 25.
4. Dalhamn, T., Friberg, L., Acta Pharmacol. et Toxicol., 1954, v10, 199.
5. Vargas, R., Cafruny, E. J., J. Pharm. & Exp. Therap., 1962, v135, 112.
6. Perry, H. M., Jr., Tipton, I. H., Schroeder, H. A., Steiner, R. L., Cook, M. J., J. Chron. Dis., 1961, v14, 259.
7. Cotzias, G. C., Borg, D. C., Selleck, B., Am. J. Physiol., 1961, v201, 927.
8. Perry, H. M., Jr., Perry, E. F., J. Clin. Invest., 1959, v38, 1452.
9. Kagi, H. R., Vallee, B. L., J. Biol. Chem., 1961, v236, 2435.
10. Piscator, M., Nord, Hyg. T., 1965, v45, 76.
11. Jacobs, E. E., Jacob, M., Sanadi, D. R., Bradley, L. B., J. Biol. Chem., 1956, v223, 147.

Received July 6, 1965. P.S.E.B.M., 1965, v120.

Effect of CCl_4, Dimethylnitrosamine, and Thioacetamide on Hepatic DPNH and TPNH Cytochrome C Reductase.* (30660)

PHILIP D. CLEVELAND AND EDWARD A. SMUCKLER (Introduced by E. C. Alvord)
Department of Pathology, University of Washington, Seattle

Oral administration of carbon tetrachloride (CCl_4) to rats results in an early alteration in the morphology of the endoplasmic reticulum of the liver, a profound decrease in protein synthesis, and an inactivation of glucose 6-phosphatase and aminopyrine demethylating enzyme(1,2,3,10). In contrast the activity of diphosphopyridine nucleotide (DPNH) cytochrome C reductase is increased in CCl_4 poisoning. The following experiments were undertaken to see if a similar increase in activity occurs in poisoning with dimethylnitrosamine (DMNA) and thioacetamide (TIA), both of which have similar effects on liver protein synthesis compared to CCl_4, and to analyze for changes in the triphosphopyridine nucleotide (TPNH) cytochrome C reductase activity(4,5). The experiments were so designed that dosage levels of DMNA and TIA resulted in a depression of amino acid incorporation of similar magnitude to the dose of CCl_4 employed(5).

Materials and methods. Thirty-six male Sprague-Dawley rats weighing 100-250 g were fasted overnight prior to use. CCl_4 was dissolved in an equal amount of mineral oil, and DMNA or TIA in water. The poisons were administered without anesthesia by stomach tube in doses chosen on the basis of preliminary experiments to be equitoxic, producing a similar pattern of histological change and a depression of protein synthesis of about 50% as measured by *in vivo* amino acid incorporation into liver protein. Dosages employed were as follows: 0.25 ml CCl_4/100 g body weight in an equal volume of mineral oil; 20 mg TIA/100 g body weight; 5 mg DMNA/100 g body weight. Controls received mineral oil or water alone. Two hours following administration of CCl_4 or DMNA and 6 hours following administration of TIA the animals were sacrificed by a sharp blow to the head, the body cavity quickly opened, and the liver perfused *via* the aorta with ice-cold 0.85% saline until grossly free of blood. The livers were then isolated, weighed, and minced in 3 volumes of ice-cold 0.15 M sucrose, 0.005 M $MgCl_2$, 0.0025 M KCl, 0.005 M mercaptoethanol, 0.1 M Tris pH 7.3. The minced livers were transferred to homogenizers fitted with teflon pestles, and disintegrated with 8 strokes while the tubes were immersed in an ice bath. The crude homogenates were centrifuged at 0-4°C for 10 minutes at 12,000 *g* in a Servall SS1 centrifuge. The resulting supernatant fluid

* This investigation was supported in part by U.S.P.H.S. Grant HE-03174 and AM-08686.