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Effect of cadmium or magnesium on calcium-dependent central function that reduces blood pressure

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Abstract The effect of intracerebroventricular (i.c.v.) administration of cadmium or magnesium on central calcium-dependent blood pressure regulation was investigated. The systolic blood pressure of spontaneously hypertensive rats (SHR; male, 13 weeks of age) decreased following i.c.v. administration of cadmium chloride (20 nmol/rat), and increased following i.c.v. administration of magnesium chloride (20, 600, and 1200 nmol/rat). The hypotensive effect of cadmium was suppressed by i.c.v. administration of W-7 (a calmodulin antagonist, 30 μg/rat). Taking into consideration these results with our previous reports, it is suggested that cadmium binds to the calcium-binding sites of calmodulin and activates calcium/calmodulin-dependent enzymes in a disorderly manner, whereas magnesium does not. Therefore, cadmium increases dopamine synthesis in the brain via a calmodulin-dependent system, and the resultant increase in dopamine levels inhibits sympathetic nerve activity and reduces blood pressure in SHR.

Key words Blood pressure · Calcium/calmodulin · Cadmium · Dopamine synthesis in brain · Magnesium · Spontaneously hypertensive rats (SHR)

Introduction

We have previously suggested that calcium plays two separate roles in the regulation of blood pressure, through the peripheral and central systems. (1) Serum calcium directly increases blood pressure as a result of its effect on the vasculature. (2) Some of the serum calcium is transported to the brain where it increases dopamine (DA) synthesis through a calmodulin-dependent system, and the resultant increase in DA levels in the brain inhibits sympathetic nerve activity via the D_2 receptor and reduces blood pressure (Sutoo et al. 1987, 1988, 1990b; Sutoo and Akiyama 1997, 1999). The low serum and brain calcium levels seen in spontaneously hypertensive rats (SHR) may result from a decrease in the availability of bone calcium, which results in a decrease in DA synthesis in the brain, with the low levels of DA in the brain producing an increase in blood pressure (Sutoo et al. 1993). Also, the hypertension in SHR was improved by the intracerebroventricular (i.c.v.) administration of calcium chloride (Sutoo and Akiyama 1999) and exercise which elevates serum calcium, brain calcium, and brain DA levels (Akiyama and Sutoo 1999).

Previous behavioral tests were performed in mice (Sutoo et al. 1985, 1986b), and chemical tests were done using ^{1}H-nuclear magnetic resonance spectroscopy (^{1}H-NMR; Sutoo et al. 1986a, 1989a; Akiyama et al. 1990). It was suggested that cadmium binds to all four calcium-binding sites of calmodulin and activates calcium/calmodulin-dependent enzymes in a disorderly manner, whereas magnesium does not bind completely to the calcium-binding sites and therefore does not activate the calcium/calmodulin-dependent enzymes. Hence, we have postulated that when cadmium enters the brain of animals, it reduces blood pressure and affects other physiological functions via a calmodulin-dependent system, whereas magnesium does not affect this pathway. In the present study, the effects of i.c.v. administration of cadmium and magnesium on the blood pressure in SHR was investigated to verify this hypothesis.

Materials and methods

Male SHR, 12 weeks of age, provided by Charles River Japan (Kanagawa, Japan), were housed at room temperature (22 ± 2 °C) in our animal center for 1 week before the commencement of the experiments, under a 12: 12 h light/dark schedule. Food and water were provided ad libitum. For the i.c.v. injection, the rats were anesthetized with pentobarbital sodium and a stainless-steel cannula (22 gauge) was stereotaxically implanted into the lateral

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cerebral ventricle; 1 mm posterior to the bregma, 1.2 mm lateral to the midline, and 4 mm below the surface of the skull. The cannula was anchored to the skull with dental acrylic cement. The experiments were performed in conscious rats 4 days after the implantation of the cannula.

Systolic blood pressure in the conscious, warmed and restrained rats was determined by the tail-cuff method, using a programmed sphygmomanometer (BP-98A; Softron, Tokyo, Japan). The animals were restrained for 5 min using a temperature-controlled warming holder (37 °C), before the systolic blood pressure was measured. Each estimation was the average of three recordings taken at 1 min intervals. Firstly, the SHR were administered an i.c.v. injection of saline, cadmium chloride (20 nmol/rat) or magnesium chloride (20, 600, and 1200 nmol/rat) alone, and the systolic blood pressure was monitored at 15 min intervals from 15 min before to 2 h after the injection. Next, the effects of i.c.v. administration of W-7 [N-(6-aminohexyl)-5-chloro-1-naphthalenesulfonamide] (a calmodulin antagonist, 30 μg/rat) on the systolic blood pressure response elicited by cadmium were analyzed; the SHR were administered an i.c.v. injection of cadmium chloride together with saline or W-7, and the systolic blood pressure was monitored. Cadmium chloride, magnesium chloride, and W-7 were dissolved in saline, and the dosages were determined in a preliminary experiment which was carried out based on previous reports (Sutoo et al. 1986b, 1987). The i.c.v. injection was performed in conscious animals using an injection volume of 10 μl/rat. Animals received humane care in compliance with the 'Guiding principles for the care and use of laboratory animals' formulated by the Japanese Pharmacological Society.

Results

The blood pressure in the SHR did not show any changes following i.c.v. injection of saline (data was not shown). The systolic blood pressure in the SHR decreased gradually following i.c.v. administration of cadmium chloride (20 nmol/rat) until 30 min after the injection, remained at a plateau level until 75 min after the injection, and subsequently returned slowly to the basal level. The blood pressure decreased significantly, by 17–19 mmHg ($P < 0.05$), 30–75 min after the administration of cadmium. However, the administration of magnesium chloride (20 nmol/rat) rapidly increased the systolic blood pressure, by 18 mmHg ($P < 0.05$), immediately after the injection, and this response was maintained until 75 min after the injection (Fig. 1). Even at higher concentrations (600 and 1200 nmol/rat), magnesium chloride induced a similar response, and did not decrease the blood pressure (data not shown). Administration of W-7 (30 μg/rat) suppressed the hypotensive response induced by cadmium chloride in SHR, while administration of W-7 alone did not significantly affect the systolic blood pressure (Fig. 2). The hypotensive response elicited by cadmium, and the suppressant effect of W-7 on this response, were practically identical to the previously reported results of a study using calcium (Sutoo et al. 1987).

Discussion

Different effects of cadmium on blood pressure, i.e., depressor and pressor, have been reported by many

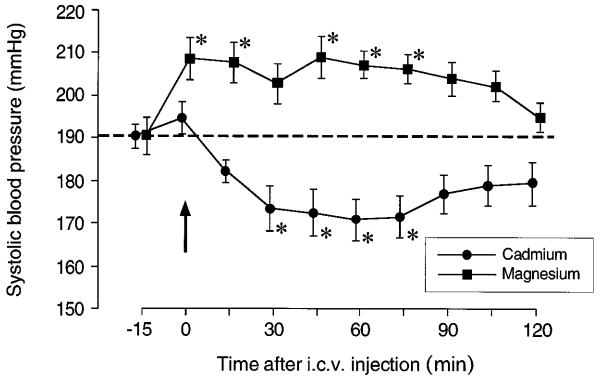

Fig. 1 Effect of intracerebroventricular (i.c.v.) administration of cadmium chloride and magnesium chloride on the systolic blood pressure in spontaneously hypertensive rats (SHR). Cadmium chloride (20 nmol/rat) or magnesium chloride (20 nmol/rat) was injected i.c.v. at 0 min (*arrow*). Results are expressed as mean ± SEM of 10 rats. The *dotted line* indicates the preinjection level. $*P < 0.05$ compared with the preinjection level by Dunnett's *t*-test

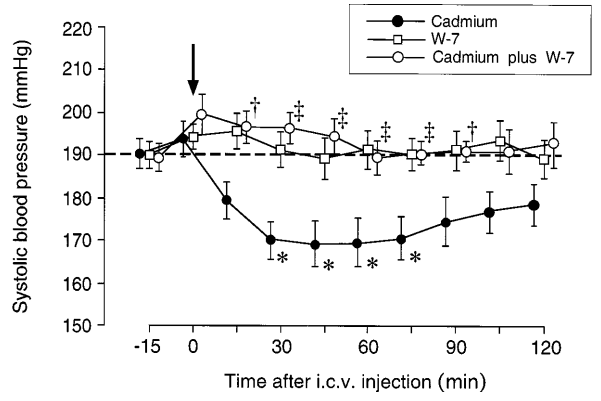

Fig. 2 Effect of i.c.v. administration of W-7 on the systolic blood pressure response elicited by cadmium chloride administration in SHR. The animals were administered an i.c.v. injection of cadmium chloride (20 nmol/rat), W-7 (30 μg/rat), or a mixture of both cadmium chloride and W-7, at time 0 (*arrow*). Results are expressed as mean ± SEM of 10 rats. The *dotted line* indicates the preinjection level. $*P < 0.05$ compared with the preinjection level by Dunnett's *t*-test. $\dagger P < 0.05$, $\ddagger P < 0.01$ compared with the cadmium-treated group, during the same period by Newman-Keuls *t*-test

laboratories carrying out studies under different experimental conditions (Sutoo and Akiyama 1997). Thus, cadmium may be considered to affect blood pressure through several separate pathways or mechanisms. The following mechanisms for the increase in blood pressure following cadmium administration have been proposed based on the results of animal experiments in several laboratories: sodium retention (Perry et al. 1971), increase in plasma-renin levels (Perry and Erlanger 1973), and stimulation of adrenal catecholamine release (Hart and Borowitz 1974). However, the mechanism by which cadmium reduces blood pressure has not yet been clarified.

In previous investigations, our laboratory examined the role of calcium in brain functions. Studies have

demonstrated that calcium activates tyrosine hydroxylase in the brain through a calmodulin-dependent system, resulting in elevation of the levels of DA which regulates various functions of the brain (Sutoo et al. 1985, 1989b). This concept was developed in an attempt to elucidate the mechanism by which calcium reduces blood pressure; it was demonstrated that the i.c.v. injection of calcium chloride depressed the mean arterial pressures in conscious Wistar rats, as well as the i.c.v. injection of DA, and that the effect of calcium was abolished by W-7-methyltyrosine (MPT, an inhibitor of tyrosine hydroxylase) and hexamethonium (a ganglion blocker; see Sutoo et al. 1987; Sutoo and Akiyama 1991). These findings suggested that calcium activates calmodulin-dependent DA-synthesis in the brain, and the resultant increase in DA levels in the brain decreases blood pressure through peripheral sympathetic inhibition.

In SHR, the serum levels of calcium, and the levels of DA, tyrosine hydroxylase, and calmodulin in the neostriatum were lower than those in normotensive Wistar Kyoto rats (the parent strain of SHR; see Akiyama et al. 1992; Sutoo et al. 1993). Also, the hypertension in SHR was rectified by i.c.v. administration of calcium chloride; this effect of calcium was inhibited by MPT, hexamethonium and eticlopride (dopamine D_2 receptor antagonist; Kusano et al. 1993; Sutoo and Akiyama 1999). These findings suggested that the decreased serum calcium levels seen in SHR may result in lower brain calcium levels and reduced calcium/calmodulin-dependent DA synthesis in the brain, and that the low levels of tyrosine hydroxylase and calmodulin synergistically reduce DA synthesis. The resultant reduction in brain dopaminergic function in SHR increases the blood pressure.

In the present study, the systolic blood pressure in SHR was reduced following i.c.v. administration of cadmium chloride, as well as that following the administration of calcium chloride. Also, the ability of cadmium to reduce blood pressure in SHR was inhibited by the administration of W-7. W-7 is the most suitable compound because of its very high affinity for calmodulin and low toxicity to other cellular constituents (Asano and Hidaka 1984). The findings suggest that the reduction in blood pressure noted following cadmium administration in SHR occurs via a calmodulin-dependent system in the brain. However, magnesium chloride does not activate this system and thus does not reduce the systolic blood pressure in SHR.

^{1}H-NMR spectra indicated that Cd^{2+} can bind to all four calcium-binding sites and induces considerable conformational change in calmodulin, as does Ca^{2+}, and thus confuses activation or modulation of calmodulin-dependent functions. However, Mg^{2+}-induced conformational change is different from Ca^{2+}- or Cd^{2+}-induced conformational changes, and thus Mg^{2+} does not confuse calmodulin-dependent functions (Sutoo et al. 1986a, 1989a; Sutoo 1994). Also, W-7 was found to affect the spectra of Ca^{2+}- and Cd^{2+}-saturated calmodulin in a very similar manner; namely, it inhibits calmodulin activation by Cd^{2+}, as it does calmodulin activation by Ca^{2+} (Akiyama and Sutoo 1988; Akiyama et al. 1990).

Dopamine synthesis in the brain is enhanced by a calcium/calmodulin-dependent system, and the calmodulin-dependent system is activated by both cadmium as well as calcium. Thus, the DA levels in the neostriatum and nucleus accumbens in mice were found to be increased at 30 min after i.c.v. administration of cadmium chloride (25 nmol/mouse), compared to those in a control group administered saline, and this ability of cadmium was inhibited by W-7 (Sutoo et al. 1990a). Suzuki et al. (1985) also reported that phosphodiesterase activity was enhanced by cadmium via calmodulin. It is suggested, considering these findings and the ability of the brain DA to reduce blood pressure, that cadmium binds to calmodulin and activates calmodulin-dependent enzymes, while magnesium does not, and that cadmium leads to increased DA synthesis in the brain and the resultant increase in DA levels reduces blood pressure in SHR.

High levels of calcium and magnesium ions exist in organisms, and the two metal ions are clearly distinguished by calmodulin. Cadmium is naturally present in extremely small quantities in the ecosystem, and calmodulin does not have the intrinsic ability to distinguish between calcium and cadmium. Also, calcium channels mediate cadmium uptake in a variety of tissues (Hinkle et al. 1987; Shibuya and Douglas 1992; Friedman and Gesek 1994; Hinkle and Osborne 1994). When cadmium is released into the ecosystem, it is accidentally taken up by organisms and activates calmodulin-dependent functions. We believe that this is one of the mechanisms of cadmium poisoning. Incidentally, it was confirmed in the present study that the abnormal physiology induced by i.c.v. administration of cadmium, such as hypersensitivity and hyperkinesia, were abolished together with a hypotensive response by the i.c.v. administration of W-7. The hypertensive effect of magnesium administration in SHR should also be investigated from the viewpoint of another mechanism or pathway.

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