Opposite Effects of Calcium and Magnesium on the Central Blood Pressure Regulation in the Spontaneously Hypertensive Rats

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ABSTRACT—The effects of intracerebroventricular administration of calcium or magnesium on the blood pressure regulation in the brain were investigated. The systolic blood pressure in spontaneously hypertensive rats (male, 13-week-old) was decreased by calcium chloride (100 μg/rat) and increased by magnesium chloride (20, 100 or 500 μg/rat). The depressor response induced by calcium was inhibited by magnesium chloride in a dose-dependent manner. Combining these results with those previously reported, it is suggested that magnesium inhibits the ability of calcium to reduce blood pressure through calmodulin- and dopamine-dependent functions in the brain.

Keywords: Blood pressure in spontaneously hypertensive rats, Calcium/calmodulin-dependent dopamine synthesis, Magnesium

We have previously suggested that calcium ions have two separate roles in the regulation of blood pressure through the peripheral and central systems. Serum calcium directly increases blood pressure as a result of its effect on the vasculature. However, part of the serum calcium is transported to the brain, causing an increase in dopamine (DA) synthesis through a calmodulin-dependent system; subsequently, the increased level of DA inhibits sympathetic nerve activity via the D₂ receptor and reduces blood pressure (1, 2). The low serum calcium level observed in spontaneously hypertensive rats (SHR) may result from a decrease in DA synthesis in the brain, and therefore, a low level of brain DA, resulting in an increase in blood pressure (3). Moreover, the hypertension in SHR was alleviated following the intracerebroventricular (i.c.v.) administration of calcium chloride (2) or DA (4).

On the other hand, it was observed by ¹H-nuclear magnetic resonance (¹H-NMR) spectroscopy that magnesium does not activate calcium-free calmodulin, and inhibits interface sites of calmodulin, as a calmodulin antagonist does (5). Also, DA release was inhibited by magnesium (6–9). Therefore, we have postulated that magnesium inhibits the ability of calcium to reduce blood pressure through calmodulin- and DA-dependent functions in the brain. In the present study, the effect of i.c.v. administration of magnesium on the calcium-dependent blood pressure response in SHR was investigated to verify this hypothesis.

Male SHR (12-week-old) were provided by Charles River Japan (Kanagawa). They were housed at room temperature (22 ± 2°C) in our animal center for 1 week before the commencement of experiments, under a 12-h light/dark condition. Food and water were provided ad libitum. For i.c.v. injection, the rats were anesthetized with pentobarbital sodium (50 mg/kg, i.p.) and then a stainless steel cannula (22 gauge) was stereotaxically implanted into the lateral cerebral ventricle, at the coordinates 1 mm posterior to the bregma, 1.2 mm lateral from the midline and 4 mm below the surface of the skull. The cannula was anchored to the skull with dental acrylic cement. The location of the ventricular cannula was confirmed by staining the lateral ventricle with bromophenol blue solution injected after the experiments. The experiments were performed in conscious rats 4 days after the cannula implantation. Before the experiment, the systolic blood pressure was measured in all rats for 3 days to allow them to adapt to the measuring environment and to confirm the baseline values. The animals received humane care in compliance with the Guiding Principles for the Care and Use of Laboratory Animals formulated by The Japanese Pharmacological Society.

The systolic blood pressure in conscious, warmed and restrained rats was determined by the tail-cuff method using a programmed sphygmomanometer (BP-98A; Softron, Tokyo). The animals were restrained for 5 min using a temperature-controlled warming holder (37°C) designed
for rats, and the systolic blood pressure was measured. Each estimation was the average of three recordings taken at 1-min intervals. First, SHR were administered an i.c.v. injection of saline, calcium chloride (100 μg/rat), or magnesium chloride (20, 100 and 500 μg/rat) alone, and the systolic blood pressure was monitored at 15-min intervals from 15 min before to 2 h after i.c.v. injection. Next, the effect of i.c.v. administration of magnesium chloride on the systolic blood pressure response elicited by calcium chloride administration was investigated. SHR were administered an i.c.v. injection of a mixture solution of calcium chloride (100 μg/rat) and magnesium chloride (20, 100 or 500 μg/rat), and the systolic blood pressure was monitored. All chemicals were dissolved in saline, and their dosages were determined in a preliminary experiment carried out based on previous reports (2, 10, 11). The drugs were administered to conscious animals at 10 μl/rat. Data for the same period for comparisons of multiple groups were analyzed using ANOVA and Dunnett’s test.

The mean ± S.E.M. of systolic blood pressure in SHR was 190 ± 3 mmHg. The systolic blood pressure in SHR decreased following i.c.v. administration of calcium chloride, with the minimum value being reached 30 min after the administration, and thereafter returned slowly to the baseline value. The blood pressure decreased significantly 15 – 90 min after the administration of calcium chloride, by 14 – 38 mmHg (P<0.05 – 0.01), compared with the saline-treated group (Fig. 1). In the SHR group pretreated with magnesium chloride, the reverse response was observed, i.e., the systolic blood pressure increased at any dose. However, the vasopressor activity of magnesium did not depend on dose. The blood pressure increased significantly from immediately after to 90 min after the administration of magnesium chloride, by 13 – 31 mmHg (P<0.05 – 0.01), compared with the saline-treated group (Fig. 1). The ability of calcium chloride to reduce blood pressure was inhibited by the i.c.v. administration of magnesium chloride in a dose-dependent manner. The hypotensive response induced by calcium chloride was completely abolished by the administration of 500 μg/rat of magnesium chloride (Fig. 2).

In the present study, the systolic blood pressure in SHR decreased following i.c.v. administration of calcium chloride, as well as following the administration of DA, while it increased following i.c.v. administration of magnesium chloride. Moreover, the ability of calcium to reduce blood pressure was significantly inhibited by i.c.v. administration of magnesium chloride. On the other hand, in a previous biochemical test, brain DA level significantly increased following i.c.v. administration of magnesium chloride, as well as i.c.v. administration of calcium chloride (10). These findings suggest that DA synthesis in the brain is enhanced by not only calcium but also magnesium and that the function of increased DA is inactivated by magnesium. These findings are supported by previous reports. Tyrosine hydroxylase was activated and DA synthesis was enhanced by magnesium via cAMP-dependent protein kinase (12, 13). Dopamine release stimulated by N-methyl-D-aspartate (6, 7), nitric oxide (8) or potassium chloride (8) was inhibited by magnesium. Moreover, inhibition of DA release by adenosine was mediated by the adenosine A1 receptor, and this inhibition was enhanced by magnesium (9). It must be investigated in the future whether or not the sites of DA action were significantly activated or inhibited by magnesium.

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**Fig. 1.** Effect of calcium chloride or magnesium chloride administration on the systolic blood pressure in SHR. Saline, calcium chloride (100 μg/rat) or magnesium chloride (20, 100 or 500 μg/rat) was injected intracerebroventricularly (i.c.v.) at 0 min. Results are expressed as the mean ± S.E.M. The dotted line indicates the pre-injection level. *P<0.05, **P<0.01, compared with saline-treated group, during the same period by Dunnett’s test.

**Fig. 2.** Effect of magnesium chloride administration on the systolic blood pressure response elicited by calcium chloride in SHR. The animals were administered an intracerebroventricular (i.c.v.) injection of calcium chloride (100 μg/rat) or a mixture of both calcium chloride (100 μg/rat) and magnesium chloride (20, 100 or 500 μg/rat), at time 0. Results are expressed as the mean ± S.E.M. The dotted line indicates the preinjection level. *P<0.05, **P<0.01, compared with calcium-treated group, during the same period by Dunnett’s test.
synthesis activated by calcium, DA synthesis activated by magnesium and DA release inhibited by magnesium are the same. In the present study, although the vasopressor activity of magnesium did not depend on dose, magnesium inhibited the ability of calcium to reduce blood pressure in a dose-dependent manner. We think that the direct action of magnesium on blood pressure and indirect action of magnesium on calcium-dependent response of blood pressure may result from different mechanisms or pathways.

It is generally accepted that calmodulin regulation of many intracellular calcium-dependent functions involves a conformational change in calmodulin that accompanies calcium binding. NMR spectroscopic analysis is a prominent method used in studies on conformational changes of proteins. Previous $^1$H-NMR studies suggested that magnesium does not bind completely to the calcium-binding sites and therefore, does not activate the calmodulin-dependent enzymes (14). Also, magnesium affects interface sites of calmodulin with target enzymes, as a calmodulin antagonist does (5).

When ethanol is injected i.p. at a dose of 4.5 g/kg, mice lose their righting reflex and lapse into a state resembling sleep for approximately 1 h. The duration of ethanol-induced sleeplike state (ethanol-induced sleeping time) was prolonged following i.c.v. administration of DA. The administration of calcium chloride also prolonged the ethanol-induced sleeping time. The ability of calcium to prolong ethanol-induced sleeping time was abolished by calmodulin antagonists (W-7 and trifluoperazine) or an inhibitor of tyrosine hydroxylase ($\alpha$-methyltyrosine). However, magnesium chloride did not affect the duration of the ethanol-induced sleeping time (15). These results suggest that calcium activates tyrosine hydroxylase in the brain via a calmodulin-dependent pathway, leading to increased levels of DA in the brain that may subsequently prolong the ethanol-induced sleeping time. However, magnesium chloride does not significantly activate calmodulin. Moreover, i.c.v. administration of magnesium chloride abolished the ability of calcium to prolong the ethanol-induced sleeping time (10) as well as the calcium-dependent response of blood pressure which is shown in the present study.

In conclusion, it is suggested that calcium causes an increase in brain DA synthesis via a calmodulin-dependent system, and the subsequently increased level of DA inhibits sympathetic nerve activity and reduces blood pressure in SHR. Magnesium inhibits DA release or calmodulin activity and thus reduces the calcium-dependent response of blood pressure in the brain.

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