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Hypertension in epileptic mice: a phenomenon related to reduction of Ca^{2+}-dependent catecholamine synthesis in the brain

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Abstract

The possible complication of hypertension and epilepsy was investigated through the response in epileptic El mice. The systolic blood pressure in El mice (male, 8 weeks of age) and that in normal ddY mice (the parent strain of El mice) were compared by a tail-cuff method, using a programmed sphygmomanometer. The systolic blood pressure in El mice (120.5 ± 5.6 mm Hg) was 28% ($P < 0.01$) higher than that in ddY mice (93.9 ± 5.3 mm Hg). The higher systolic blood pressure in El mice was lowered by the acute intracerebroventricular administration of $CaCl_2$ (10 μmol/kg, 30 min before measurement) or dopamine (30 nmol/mouse, 15 min before measurement), and was also improved by the chronic oral supplementation with 1.2% calcium (Ca^{2+}) solution. Combining these results with those in our previous reports, where it is stated that lowering of Ca^{2+}-calmodulin-dependent catecholamine synthesis increases the susceptibility to epileptic convulsions, we suggest that the increase in susceptibility to epileptic convulsion and occurrence of hypertension in El mice may be linked and that the two diseases may be associated.

Keywords: Blood pressure; Ca^{2+}-calmodulin; Dopamine; Epilepsy; (El mouse)

1. Introduction

We have investigated the role of Ca^{2+} in biogenic amine synthesis in the brain and have suggested that Ca^{2+} activates biogenic amine-synthesizing enzymes, i.e., tyrosine hydroxylase and tryptophan hydroxylase, through an intracerebral calmodulin-dependent function (Sutoo et al., 1985, 1989). These studies have been applied individually to elucidate the expression mechanisms of hypertension and epileptic convulsions. Conclusions derived from animal experiments on these diseases have many points in common.

In experiments on blood pressure, we found that intracerebroventricular (i.c.v.) administration of $CaCl_2$ and oral intake of $CaCl_2$ produced a hypotensive response in conscious rats. The intracerebroventricular administration of dopamine similarly produced a hypotensive response. This effect of Ca^{2+} was abolished by the administration of a calmodulin antagonist (*N*-(6-aminohexyl)-5-chloro-1-naphthalenesulfonamide (W-7)) or an inhibitor of catecholamine-synthesizing enzyme (α-methyltyrosine). Therefore, it was suggested that Ca^{2+} activates the catecholamine-synthesizing enzyme through the Ca^{2+}-calmodulin-dependent system in the brain, and the subsequent increase in the amount of catecholamine (especially dopamine) produces the depressor response of blood pressure (Sutoo et al., 1987a, 1990a). Also, a part of the serum Ca^{2+} is transported to the brain, leading to the reduction of blood pressure (Sutoo et al., 1988). This idea was developed to elucidate the mechanism of hypertension in spontaneously hypertensive rats (SHR), and we arrived at the conclusion that the decrease of the serum Ca^{2+} level in SHR causes a decrease in dopamine synthesis in the brain and the subsequent lowering of brain dopamine level produces an increase in blood pressure (Sutoo et al., 1993).

In experiments on epilepsy, on the other hand, we found that a significant reduction in the serum Ca^{2+} level of epileptic El mice, which may be due to a decrease in the availability of bone Ca^{2+}, produces a lowering of brain calmodulin activity and the subsequent decrease in catecholamine (especially dopamine) synthesis. Lowering of the brain dopamine level increased the susceptibility to epileptic convulsions and

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induced abnormal behavior in El mice, and these situations were improved by i.c.v. administration of $CaCl_2$ or dopamine (Sutoo et al., 1992a,b).

Thus, the conclusions obtained from animal experiments regarding each disease have many points of similarity, and these findings lead to the possibility that hypertensive complications may be observed in El mice. This study was carried out to confirm this hypothesis, and the blood pressure in El mice was compared with that in normal mice.

2. Materials and methods

2.1. Animals

El mice were received from Dr. Masaro Nakagawa of the National Institute of Health (Tokyo, Japan) and were subsequently bred at our animal center. The El mouse is an inbred strain with convulsive tendencies and was derived from the ddY mouse (Imaizumi et al., 1959). Violent tonic-clonic convulsions occur in adult El mice as a result of postural stimulation and can be produced by tossing the animals several times in the air. Electroencephalograms (EEG) were recorded for El mice during convulsive seizures, and the spike discharges were found to indicate epileptic convulsions (Suzuki, 1976). El mice were kept in groups of 8–11 in stainless steel cages at 22 ± 2° C and were exposed to a 12-h light/dark schedule. All animals were fed general mouse feed (MF; Oriental Yeast, Tokyo, Japan). Male mice were tested when they reached 8 weeks of age. Although non-stimulated El mice did not exhibit seizures, stimulated El mice readily exhibited seizures even on slight stimulation such as handling for blood pressure measurement. In this study, therefore, non-stimulated El mice, i.e., those which had not exhibited seizures, were used. The disorders of serum Ca^{2+} and brain dopamine in non-stimulated El mice were already confirmed to be similar to those seen during the resting stage of stimulated El mice.

Male ddY mice, the parent strain of El mice, were used as controls. Three-week-old ddY mice were provided by Doken (Ibaraki, Japan). They were housed under identical conditions for 5 weeks and then analyzed.

All animals received humane care in compliance with the 'Guiding Principles for the Care and Use of Laboratory Animals' formulated by the Japanese Pharmacological Society and the protocol was approved by the International Symposium on Epileptic El Mouse (Tokyo, 1992).

2.2. Measurement of blood pressure

Systolic blood pressure was determined in conscious, warmed and restrained mice by a tail-cuff method, using a programmed sphygmomanometer (MK-1000, Muromachi Kikai Co., Tokyo, Japan). The animals were restrained for 5 min, using a plastic holder designed for mice, in a temperature-controlled warming chamber (35° C). Restrained mice were transferred speedily to another chamber maintained at 33° C for measurement. Each estimation was the average of three recordings taken at 1-min intervals.

Systolic blood pressures in El and ddY mice were compared among groups of animals pretreated with test substances. The groups were as follows: (a) ddY mice injected i.c.v. with physiological saline 15 min before measurement of blood pressure; (b) El mice under conditions identical to those in (a); (c) ddY mice injected i.c.v. with $CaCl_2$ saline solution (10 μmol/kg) 30 min before measurement; (d) El mice under conditions identical to those in (c); (e) El mice injected i.c.v. with dopamine (30 nmol/mouse) 15 min before measurement. The intracerebroventricular administration of $CaCl_2$ and dopamine, whose dosages and conditions were based on our previous studies (Sutoo et al., 1985, 1992a), was performed in conscious mice, using an injection volume of 5 μl/mouse. The point of injection was 1 mm lateral to bregma and the injection needle (27 gauge) was pushed through the bone cover to the 3 mm depth. Data were analyzed using the analysis of variance (ANOVA) and Newman-Keuls t-test for subsequent comparisons of mouse groups.

As demonstrated in the Results section, the systolic blood pressure in El mice was higher than that in ddY mice. Also, the systolic blood pressure in El mice after acute $CaCl_2$ administration was equivalent to that in ddY mice without $CaCl_2$ treatment. In the next step of the investigation, therefore, the effect of continuous oral intake of Ca^{2+} on the systolic blood pressure in El mice was investigated. Two mouse groups were given separately and freely either 1.2% Ca^{2+} solution or tap water as drinking water for 16 days, and systolic blood pressure was monitored every day during this period. Ca^{2+} concentration was determined in a pilot test. The data were analyzed using Student's t-test.

3. Results

The systolic blood pressure in ddY mice was 93.9 ± 5.3 mm Hg (11) (mean ± S.E.M. (n)), and that in El mice was 120.5 ± 5.6 mm Hg (10) (Fig. 1). This level in El mice was significantly higher by 28% ($P < 0.01$) than that in ddY mice. Also, the higher systolic blood pressure in El mice was significantly lowered by the acute i.c.v. administration of $CaCl_2$ (10 μmol/kg) or dopamine (30 nmol/mouse) to equal that in ddY mice pretreated with saline, i.e., 96.0 ± 3.8 mm Hg or 93.3 ± 2.9 mm Hg, respectively. The systolic blood pressure in ddY mice was decreased slightly by the i.c.v. pretreatment with $CaCl_2$, but it was not significantly changed.

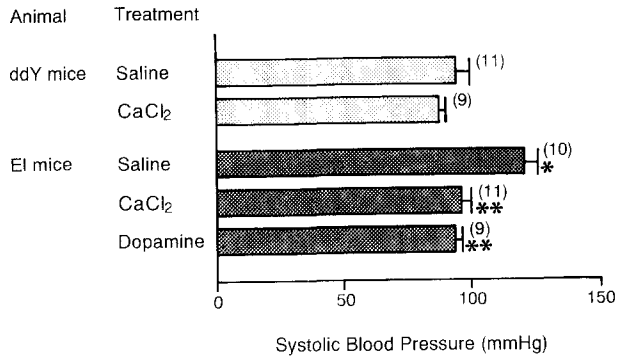

Fig. 1. Systolic blood pressures in El and ddY mice, and the effect of intracerebroventricular administration of Ca^{2+} or dopamine on them. $CaCl_2$ (10 μmol/kg) or dopamine (30 nmol/mouse) was injected intracerebroventricularly at 30 min or 15 min, respectively, before the measurement of blood pressure. All intracerebroventricular injection volumes were 5 μl. Each value represents the mean ± S.E.M. (n). $^{*}P < 0.01$ compared with saline-treated ddY mice group, using Newman-Keuls t-test: $^{**}P < 0.01$ compared with saline-treated El mice group, using Newman-Keuls t-test.

As shown in Fig. 2, the higher blood pressure in El mice was also lowered by the chronic oral supplementation with Ca^{2+}. Namely, the systolic blood pressure in El mice was lowered gradually with supplementation by 1.2% Ca^{2+} solution, and changed significantly from the 8th day. At the 16th day, the systolic blood pressure (101.5 ± 3.8 mm Hg (9)) was lower by approximately 14% ($P < 0.01$) than that in the control El mice group given tap water (117.6 ± 2.4 mm Hg (9)). The body weight of El mice given Ca^{2+} solution was less by approximately 5% than that in the control El mice at the 16th day; however, it was not significantly changed.

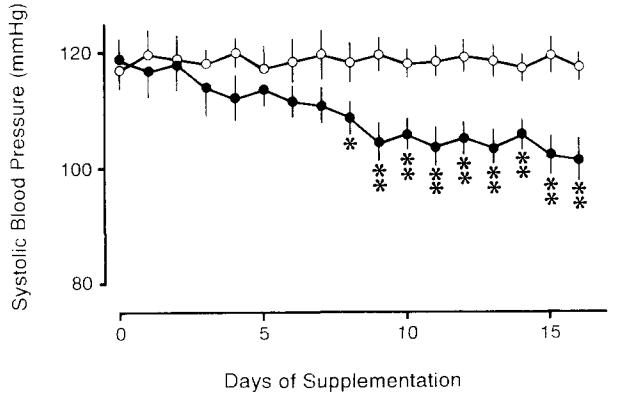

Fig. 2. The effect of continuous oral intake of $CaCl_2$ solution on systolic blood pressure in El mice. Whereas the control mouse group (○) was given tap water, the experimental mouse group (●) was given 1.2% Ca^{2+} solution to drink ad libitum, and the systolic blood pressure was measured every day. The vertical lines indicate the S.E.M. of 9 experiments. $^{*}P < 0.05$, $^{**}P < 0.01$ compared with control group, using Student's t-test.

4. Discussion

The role of Ca^{2+} in blood pressure regulation has been investigated in many fields, from molecular biological studies to clinical studies. Numerous investigators have suggested that Ca^{2+} is required for the contraction of vascular smooth muscles, and that i.v. administration of Ca^{2+} elevates blood pressure. In other words, greater amounts of serum Ca^{2+} flow into smooth muscle cells and induce contractions. Subsequently, this increases vascular resistance and produces a pressor response. On the contrary, it was reported that hypertensive subjects had a significantly lower concentration of serum Ca^{2+} (McCarron, 1982a; Folsom et al., 1986), and a lower daily Ca^{2+} ingestion than normotensive controls (McCarron et al., 1982; Harlan et al., 1984). Also, experimental studies have demonstrated that a significantly higher blood pressure is seen in Ca^{2+}-deprived rats (Belizan et al., 1981), and a blood-pressure-lowering response to an increase in Ca^{2+} intake is shown in normotensive and spontaneously hypertensive rats (Ayachi, 1979; McCarron, 1982b). Furthermore, results of several human intervention studies suggest that an increase in Ca^{2+} intake is associated with a decrease in blood pressure in hypertensive and normotensive men and women (Belizan et al., 1983; Grobbee and Hofman, 1986; Strazzullo et al., 1986; Lyle et al., 1987).

The complex interrelationship between these two apparently opposite effects of Ca^{2+} on the regulation of blood pressure is a very important problem. We have investigated the role of Ca^{2+} in the regulation of blood pressure and have reported the following (Sutoo et al., 1987a, 1988, 1990a, 1993): (1) Intracerebroventricular administration of $CaCl_2$ activates dopamine synthesis in the brain through a calmodulin-dependent system, and an increase in the dopamine level produces a long-lasting hypotensive response. (2) A part of serum Ca^{2+} is transported to the brain, which reduces blood pressure through a calmodulin-dependent dopamine-synthesizing system. Both intraperitoneally injected and ingested, Ca^{2+} also reduces blood pressure via this pathway. (3) Serum Ca^{2+} levels and neostriatal dopamine levels in SHR were lower than those in normotensive Wistar Kyoto rats (WKY, the parent strain of SHR); therefore hypertension in SHR may be due to the reduction of Ca^{2+}-calmodulin-dependent dopamine synthesis in the brain. In the light of these findings, we suggested that Ca^{2+} activates tyrosine hydroxylase in a calmodulin-dependent manner, and activated neostriatal dopaminergic neurons influence brain regions which have regulated blood pressure, such as the brainstem or the hypothalamus, and continuously inhibit peripheral sympathetic nerves, to produce hypotension.

On the other hand, we reported that the serum

Ca^{2+} level in El mice was significantly lower than that in ddY mice (Sutoo et al., 1987b). The lower serum Ca^{2+} level in El mice may be due to a decrease in the availability of bone Ca^{2+}. The dopamine levels in the neostriatum and nucleus accumbens septi of El mice were also 11–15% lower than those in ddY mice; however, after i.c.v. administration of $CaCl_2$, these levels were equivalent to those in ddY mice without $CaCl_2$ treatment (Sutoo et al., 1990b). We suggested, therefore, that central nervous function, especially dopamine synthesis, could be altered by the lower Ca^{2+} levels in El mice. This finding is in agreement with a previous finding that the dopamine level was abnormally low during the resting stage of El mice who had exhibited seizures (Hiramatsu, 1981). A similar result was also reported for the spontaneously epileptic rat (Hara et al., 1993). We thought that the reduction of Ca^{2+}-dependent dopamine synthesis in the brain might increase the susceptibility to epileptic convulsions and induce abnormal behavior. From conclusions derived from two serial animal experiments on blood pressure and epilepsy, we arrived at the hypothesis that blood pressure in El mice may be higher than that in normal mice.

In the present study, in fact, the systolic blood pressure in El mice was approximately 28% higher than that in ddY mice. The higher systolic blood pressure in El mice was lowered by the acute i.c.v. administration of $CaCl_2$ or dopamine and by the chronic oral administration of $CaCl_2$. The result may be due to two possible causes: (a) blood pressure in El mice is originally high compared with that in ddY mice; or (b) blood pressure in El mice is increased during handling for blood pressure measurement while that in ddY mice is not affected during handling. Either way, however, it may be safely said that hypertension in El mice is related to Ca^{2+} and dopamine in the brain. In cause (a), the same mechanism as that of hypertension in SHR which was suggested in a previous report (Sutoo et al., 1993) was thought to be involved. Namely, the decrease of the serum Ca^{2+} level in El mice causes a decrease in Ca^{2+}-calmodulin-dependent dopamine synthesis in the brain and a subsequent lowering of brain dopamine level produces an increase in blood pressure. On the other hand, an increase in blood pressure during epileptic seizures has been reported from clinical studies in adults and children (White et al., 1961; Perlman and Volpe, 1983; Greisen, 1986). In the present study, convulsions were not seen among El mice used in our experiments during, or just before, blood pressure measurement. However, it is undeniable that stress in El mice during handling for blood pressure measurement was greater than in ddY mice because hypersensitivity has been observed in the general behavior of El mice. Also, it was observed previously that stress changes the Ca^{2+}-calmodulin-dependent dopamine synthesis in the brain, which successively induces behavioral changes (Sutoo et al., 1991). In cause (b), therefore, blood pressure in El mice may be increased through a change of the Ca^{2+}-dependent dopamine synthesis by stress induced during handling.

Although i.c.v. injection of $CaCl_2$ (30 μmol/kg) produced the depressor response of mean arterial pressure in the conscious normotensive Wistar rat in a previous study (Sutoo et al., 1987a), $CaCl_2$ (10 μmol/kg) did not significantly change the systolic blood pressure in the conscious normotensive ddY mice in this study. We think that the difference in results between the two experiments originates from differences in conditions such as animals used, $CaCl_2$ concentration and method of blood pressure measurement. In the present study, non-stimulated El mice were used because stimulated El mice exhibited seizures during handling for blood pressure measurement. However, the disorders of serum Ca^{2+} and brain dopamine in non-stimulated El mice have already been confirmed to be similar to those during the resting stage of stimulated El mice. Also, it was observed that behavioral disorders other than seizures (such as irritability, restlessness, fearfulness, vocalization, reactivity, touch response, and startle response) in non-stimulated El mice are more severe than those in stimulated El mice (Sutoo et al., 1992a). We must note that, at the concentration used, Ca^{2+} exerts non-specific membrane effects. In addition, the effect of serotonin on the hypertension in El mice should also be confirmed in future because abnormal behavior in El mice was eliminated by i.c.v. injection of serotonin as well as Ca^{2+} or dopamine (Sutoo et al., 1992a).

We suggested previously that the Ca^{2+}-dependent catecholamine synthesis disorder of the brain in El mice increased susceptibility to epileptic convulsions and induced various abnormal behaviors. Based on the results in the present study, we also suggest that the disorder of this pathway produces hypertension in El mice. Trompeter et al. (1982) reported, based on case records of 45 children with hypertension in their hospital, that convulsions were the most common neurological complication associated with severe arterial hypertension, occurring in 42 (92%) of the 45 children. Although the relationship between both diseases should be investigated more thoroughly from various aspects, e.g., whether the same structures of the central nervous system are responsible for the two diseases, we think that the conclusion derived from this study could elucidate one of the possible mechanisms of the neurological complications of hypertension.

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