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Intracerebroventricular Infusion of Hypertonic Saline Raises Blood Pressure by Activating the Sympathetic Nervous System

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ABSTRACT

Salt and angiotensins are known to elevate blood pressure whether alone or in combination and pressor effects of these substances are closely related to changes in activity of the sympathetic nervous system. The present study was performed to examine the effect of chemical sympathectomy with guanethidine on blood pressure (BP) change and humoral factors in rats which received continuous and concomitant infusion of intracerebroventricular (i.c.v.) hypertonic NaCl with intravenous (i.v.) angiotensin II (Ang II), both at subpressor doses for 7 days. Male rats were divided into 3 groups which received the following infusions using an osmotic minipump at a rate of 1 μl/min: Group 1 (n = 11), 0.15 M NaCl i.c.v. and Ang II (5.4 pmol/kg/min) i.v.; Group 2 (n = 9), 0.8 M NaCl i.c.v. and Ang II i.v.; Group 3 (n = 5), 0.8 M NaCl i.c.v. and Ang II i.v. with daily i.p. injection of guanethidine (40 mg/kg). Significant increase in b.p. was observed only in Group 2 (from 103 ± 3 mmHg to 132 ± 5 mmHg on day 7, $p < 0.001$). Addition of i.p. guanethidine to i.c.v. infusion of 0.8 M NaCl and the subpressor dose of Ang II completely prevented increase in the b.p., suggesting that the integrity of the sympathetic nervous system is necessary for the development of b.p. elevation in response to i.c.v. hypertonic NaCl plus i.v. Ang II.

INTRODUCTION

There is a huge body of evidence that salt (sodium chloride, NaCl) is mandatory for the maintenance of circulation, but hypertension develops due to excessive salt intake in susceptible animals and humans or in the presence of other precipitating factors. In addition to the volume retaining effect through the kidney, NaCl has pressor effects through the central nervous system. In both situations, NaCl has interactions with many pressor and depressor systems and among them, interactions with the renin–angiotensin system and the sympathetic nervous system have been widely investigated. In this paper, we will briefly review mechanisms of pressor effect by intracerebroventricular (i.c.v.) administration of NaCl and focus on a series of our own studies on the interaction of centrally loaded NaCl with intravenously given angiotensin II (Ang II) and the important role played by the sympathetic nervous system.

EFFECT OF ACUTE I.C.V. ADMINISTRATION OF HYPERTONIC NaCl ON B.P. AND HORMONAL SYSTEMS

Andersson et al. (1972) were the first to report that infusion of hypertonic NaCl into the third ventricle of the goat resulted in a significant increase in b.p. associated with natriuresis and antidiuresis with a reduction of plasma renin activity (PRA). This study was the first to relate NaCl with b.p. control through the central nervous system and i.c.v. administration of hypertonic NaCl had been regarded as a pressor stimulus. The pressor response to acute i.c.v. administration of hypertonic NaCl was reported to be due to combined activation of various pressor systems as listed in Table 1.

Their findings were followed by Chiu and Sawyer (1974) who showed that pressor and natriuretic responses to administration of hypertonic NaCl into the third ventricle were abolished by the pretreatment with guanethidine in the cat. This result showed the importance of the sympathetic nervous system in the blood pressure elevation in response to acute solitary i.c.v. infusion of hypertonic NaCl. Takishita and Ferrario (1983) reported that the pressor response to i.c.v. NaCl administration was mainly due to sympathetic hyperactivity. Bealer (1983) showed that the hypothalamus was one of the brain areas which were involved in the pressor response because the destruction of the anterior wall of the third ventricle attenuated this pressor response. Kawano and Ferrario (1984) demonstrated that i.c.v. NaCl administration increased norepine-

TABLE 1

Studies on the pressor response to acute i.c.v. administration of hypertonic NaCl in various animals.

1972	Andersson et al.	I.c.v. hypertonic NaCl infusion causes b.p. elevation and antidiuresis (gout).
1974	Chiu and Sawyer	Pressor and natriuretic responses to administration of hypertonic NaCl into the 3rd ventricle are abolished by the pretreatment with guanethidine (cat).
1983	Takishita and Ferrario	Pressor response to i.c.v. NaCl administration is mainly due to sympathetic hyperactivity and is not derived from suppression of the brain renin angiotensin system (dog).
1983	Bealer	Destruction of the anteroventral wall of the third ventricle in the hypothalamus attenuates pressor and natriuretic responses to i.c.v. NaCl administration (rat).
1984	Kawano and Ferrario	I.c.v. NaCl administration increases norepinephrine
1984	Sasaki et al.	B.p. elevation in response to hypertonic NaCl administration into the cisterna magna is mainly due to the vasopressin system (rat).
1989	Katahira et al.	Pressor response to i.c.v. (lateral ventricle) administration of hypertonic NaCl is associated with a reduction of norepinephrine release in the ventrolateral medulla (rat).
1991	Katahira et al.	Pressor response to i.c.v. administration of hypertonic NaCl causes releases of certain amino acid neurotransmitters in the ventrolateral medulla (rat).

I.c.v.: intracerebroventricular; b.p.: blood pressure; VLM: the ventrolateral medulla.

phrine, vasopressin and cortisol and reduces plasma renin activity and renal sympathetic activity. On the other hand, Sasaki et al., (1984) showed that b.p. elevation in response to hypertonic NaCl into the cisterna magna was mainly due to the activation of the vasopressin system. We reported that pressor response to administration of hypertonic NaCl into the lateral ventricle of the rat was associated with a reduction of norepinephrine release (Katahira et al., 1989b) and an increase in the release of glutamate in the ventrolateral medulla oblongata.

TABLE 2

Studies on the pressor response to long-term i.c.v. administration of hypertonic NaCl in various animals

1984	Miyajima and Bunag	B.p. elevation due to continuous i.c.v. (3rd ventricle) administration of hypertonic NaCl is caused by the suppression of sympatho-inhibitory action of the anterior hypothalamus (rat).
1986	Soltis and Bohr	Continuous i.c.v. infusion of hypertonic NaCl induces hypertension by enhancing mineralocorticoid action of DOCA without nephrectomy and oral salt loading (rat).
1989	Katahira et al.	Concomitant infusion of subpressor doses of i.c.v. hypertonic NaCl and i.v. angiotensin II synergistically raises b.p. (rat).
1990	Katahira et al.	B.p. elevation of Dahl-S rat due to oral NaCl loading exceeds one due to i.c.v. Hypertonic NaCl (Dahl rat).
1991	Kawano et al.	B.p. elevation due to i.c.v. NaCl is more dependent on the sympathetic nervous system rather than on the vasopressin system (rat).

I.c.v.: intracerebroventricular; b.p.: blood pressure; DOCA: deoxycorticosterone acetate.

EFFECT OF LONG-TERM I.C.V. ADMINISTRATION OF HYPERTONIC NaCl ON B.P. AND HORMONAL SYSTEMS

Table 2 lists studies on the pressor response to long-term i.c.v. administration of hypertonic NaCl in various animals. Miyajima and Bunag (1984) reported that continuous b.p. elevation was noted in response to prolonged administration of hypertonic NaCl into the third ventricle of the rat and suggested that the cause of the b.p. elevation was the suppression of sympatho-inhibitory action of the anterior hypothalamus resulting in the sympathetic activation. This was a new experimental hypertensive model in which NaCl played an important role in the central nervous system and this procedure has been considered as selective NaCl loading, which was restricted to the central nervous system. Another hypertensive model was reported by Soltis and Bohr (1986) who showed that continuous i.c.v. infusion of hypertonic NaCl induced hypertension by enhancing mineralocorticoid action of deoxycorticosterone acetate (DOCA) without preceding nephrectomy and oral salt loading. They showed that the vascular

responsiveness to exogenous pressor substances was exaggerated in this model.

The Dahl salt-sensitive rat is a genetically determined hypertensive model and the b.p. is raised drastically by a diet containing 8% NaCl. Akahoshi et al. (1986) demonstrated that solitary i.c.v. administration of hypertonic NaCl into Dahl-salt sensitive rats caused b.p. elevation without oral salt loading showing that the central nervous system plays a role in the development of salt-sensitive hypertension. However, in 1990, we showed that b.p. elevation of Dahl-S rat due to oral NaCl loading was noted to a similar extent regardless of kinds of i.c.v. solution suggesting that central sodium loading was not the major component of b.p. elevation seen in Dahl rats (Katahira et al., 1990). In support of our report, Nakamura and Cowley (1989) showed that b.p. elevation of Dahl-S rat in response to high sodium diet preceded, but not followed, the elevation of sodium concentration of cerebrospinal fluid. Kawano et al. (1991) also demonstrated that b.p. elevation due to chronic i.c.v. NaCl was more dependent on the sympathetic nervous system rather than on the vasopressin system.

SYNERGISTIC B.P. ELEVATION BY I.C.V. NaCl INFUSION AND SUBPRESSOR ANGIOTENSINS IN RATS

Angiotensin II (Ang II), a potent pressor substance of biological origin, is known to have a pressor effect mainly by a direct vasoconstrictive action. It is also known to elevate the b.p. by facilitating the sympathetic nervous system peripherally as well as centrally. Thus the effects of i.c.v. NaCl infusion and i.v. angiotensins on the b.p. both appear to be exerted via the sympathetic nervous system. In 1989, we demonstrated in the rat that continuous and concomitant infusion of subpressor doses of i.c.v. hypertonic NaCl and i.v. angiotensin II synergistically raised b.p. (Katahira et al., 1989a).

Firstly, we confirmed the pressor effect of solitary hypertonic NaCl infusion into the lateral ventricle of the rat. Significant b.p. elevation was obtained only by 1.5 M NaCl at a rate of 5 μl/h starting 8th day of infusion but not by 0.8 M NaCl at the same rate. This finding was consistent with the report of Soltis and Bohr (1986) that infusion of 0.8 M NaCl into the lateral ventricle of rats did not have a marked effect on the b.p. The continuous i.c.v. infusion of hypertonic NaCl resulted in suppression of plasma renin activity as well as plasma aldosterone concentration even in the dose of NaCl which did not cause b.p. elevation. Therefore, i.c.v. infusion of this lower dose of hypertonic NaCl was physiologically significant though it was devoid of pressor effect. The mechanism of the reduction of PRA by acute i.c.v. infusion of hypertonic NaCl is thought to be due to suppression of the renal sympathetic activity and increasing plasma vasopressin.

Secondly, we combined i.c.v. infusion of a lower dose of hypertonic NaCl (0.8 M) with a known subpressor dose of intravenous (i.v.) angiotensin II (5.4 pmol/kg/min). Either one of them did not cause any b.p. change, but when they were given concomitantly, b.p. was elevated starting from day 2 of the treatment demonstrating the synergistic pressor effect of i.c.v. NaCl with i.v. angiotensin II. In a separate series of experiment (Mikami et al., 1988), we examined the effects of combination of i.v. angiotensin III with i.c.v. NaCl on the b.p. and plasma renin and aldosterone. Synergistic pressor effect was noted but the magnitude of b.p. change was much less than that caused by the previous combination though hormonal effect was comparable.

Figure 1 illustrates effects of various pharmacologic blockade of the known pressor systems on the b.p. of rats treated with these combinations of i.c.v. NaCl and i.v. Ang II. B.p. reduction was noted most prominently with hexamethonium, a ganglion blocker, in groups where significant b.p. elevation was noted at the end of infusion. These results suggest that in these models, hyperactivity of the sympathetic nervous system, but neither angiotensin or vasopressin, was responsible for the elevated b.p.

Thirdly, we tried to determine which of the concentration of NaCl or the total amount of NaCl was responsible for the b.p. change in combination with subpressor dose of i.v. Ang II. In the presence of i.v. Ang II, only 0.8 M NaCl caused a significant b.p. elevation indicating that hypertonicity of NaCl is a requisite for the pressor effect.

THE IMPORTANCE OF THE SYMPATHETIC NERVOUS SYSTEM IN THE DEVELOPMENT OF HYPERTENSION DUE TO NaCl–ANGIOTENSIN SYNERGISM

On these historical backgrounds, we tried to determine the role of the sympathetic nervous system in the development of hypertension induced by the combination of i.c.v. hypertonic NaCl with i.v. Ang II, either one of them when given alone does not elevate b.p.

Methods

Twenty-five male Wistar rats weighing 300–350 g were used. They were housed in individual cages in a temperature controlled room with a 12 h dark/light

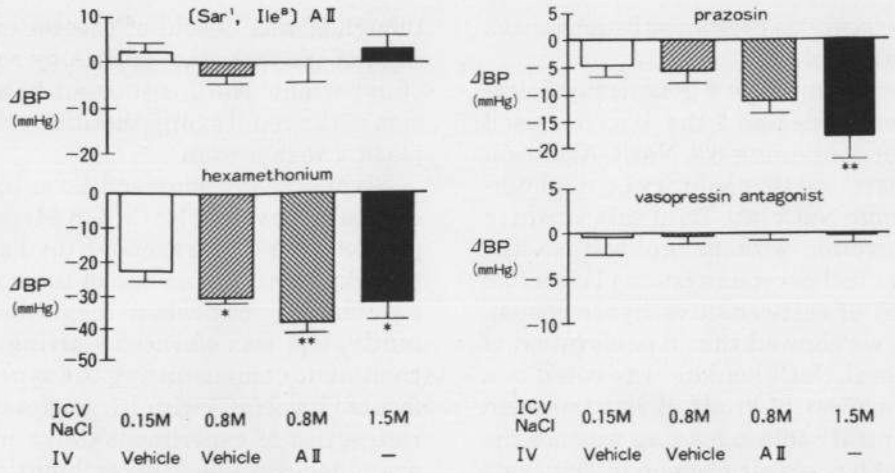

Fig. 1. Blood pressure changes due to blockade of pressor systems in conscious rats on chronic infusions of i.c.v. hypertonic NaCl with or without i.v. Ang II. Values are means±SE; Vehicle: 0.15 M NaCl; [Sar[1], Ile[8]]Ang II (300 ng/min i.v. for 10 min); prazosin (1 μg i.v.); hexamethonium (20 mg/kg, i.v.); vasopressin antagonist for vascular (V_1) receptor (10 μg/kg, i.v.); Ang II was given in a subpressor dose of 5.4 pmol/kg/min. Infusions of 0.15 M and 0.8 M NaCl i.c.v. were done at a rate of 1 μl/h for 7 days and that of 1.5 M NaCl was done at 5 μl/h for 14 days. *P <0.05, **P <0.01 vs. vehicle control.

cycle and were given free access to water and standard rat chow. Under pentobarbital anesthesia, a catheter for continuous infusion of NaCl solutions into the lateral cerebroventricle was implanted using a stereotaxic apparatus. A central catheter made of 27 G needle was inserted into the right lateral ventricle with stereotaxic coordinates AP = 1.3 mm, L = 2.1 mm and H = 3.6 mm and connected to an osmotic minipump which contained either 0.8 M or 0.15 M NaCl with a delivery rate of 1 μl/h for 7 days. A polyethylene catheter (PE-60) was inserted into the left femoral vein and connected to another osmotic minipump which contained Ang II or 0.15 M NaCl.

Experimental protocol

Rats were randomly divided into 3 groups which received the following infusions: Group 1 (n = 11), 0.15 M NaCl i.c.v. and Ang II i.v. (5.4 pmol/kg/min, dissolved in 0.15 M NaCl); Group 2 (n = 9), 0.8 M NaCl i.c.v. and Ang II i.v.; Group 3 (n = 5), 0.8 M NaCl i.c.v. and Ang II i.v. concomitantly with daily intraperitoneal (i.p.) injection of guanethidine (40 mg/kg, according to Nielsen, 1977); Group 4 (n = 9), 0.15 M NaCl i.c.v. and 0.15 M NaCl i.v. (vehicle control). B.p. was measured by a standard tail-cuff method. The base-line systolic b.p. was determined as an average of 3 measurements. After surgery, systolic b.p. was measured every day during the infusion period. At the end of the experiment, blood samples were collected for measurements of PRA and PAC. Angiotensin II was purchased from Peptide Inc., Osaka, Japan. Guanethidine was supplied by Ciba-Geigy Pharmaceutical Co. Ltd., Basel, Switzerland.

B.p. responses of the 3 groups are depicted in Fig. 2. In Group 1, b.p. did not increase significantly during infusion period confirming that this dose of Ang II was subpressor. A significant increase in b.p. was observed only in Group 2 which received concomitant infusion of i.c.v. hypertonic NaCl and i.v. Ang II. B.p. rose from 102 ± 3 mmHg at the base-line to 132 ± 5 mmHg on day 7. In Group 3 which received additional daily intraperitoneal administration of guanethidine to i.c.v. infusion of 0.8 M NaCl and the subpressor dose of Ang II, the increase in the b.p.

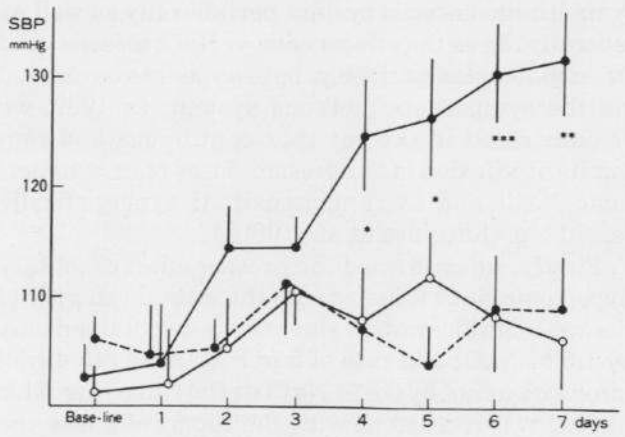

Fig. 2. Prevention by intraperitoneal administration of guanethidine (40 mg/kg/day) of increase in blood pressure in response to concomitant infusion of i.c.v. 0.8 M NaCl and i.v. Ang II (5.4 pmol/kg/min). Hollow circles/solid line: group 1, i.c.v. 0.15 M NaCl and i.v. Ang II (n = 11); filled circles/solid line: group 2, i.c.v. 0.8 M NaCl and i.v. Ang II (n = 9); filled circles/broken line: group 3, i.c.v. 0.8 M NaCl and i.v. Ang II + guanethidine (n = 5). *P <0.05, **P <0.01, ***P <0.005 vs. guanethidine group. (With permission from the publisher).

TABLE 3

Effects of guanethidine in concomitant infusions of i.c.v. NaCl with i.v. angiotensin II on PRA and PAC in rats.

Group	n	i.c.v. NaCl	i.v.	i.p.	PRA (ng/ml/h)	PAC (pg/ml)
1	11	0.15 M	Ang II	none	1.1±0.3**	744±65***
2	9	0.8 M	Ang II	none	0.4±0.1***	361±31*
3	5	0.8 M	Ang II	Guanethidine	0.5±0.3**	333±49
4	9	0.15 M	Vehicle	none	2.7±0.2	273±32

Values are means±SE. PRA, plasma renin activity; PAC, plasma aldosterone concentration; Vehicle, 0.15 M NaCl; Ang II, angiotensin II; Ang II was given at a subpressor dose of 5.4 pmol/kg/min. Solutions were infused with an osmotic minipump at a rate of 1 μl/h for 7 days. Guanethidine given i.p. 40 mg/kg/day. $^{*}P<0.05$, $^{**}P<0.005$, $^{***}P<0.001$, vs. Group 4.

was completely prevented. As shown in Table 3, Group 1 which received i.v. Ang II had an increase in the level of PAC with a suppression of PRA as compared with the vehicle control. In Group 2, concomitant i.c.v. infusion of hypertonic saline with i.v. Ang II lowered PRA and PAC significantly. The levels of PRA and PAC in Group 3 which received an additional administration of guanethidine to i.c.v. hypertonic NaCl plus i.v. Ang II were essentially the same as in the Group 2.

The results from the present and previous studies show that chemical sympathectomy with guanethidine prevented the synergistic pressor effect of i.c.v. hypertonic saline and i.v. Ang II suggesting that the b.p. elevation was mainly due to the hyperactivity of the sympathetic nervous system. Thus the presence of the intact sympathetic nervous system is necessary for the development of b.p. elevation in this model. In conclusion, the sodium status in the central nervous system is important in the regulation of the b.p., which is closely related to the activity of the sympathetic nervous system.

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