

Amiloride-sensitive Na^+ Channels Are Not Involved in the Cardiovascular Responses to Increased Na^+ Concentration in Cerebrospinal Fluid

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ABSTRACT

This study was undertaken to investigate the relationship between the Na^+ channels of the cardiovascular regulation center and the responses to increased Na^+ concentration in the cerebrospinal fluid (CSF), by observing the effects of *icv* administration of the agents affecting Na^+ transport.

Icv infusion of 200 μl of 1 M NaCl produced hypertension and bradycardia in the urethane-anesthetized rabbit, and the bradycardia was inhibited and reversed to tachycardia by vagotomy. Amiloride, a Na^+ transport inhibitor, produced hypertension and bradycardia, which were not altered by vagotomy, and it did not affect the NaCl-induced responses. Benzamil, a derivative of amiloride with higher specificity, neither produced any cardiovascular effects by itself, nor affected the NaCl-induced responses.

In vagotomized rabbits, *icv* amiloride reversed the NaCl-induced tachycardia to a bradycardia, but the bradycardiac effect was not altered by pretreating with NaCl.

This study showed that although amiloride and benzamil slightly differ in their cardiovascular action, neither of them did affect the NaCl-induced responses. We suggest that the Na^+ channels which are sensitive to amiloride or benzamil in the cardiovascular regulation center are not involved in the NaCl-induced response.

Key Words: Amiloride, Benzamil, Na^+ channel, Cerebrospinal fluid

INTRODUCTION

Since Verney (1947) reported that infusion of hypertonic NaCl into internal carotid artery releases antidiuretic hormone, many investigators reported that central Na^+ is responsible for the regulation of body fluid (Andersson *et al.*, 1972), thirst (McKinley *et al.*, 1974), the release of vasopressin (Wang *et al.*, 1982), and the regulation of kidney (Chiu and Sawyer, 1974; Zucker, 1974). It has been reported that increase in Na^+ concentration in cerebrospinal fluid ($[\text{Na}^+]_{\text{CSF}}$) may directly stimulate the regulation center of blood

pressure (Bunag and Miyajima, 1984a, 1984b; Lee *et al.*, 1986), and that chronic infusion of hypertonic NaCl increases blood pressure, heart rate, and sympathetic nerve activity, which are not related to increased osmotic pressure, but ascribed to the increased concentration of Na^+ in CSF (Wei *et al.*, 1979). As for the mechanism of the *icv* NaCl-induced hypertension, however, no clear consensus has been reached in spite of numerous observations. Thus, Thrasher *et al.*, (1980) and Baik *et al.*, (1991) suggested that vasopressin released in response to increased $[\text{Na}^+]_{\text{CSF}}$ may cause the hypertension, based on the observations that vasopressin antagonist prevented the *icv* NaCl effects. In contrast, other investigators suggested that direct sympathetic stimulation may be

responsible for the hypertension, since guanethidine abolished the hypertensive response to increased $[Na^+]_{CSF}$ and since increased sympathetic activity was ascertained in dogs (Takishita and Ferrario, 1983) and in cats (Chiu and Sawyer, 1974). Bunag and Miyajima (1984a, 1990) claimed that both sympathetic nervous and vaso-pressinergic systems are involved in the hypertension, but they suggested that the activation of the former is followed by the latter.

This study is designed to clarify the mechanism involved in the cardiovascular effects of *icv* NaCl-infusion by observing the effects of Na^+ transport inhibitors, amiloride (Clark *et al.*, 1992) and benzamil (Janis *et al.*, 1987; Kleyman and Cragoe, 1988) and their influence upon the *icv* NaCl-induced hypertension. The rationale is that the increased Na^+ entry into the cell as an inevitable consequences of increased $[Na^+]_{CSF}$ may underlie the Na^+ -induced response, and thus, it is speculated that modulation of the Na^+ channel will lead to modification of the response.

METHOD

Rabbits of either sex weighing 1.8~2.2 kg were anesthetized with 1 g/kg urethane *s.c.* The animal was fastened prone with its head extended and the trachea was cannulated. Blood pressure was taken from the left femoral artery and recorded on a recorder (Gould model 3400) through pressure transducer (Statham P231D). Heart rate was simultaneously recorded along with blood pressure by means of biotachometer and expressed as beats per minute (mean \pm SE). The osmotic pressures of NaCl and sucrose solution were checked by osmometer (Advanced Wide-Range Osmometer 3WII).

Drug administration

For *icv* administration, polyethylene tube (2.5 cm in length, 22G) were inserted into a lateral ventricle and fixed with glue. Drugs were injected through this tube, while hypertonic NaCl solution was slowly infused with infusion pump (Harvard) at a rate of 40 μ l/min for 5 min (total volume: 200 μ l). The volume of the other drugs administered *icv* was less than 100 μ l. For *iv* administration, 21G

needle was inserted into left ear vein and the volume administered was less than 1.0 ml.

Results and statistics

Blood pressure was expressed as mean arterial pressure (diastolic pressure + 1/3 pulse pressure). The significance of the difference between groups was tested by Student's *t*-test.

Drugs

Amiloride hydrochloride and benzamil hydrochloride were purchased from RBI, and sucrose from Sigma. All of these drugs were dissolved in 0.9% NaCl solution right before use.

RESULTS

Effects of *icv* NaCl

Infusion of iso-osmotic (0.15 M) NaCl did not affect the blood pressure and heart rate. But higher concentration of NaCl produced hypertension and bradycardia. With 0.5 M NaCl the blood pressure was increased by 15 ± 2.4 mmHg, 30~40 sec after starting infusion. The infusion was continued for 5 min. After the infusion was stopped, the blood pressure slowly recovered to

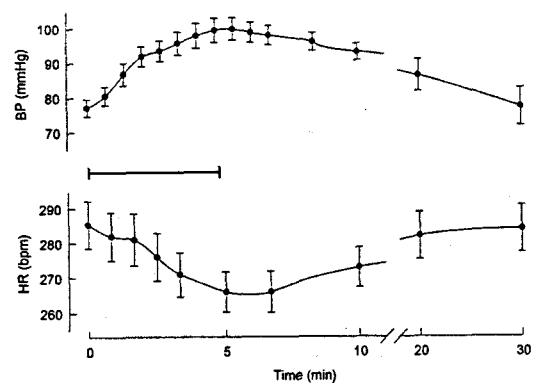


Fig. 1. Changes of arterial blood pressure (BP) and heart rate (HR) induced by *icv* infusion of 1M NaCl in rabbits. Horizontal bar indicates the duration of infusion. Each dot represents mean \pm S.E.M. of 20 experiments.

normal. The higher the NaCl concentration, the greater the blood pressure increased. In contrast to blood pressure, heart rate was reduced as the concentration of NaCl was increased. Thus, infusion of 1 M NaCl increased the blood pressure by 23 ± 1.4 mmHg ($n=20$), while it reduced heart rate by 24 ± 3.2 bpm (Fig. 1). Since infusion of concentrated NaCl solution higher than 1.5 M produced severe arrhythmia and tachypnea, 1 M NaCl was employed in this study. To rule out the possibility of osmolar effects of hypertonic saline on the cardiovascular system, we administered iso-osmotic sucrose *icv*; however, no significant changes were observed ($n=16$).

After vagotomy, 1 M NaCl increased the blood pressure by 20 ± 2.0 mmHg, not significantly differing from those of the unvagotomized rabbits, while the heart rate was reversed to an increase by 11 ± 1.4 bpm ($n=12$, Table 1).

Effects of *icv* amiloride and benzamil

Amiloride in doses up to $10 \mu\text{g}/\text{kg}$ did not produce significant changes in blood pressure and heart rate. However, $30 \mu\text{g}/\text{kg}$ amiloride significantly increased the blood pressure by 11 ± 2.5 mmHg ($n=13$), $100 \mu\text{g}/\text{kg}$ by 16 ± 2.0 mmHg ($n=8$), and $300 \mu\text{g}/\text{kg}$ by 14 ± 2.8 mmHg. The heart rate was decreased significantly with 30, 100 and $300 \mu\text{g}/\text{kg}$ (Fig. 2). In vagotomized rabbits ($n=6$), hypertensive effects of amiloride tended to be increased, but not significantly (Table 1). To test whether these effects of amiloride are of central origin, we administered $30 \mu\text{g}/\text{kg}$ amiloride intravenously, but no significant changes were observed ($n=4$).

In contrast to amiloride, benzamil in doses up to *icv* $100 \mu\text{g}/\text{kg}$ did not affect blood pressure, but the heart rate was slightly decreased only with $100 \mu\text{g}/\text{kg}$ (Fig. 2).

The influences of pretreatment with amiloride and benzamil on the 1 M NaCl-induced hypertensive and bradycardiac effects

In observing the influences of these drugs on the 1 M NaCl-induced cardiovascular responses,

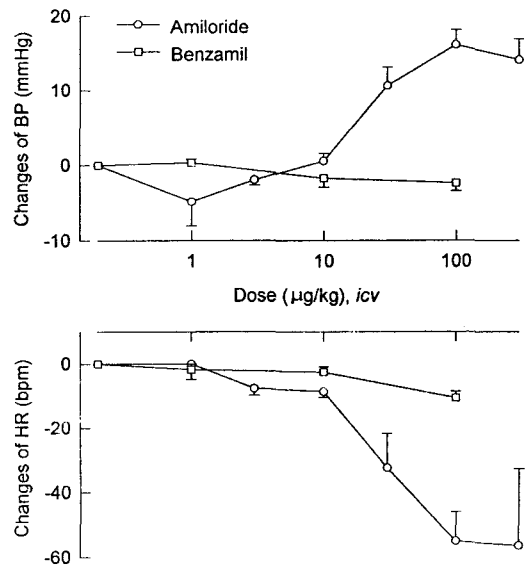


Fig. 2. Effects of *icv* amiloride and benzamil on blood pressure and heart rate. Each dot represents mean \pm S.E.M. of 8~12 rabbits.

Table 1. Effects of *icv* $30 \mu\text{g}/\text{kg}$ amiloride and vagotomy on the blood pressure (BP) and heart rate (HR) responses induced by *icv* 1 M NaCl in rabbits

PreTx	Drugs	Control			After vagotomy		
		n	dBP(mmHg)	dHR(bpm)	n	dBP(mmHg)	dHR(bpm)
-	NaCl	20	23 ± 1.4	-24 ± 3.2	12	20 ± 2.0	$11 \pm 1.4^{**}$
Amiloride	NaCl [†]	8	24 ± 2.4	-23 ± 6.9	4	19 ± 2.1	$-6 \pm 2.4^*$
-	Amiloride	13	11 ± 2.5	-33 ± 11	6	14 ± 1.7	-25 ± 4.1
NaCl	Amiloride ^{††}	-	-	-	5	19 ± 4.2	-19 ± 1.3

Mean \pm S.E.M. [†]: Net effects of NaCl after pretreatment with amiloride. ^{††}: Net effects of amiloride after NaCl-pretreatment. Asterisks indicate significant differences between control and vagotomized groups (* $p < 0.05$; ** < 0.01). PreTx; pretreatment; bpm: beats per min

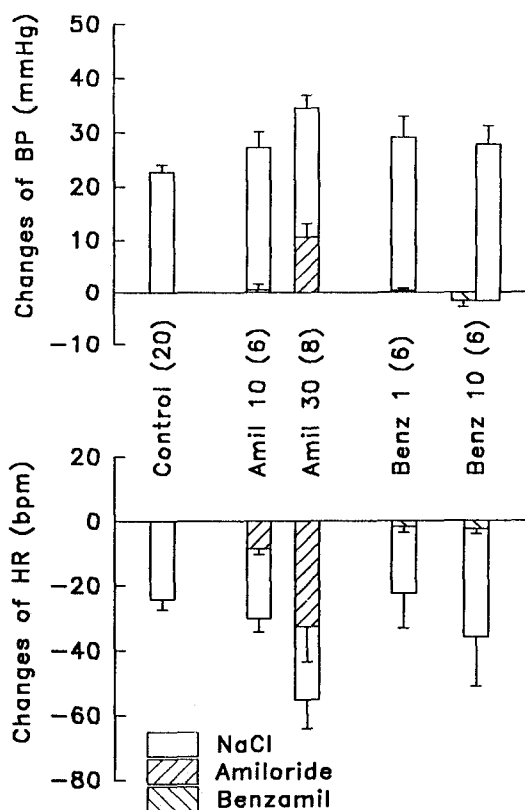


Fig. 3. The influence of amiloride and benzamil on the 1 M NaCl-induced hypertensive and bradycardiac effects in rabbits with intact vagus. The blank columns represent the net effects of 1 M NaCl. *Upper panel:* The shaded columns show the effects of pretreatment of the indicated drugs on blood pressure. *Lower Panel:* The shaded columns show the effects of the pretreatment on heart rate. Numrals in parentheses indicate the number of experiments. For details see text.

we selected two doses for each agent: one that produces cardiovascular changes and the other ineffective dose.

After pretreatment with 10 $\mu\text{g}/\text{kg}$ amiloride, which does not produce any significant changes, NaCl increased the blood pressure by 27 ± 2.8 mmHg, not differing from that of NaCl alone. 30 $\mu\text{g}/\text{kg}$ amiloride *icv* produced hypertension and bradycardia as mentioned previously, and the following NaCl infusion further increased the blood pressure by 24 ± 2.4 mmHg, a magnitude not dif-

ferent from that of 10 $\mu\text{g}/\text{kg}$ -pretreated group as well as from that of NaCl alone. The bradycardia, as well as the hypertension, induced by NaCl was not affected by 30 $\mu\text{g}/\text{kg}$ amiloride (Table 1 and Fig. 3).

In vagotomized rabbits, 30 $\mu\text{g}/\text{kg}$ amiloride did not affect the NaCl-induced hypertension. In contrast to blood pressure, it reduced the heart rate by 6 ± 2.4 bpm. The administration of 30 $\mu\text{g}/\text{kg}$ amiloride following NaCl-pretreatment produced hypertension and bradycardia, also not significantly different from the responses of amiloride alone (Table 1).

Benzamil 1 and 10 $\mu\text{g}/\text{kg}$ *icv* did not affect blood pressure and heart rate by themselves, nor did they alter the 1 M NaCl-induced responses (Fig. 3).

DISCUSSION

Abnormalities in Na^+ metabolism and its transport have long been focused as a mechanism underlying the essential hypertension by many investigators. The derangement in CSF function induced by increased $[\text{Na}^+]_{\text{CSF}}$ is regarded as one of the most important mechanisms leading to hypertension. However, no consensus has been attained as the results varied according to the animal species used and the method employed.

The reports that infusion of hypertonic saline elevates blood pressure as well as heart rate via sympathetic nervous system (Takishita and Ferrario, 1983; Huang and Leenen, 1992) seem to be discordant with our present findings. We observed further that vagotomy not only abolished the bradycardia but also reversed it to a tachycardia, which indicates that the bradycardia is resulted as a reflex responding to elevated blood pressure via vagus nerve. It has also been known that vasopressin is closely related to the hypertension (Gruber *et al.*, 1987) and that the vasopressin-induced vascular response is affected by either baroreceptor reflex or other regulatory mechanism of cardiovascular system (Knape and Zeiten, 1988). On the bases of these reports we suggest that *icv* NaCl infusion produces reflex bradycardia in response to the hypertension induced by either vasopressin or increased sympa-

thetic tone.

It is well established that amiloride, a sodium transport inhibitor (Clark *et al.*, 1992), acts on three types of channels: conductive Na⁺-specific channel, Na⁺-H⁺ antiporter system, Na⁺-Ca²⁺ exchange system (Benos, 1982; Luciani and Floreani, 1985; Debetto *et al.*, 1987), and that its action sites differ according to the concentration used (Garty and Benos, 1988; Tagliatela *et al.*, 1990). That is, amiloride blocks Na⁺-specific channel in lower concentration far below micromolar range, whereas the Na⁺-H⁺ antiport system is inhibited in 1~10 micromolar and Na⁺-Ca²⁺ exchange system in millimolar concentrations. In this study, vagotomy did not affect the effects of *icv* amiloride, nor did *iv* amiloride produce any cardiovascular changes, inferring that not vagus, but another central mechanism(s) is involved in the amiloride-induced bradycardia.

It has been reported that amiloride blocks the Na⁺-dependent Ca²⁺ uptake in rat brain (Schellenberg *et al.*, 1985) and that the calcium influx through this uptake system is responsible for the release of neuro-transmitters like dopamine (Tagliatela *et al.*, 1990). On the bases of these reports, amiloride may be assumed to act on Na⁺-Ca²⁺ exchanger in the neuron involved in cardiovascular regulation, not remotely located from the cerebroventricle, leading to hypertension and to bradycardia. And we observed that amiloride did not affect the NaCl-induced hypertension and that the magnitude of bradycardia induced by amiloride in NaCl-pretreated rabbit is not different from that of amiloride alone.

It is quite plausible to assume that the increase in Na⁺ influx into the neurons involved in cardiovascular regulation following the increase in [Na⁺]_{CSF} results in excitation of sympathetic or vasopressinergic system. However, our finding that amiloride as well as benzamil did not attenuate the responses induced by NaCl-infusion suggests that Na⁺ influx into the neuron, at least through the Na⁺ channel which is sensitive to amiloride or benzamil, is not involved in the NaCl-induced cardiovascular responses. In support of this suggestion seem to be other lines of evidence that chronic infusion of 1.5 M NaCl, producing hypertension, only slightly elevates [Na⁺]_{CSF} within normal physiologic range (Kawano *et al.*, 1991) and that the increase in

[Na⁺]_{CSF} activates the central sodium receptors, leading to increase in sympathetic outflow and arterial blood pressure (Huang *et al.*, 1992; Miyajima and Bunag, 1990).

In contrast to amiloride, benzamil given *icv* did not elicit by itself any cardiovascular response. Some investigators reported that the action of benzamil is not different from that of amiloride except for the affinity being 10 times greater (Cuthbert and Edwardson, 1979; Javec *et al.*, 1991). However, Tagliatela *et al.*, (1990) and Schellenberg *et al.*, (1985) found the differences in their modes of action in inhibiting Na⁺-channel and that benzamil is more selective to the Na⁺-specific channel. It is thus likely that the differences in their action may be based on the differences in action sites or in the channel types they affect. However, further evidence should be presented. In any case, amiloride and also benzamil, a Na⁺-channel inhibitor with enhanced specificity, did not inhibit, even to the slightest degree, the cardiovascular response to *icv* NaCl infusion, strongly indicating that the Na⁺ channel sensitive to amiloride or benzamil is not involved in the NaCl-induced hypertensive responses in rabbits.

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=국문초록=

뇌척수액내 Na^+ 농도 증가에 의한 심혈관 반응과 Amiloride 민감성 Na^+ Channel과의 관계

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Na^+ 이동에 관여하는 약물을 가토 측뇌실내로 투여하여 나타난 반응을 관찰함으로써 심혈관 조절 중추의 Na^+ channel과 뇌척수액내 Na^+ 농도의 상승에 의한 심혈관 반응과의 관계를 밝히고자 하였다.

1 M NaCl 200 μl 을 urethane으로 마취된 가토의 측뇌실내로 투여시 승압과 서맥이 나타났고 이 서맥은 미주신경 절단으로 빈맥으로 역전되었다. Na^+ 이동억제제인 amiloride의 뇌실내 투여는 승압 및 서맥을 일으켰고 미주신경 절단으로 영향을 받지 않았으며 NaCl주입에 의한 심혈관계 반응에 영향을 미치지 않았다. Amiloride 유도체인 benzamil은 그 자체로도 심혈관계 반응에 아무런 영향을 미치지 않았을 뿐 아니라 NaCl의 효과에도 영향을 주지 않았다. 미주신경 절단 가토에서 amiloride는 NaCl에 의한 빈맥을 서맥으로 역전시켰으나, 그 서맥의 정도는 NaCl의 전 처치로 영향받지 않았다.

이상의 성적으로 amiloride와 benzamil이 심혈관계에 미치는 영향은 각기 다르나 그들 모두 NaCl에 의한 심혈관계 반응에는 아무런 영향을 미치지 않음을 알았다. 이로부터 심혈관 조절 중추내 amiloride나 benzamil에 민감한 Na^+ channel은 NaCl-유발 반응과는 관련이 없을 것이라 추론하였다.