Central Depressor Effects of Amino Acids in Conscious Normotensive and Two-Kidney, One-Clip Renovascular Hypertensive Rats

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Abstract The circulatory effects of intracisternal injections of amino acids were investigated in conscious normotensive control rats (NCR) and in two-kidney, one-clip renovascular hypertensive rats (RHR). Arterial pressure was measured with an indwelling catheter connected to a pressure transducer. Heart rate was counted from the arterial pulse. The intracisternal injection of glycine, γ-aminobutyric acid (GABA), taurine, serine, alanine, and sarcosine decreased blood pressure by an average of 16–30 mmHg in NCR and by an average of 32–55 mmHg in RHR. Both absolute and percent changes of depressor effects by GABA, taurine, serine, and alanine were larger in RHR than in NCR. All these amino acids also showed similar bradycardiac effects in both NCR and RHR, when compared in absolute values. The percent change of bradycardia induced by taurine and sarcosine was larger in RHR than in NCR. However, the degree of bradycardia by serine was larger in NCR than in RHR. These results suggest that serine, alanine, and sarcosine in addition to glycine, GABA, and taurine, play important roles in blood pressure control in conscious normotensive rats via central neural mechanisms and that the hypertension in renovascular hypertensive rats may involve a central abnormality.

Keywords: serine, alanine, sarcosine, intracisternal injection, two-kidney, one-clip renovascular hypertensive rats.

The pressor effects have already been observed with arginine, proline, cysteine, aspartic acid, and asparagine with concomitant bradycardia in proline and tachycardia in arginine, cysteine, aspartic acid, and asparagine, as shown in the previous study (Takemoto, 1990). With regard to the depressor effects of amino acids, GABA has been reported to show hypotensive and bradycardiac effects when applied to posterior hypothalamus (Wible et al., 1989) and intracerebroventricle

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(Persson, 1980) in conscious state, and dorsal raphe (Robinson et al., 1986) under anesthesia, while this caused contrary effects, i.e. hypertension and tachycardia at caudal ventrolateral medulla (Kihara and Kubo, 1988) and nucleus tractus solitarii (Catelli and SvED, 1988; SvED and SvED, 1989) under anesthesia. Glycine when injected into nucleus tractus solitarii (Talman and Robertson, 1989) under anesthesia and intracerebroventricle (Persson, 1980) in conscious state decreased arterial pressure and heart rate. Furthermore, dietary taurine had depressor effects in DOCA-salt (Fujita et al., 1986; Fujita and Sato, 1988) and spontaneously hypertensive rats (Nara et al., 1978; Shibata et al., 1987). However, a preliminary screening of cisternal injection of amino acids indicated a slight decrease in arterial pressure or sedation for γ-aminobutyric acid (GABA), glycine, taurine, serine, and alanine in conscious normotensive rats. Therefore, to accurately validate these depressor effects, the present study was performed. In addition to the foregoing amino acids, sarcosine, which is an N-methylglycine and is on the pathway of degradation of choline to glycine in mammals, was examined. The results showed marked hypotension in glycine, GABA, taurine, and sarcosine and marked bradycardia in glycine, GABA, serine, and alanine in conscious normotensive rats.

In addition, to compare with the circulatory effects of intracisternal injection of these amino acids between normotensive and hypertensive rats, conscious two-kidney, one-clip renovascular hypertensive rats were investigated.

METHODS

Adult male Wistar rats, 11–15 weeks old, weighing 260–380 g, were used in this study. Normotensive control rats (NCR) were intact before cannulation. Two-kidney, one-clip renovascular hypertensive rats (RHR) were prepared as follows: after rats were intraperitoneally anesthetized with thiamylal sodium (50 mg/kg), a metal clip with a gap of 0.3 mm was applied to the left renal artery. Three weeks after the clipping, a polyethylene catheter for measurement of arterial pressure was inserted into the terminal aorta through a femoral artery of the rat, which was intraperitoneally anesthetized with thiamylal sodium (50 mg/kg). Another catheter for injection of drugs was introduced into the right external jugular vein. The distal ends of both catheters were separately led to the dorsal neck subcutaneously, sutured to neck muscles and exteriorized. For central injection of the substances, a third polyethylene tube was introduced into the cisterna magna through a hole drilled in the interparietal bone and fixed on the bone with a dental cement.

After cannulation, the rat was kept separately in a plastic cage measuring 35 × 30 × 17 (depth) cm and containing wood chips. Observation was made 2–6 days after the operation. Arterial pressure was recorded through a pressure transducer connected to the arterial catheter in the rat which remained in the home cage. Heart rate was counted from arterial pulse by increasing the paper speed with another recorder.

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For intracisternal injection of amino acids, these materials were dissolved with artificial cerebrospinal fluid (ACSF). Ten microliters of amino acid solution was injected and immediately flushed with 5 μl of ACSF, because the volume of the cisternal catheter was below 3 μl. Ten micromoles of lysine and 15 μl of ACSF did not show a significant change of blood pressure (Takemoto, 1990). In addition, the blood pressure by depressor amino acids increased rapidly in a dose-dependent manner with less than 10 μmol of substances and almost recovered within 30 min after intracisternal injections (Takemoto, 1990). However, in the preliminary test it took longer for depressor amino acids to decrease and return blood pressure than for pressor acids. Thus, the concentration of injection was determined at below 10 μmol of substances for one dose, time to observe blood pressure and heart rate was for 60 min, and the interval between the injections was over four hours. After the observation was completed, the rats were intravenously anesthetized with thiopental sodium (25 mg/kg) and sacrificed. The cisternal magna was opened by removing the occipital bone and the location of the tip of the catheter was confirmed. In some rats 10 μl of trypan blue was injected before sacrificing to check the distribution of amino acids.

The following amino acids were used: GABA, glycine, serine, alanine, and sarcosine (10 μmol) and taurine (5 μmol because 10 μmol does not dissolve in ACSF).

The number of normotensive rats were seven and in the case of two-kidney, one-clip renovascular hypertensive rats, eight were used and just six in heart rate by glycine and GABA.

Data is expressed as mean ± S.D. and was analyzed by grouped t-test or by paired t-test where indicated. Criterion for statistical significance was p < 0.05. Percent change of blood pressure and heart rate between pre- and post-intracisternal injection in NCR and RHR was calculated as follows. Firstly, the maximum difference in the values of blood pressure and heart rate was gained from the observation within 60 min after injection of substances, by comparing those values before injection. Next, percent change was calculated as the ratio of the maximum difference versus the value before injection.

RESULTS

Figure 1 shows time course of arterial pressure and heart rate in NCR and RHR. After the injection of 10 μmol of glycine, GABA, and sarcosine in NCR, arterial pressure decreased promptly but recovered almost within 60 min, while the lowering effect of heart rate by these amino acids was maintained after 60 min. Intracisternal taurine (5 μmol) had a tendency to decrease both parameters in NCR. Injection of 10 μmol of serine and alanine markedly decreased heart rate and induced a biphasic pattern in arterial pressure change in NCR. Intracisternal injection of all amino acids decreased blood pressure by an average of 16–30 mmHg in NCR and by an average of 32–55 mmHg in RHR. The patterns of changes in
Fig. 1. Time course of blood pressure (circles) and heart rate (triangles) after cisternal injection of amino acids to conscious normotensive (open symbols) and two-kidney, one-clip renovascular hypertensive (closed symbols) rat. BP, blood pressure; HR, heart rate (beats/min); b, before injection. The bars indicate S.D. Gly, glycine; Tau, taurine; Ser, serine; Ala, alanine; Sar, sarcosine. At the time indicated by the arrow, each amino acid solution was intracisternally injected. Statistical significance from the value before injection is indicated by *p < 0.05, **p < 0.025 (paired t-test).
blood pressure and heart rate by intracisternal injection of these amino acids in RHR were similar to those in NCR, but the amplitude of changes in blood pressure in RHR was larger than that in NCR. The bradycardiac effect by alanine was often observed after 4 h, but not so strong.

The maximum difference in the values of blood pressure and heart rate between pre- and post-intracisternal injection in NCR and RHR is shown in Fig. 2 as percent of change. The absolute decrease in blood pressure was significantly larger in RHR in GABA, taurine, serine, and alanine. However, that in heart rate in RHR was similar to that in NCR in all acids. As shown in Fig. 2, when comparing the rate of change, larger decrease in blood pressure by GABA, taurine, serine, and alanine, and in heart rate by taurine and sarcosine were observed in RHR, while decrease in heart rate in serine was smaller in RHR.

The tips of intracisternal catheters were located around the area postrema or the caudal fourth ventricle, and trypan blue stained almost the same place, when

![Graph showing blood pressure and heart rate changes after amino acid injection.](image_url)

**Fig. 2.** Maximum percent decrease in blood pressure and heart rate after intracisternal injection of amino acids in conscious normotensive (open columns) and two-kidney, one-clip renovascular hypertensive (closed columns) rats. Gly, glycine; Tau, taurine; Ser, serine; Ala, alanine; Sar, sarcosine. **Significantly different from NCR at p<0.05, 0.025, and 0.01, respectively, by grouped t-tests.** The values, in blood pressure of NCR and RHR before injection, were 114±6.1 (mean±S.D.) and 163±8.6 mmHg in glycine, 113±6.9 and 159±17 mmHg in GABA, 111±6.6 and 166±14 mmHg in taurine, 113±8.1 and 158±9.9 mmHg in serine, 111±6.3 and 162±13 mmHg in alanine, and 109±3.6 and 176±18 mmHg in sarcosine. Those in heart rate on NCR and RHR were 360±42 and 384±66 in glycine, 372±40 and 402±49 in GABA, 348±78 and 396±59 in taurine, 372±49 and 378±66 in serine, 372±44 and 378±29 in alanine, and 354±35 and 438±56 in sarcosine.
responses by intracisternal injections of amino acids could be obtained.

DISCUSSION

In the previous study (TAKEMOTO, 1990), it was observed that intracisternal injection of amino acids, i.e., cysteine, arginine, asparagine, aspartic acid, and proline, increased arterial pressure, and intracisternal proline decreased heart rate, but cysteine, arginine, asparagine, and aspartic acid increased it in conscious rat. In this study, all amino acids used here, glycine, GABA, taurine, serine, alanine, and sarcosine, showed central depressor and bradycardiac effects in conscious NCR. The relationship between molecular structures and circulatory effects by both intracisternal pressor and depressor amino acids in NCR indicates the following tendency: the pressor and tachycardiac amino acids have larger molecular weight and polar side chain, while the depressor and bradycardiac amino acids have smaller molecular weight and aliphatic side chains. This tendency may imply the characteristic of the receptors, on which nervous terminals involve blood pressure control.

Sarcosine, N-methylglycine, is on the pathway of degradation of choline to glycine in mammals and is similar in structure to alanine, in which the difference is in site of methylation. When these amino acids were injected intracisternally, the effects were slightly different, i.e., although all of them had strong bradycardiac effect, alanine had a week depressor effect as compared with the other. If there is some enzyme to convert one another at the terminal of neuron around cisterna magna, these substances would be strongly expected to be released as neuromodulators or neurotransmitters in response to changes in circulation.

Thus far, important areas with action on blood pressure and heart rate were found in the lower brain stem, hypothalamus, forebrain, temporal cingular, and insular lobes and blood pressure is through to be controlled by specific pathways in the CNS connecting these areas with projections each containing characteristic neurotransmitters (REIS, 1984). GABA has been extensively studied as a putative neurotransmitter in regulation of circulation (PERSSON, 1980; ROBINSON et al., 1986; CATELLI and SVED, 1988; KIHARA and KUBO, 1988; SVED and SVED, 1989; WIBLE et al., 1989). Several reports have been made regarding glycine (PERSSON, 1980; TALMAN and ROBERTSON, 1989), aspartic acid (KUBO and KIHARA, 1988; SOMOGYI et al., 1989), and taurine (NARA et al., 1978; FUKITA et al., 1986; SHIBATA et al., 1987; FUJITA and SATO, 1988). There is an extensive literature on the effects of known amino acids on neurons, in vitro or in vivo, especially in anesthetized states. However, in vitro research gives little information concerning physiological function, but also almost all anesthetics themselves have the effects on circulatory system in in vivo anesthetized states. Moreover, portions of the central cardiovascular system integrate circulatory responses to behaviors in the conscious state (REIS, 1984). Therefore, the present data suggest that serine, alanine, and sarcosine may have an additional possibility as transmitters or modulators which

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control blood pressure and heart rate in the conscious state. This study from *in vivo* functional direction may have shed some additional light on the unknown neurotransmitters within the brain (McGeer et al., 1987). Confirmation of these amino acids as neurotransmitters described by McGeer et al. (1987) must await further studies from anatomical, chemical, physiological, and pharmacological approaches.

In comparing RHR with NCR in percent change of two parameters, decrease in blood pressure by GABA, taurine, serine, and alanine and that in heart rate by taurine, serine, and sarcosine in RHR were different from those in NCR (Fig. 2). In particular, intracisternal GABA in RHR lowered blood pressure to the control value in NCR. Again, the effects of intracisternal serine and alanine on blood pressure decrease were small in NCR but large in RHR. Like these, the peripheral responses of blood pressure and heart rate to centrally injected amino acids were quite different between NCR and RHR. Iriuchijima (1988) has reported that abnormal hindquarter vasoconstrictor tone is present in conscious RHR, but not in conscious NCR. This abnormal hindquarter vasoconstrictor tone may be due to resetting of the regulatory action of blood pressure in RHR (Page, 1987). The present study may provide another line of evidence for abnormality of central mechanisms in the development of peripheral hypertensive states in RHR.

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REFERENCES


