Regional Vasoconstriction and Excessive Grooming Induced by L-Arginine Injection into the Cisterna Magna of Conscious Rats

Yumi TAKEMOTO

*Department of Physiology, Hiroshima University School of Medicine,
Minami-ku, Hiroshima, 734 Japan*

Abstract Hemodynamic and behavioral changes caused by intracisternally injected L-arginine which has a pressor effect were studied in conscious rats. One week or more after inserting a cannula into the cisterna magna, electromagnetic flow probes were implanted around the superior mesenteric artery, renal artery, or terminal aorta under anesthesia. A catheter for measurement of blood pressure was inserted into the terminal aorta via a femoral artery or into the common carotid. After recovery from surgery, blood flow, arterial blood pressure, heart rate, and behaviors were observed in the conscious state. Peripheral resistance, calculated as pressure divided by flow, increased an average of 80% in the superior mesenteric artery and 60% in the renal artery when the pressure was increased by 25% following intracisternal injection of 5 μ mol of L-arginine. Hindquarter (terminal aortic) resistance showed an insignificant decrease of about 10%. The average heart rate showed no significant change. Grooming, moving, and eating all increased significantly after injection of L-arginine. Circulatory events and behaviors did not always synchronize after the injection. Intravenous chlorisondamine chloride, a ganglion blocker, significantly attenuated the pressor effect caused by intracisternal injection of L-arginine. These findings indicate that centrally injected L-arginine not only specifically triggers vasoconstriction in the viscera presumably through excitation of the regional sympathetic vasoconstrictor fibers to raise blood pressure but also centrally induces excessive grooming. However, the relationship between circulation and behavior caused by intracisternal injection of L-arginine remains unknown.

Key words: L-arginine, intracisternal injection, hemodynamics, excessive grooming, conscious rats.

Recent studies have demonstrated that many kinds of neurotransmitters or neuromodulators play important roles within the central nervous system, although

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they are assumed to be just a part of the missing neurotransmitters [1]. We have suggested previously that L-arginine, L-proline, L-cysteine, L-serine, L-alanine, and L-sarcosine in addition to L-aspartic acid, GABA, glycine, and taurine have the possibility of being neurotransmitters or modulators related to circulatory control around the medulla [2, 3]. These amino acids showed pressor or depressor effects, when they were intracisternally injected in conscious rats. After their central injection, we often observed that rats showed special behaviors. For example, rats performed excessive grooming when the blood pressure increased following intracisternal injection of L-arginine.

To characterize in detail the central cardiovascular effects of L-arginine and also to accurately validate its effects on behaviors, further observations on the hemodynamic and behavioral changes were made on conscious rats in hypertension caused by intracisternally injected L-arginine. For observation of cardiovascular changes, regional blood flow was measured with implanted electromagnetic flow probes in conscious rats, which provided information on how central stimulation with L-arginine elicits constriction or dilation of the peripheral vessels. At the same time, heart rate and arterial pressure were observed. The results showed that cisternal L-arginine specifically triggers vasoconstriction in the viscera presumably through excitation of the regional sympathetic vasoconstrictor fibers to cause elevation of blood pressure. It also increases grooming behavior. Dye infused into the cisterna magna at the end of experiment was found to be distributed mainly over the surface of the medulla and the lower cerebellum.

METHODS

In a previous study [2], 2 to 10 μ mol of L-arginine without adjustment of pH was administered to the cisternal cannula of rats 2 to 3 d after insertion of cannulae to the vessels together with that to the cisterna magna. In the present study, pH was adjusted around 7.4 to exclude the basic effects, and the duration to repair the brain damage induced by insertion of a cisternal cannula was extended to more than 1 week for further implantation and cannulation at the same time as described below. Also, the dose was decided to be 5 μ mol, because this is a sufficient dose to cause elevation of blood pressure [2].

Insertion of a cisternal cannula. Male Wistar rats were anesthetized with thiamylal sodium (50 mg/kg, I.P.) and a polyethylene cannula (sp 10 fused to sp 19, Natsume, Japan) was inserted into the cisterna magna through a hole drilled in the interparietal bone. The cannula was fixed to the bone through which two small screws were placed in position together with dental cement. After cannulation, the rat was kept separately in a transparent cage, which had wood chips on the floor and was covered with a metal wire top containing pellets and a water bottle in its indentation. The cage was placed in a light and dark cycle controlled room (6:00–18:00). Water and pellets were given *ad libitum*.

Implantation of flow probes and cannulae. One week or more after insertion

of the cisternal cannula, the rat was anesthetized again (thiamylal sodium 50 mg/kg, I.P.). An electromagnetic flow probe (Nihon Kohden type FC with internal diameter of 1–2 mm) was implanted around the superior mesenteric, left renal artery, or the terminal aorta. The technical details involved are described elsewhere [4]. The lead wire of the flow probe ending in a plug was passed under the skin and led to the dorsal neck. There the outer end of the wire was sutured to the neck muscles so that only the plug was exposed on the skin. A polyethylene catheter for arterial pressure measurement was inserted into the terminal aorta via the right femoral artery or into the aortic arch via the right common carotid artery. Another catheter was inserted into the right external jugular vein for intravenous injection. The distal end of each catheter, which was plugged with a short stainless steel wire stopper when not in use, was also led to the dorsal neck as a lead wire from the flow probe.

Materials. For intracisternal injection, L-arginine solution was dissolved in artificial cerebrospinal fluid (ACSF) and adjusted to around pH 7.4 with almost the same volume of 1 N HCl for a final concentration of 0.5 M. Ten microliters of L-arginine solution was injected and immediately flushed with 5 μ mol of ACSF.

Chlorisondamine chloride, a ganglion blocker, was intravenously injected as a bolus (2.5 mg/kg) or continuously (5.0 mg/kg). This drug was the gift of Ciba-Geigy Limited, Basel, Switzerland.

Observation of blood pressure, flow, heart rate, and behaviors. Simultaneous observation of blood pressure, flow, heart rate, and behaviors was started 2–3 d after implantation when the rat had resumed taking ample amounts of water and pellets. During the light period, observation was performed. Twenty-four rats were intracisternally injected with 5 μ mol of L-arginine. As a control, 10 μ l of ACSF was injected in the same rat 4 h and more after the amino acid injection ($n = 12$) or more than 1 h before that injection ($n = 8$). But 4 rats had no control. L-Arginine was repetitively injected to some rats.

With the rat in its home cage, an external cable from the flow meter circuit (Nihon Kohden, MFV-1100) was connected to the plug of the flow probe. A polyethylene tube from a pressure transducer was connected to the arterial catheter after the stopper was removed. Flow and pressure signals were smoothed with RC circuits with a time constant of 1 and 0.1 s, respectively, to facilitate the reading of mean values. Each flow probe was calibrated before implantation by passing known amounts of 0.9% saline through a cannulated aorta *in vivo* under pentobarbital anesthesia (50 mg/kg, I.P.). Peripheral resistance was calculated as pressure divided by flow. Flow was expressed in units of ml/(min $\cdot$ 100 g body weight), pressure in mmHg, and resistance in mmHg $\cdot$ min $\cdot$ 100 g/ml. Flow and pressure were recorded with a pen writing oscillograph.

Heart rate (beats/min) was measured by a counting device from the amplified arterial pressure signal through a Schmidt trigger. The details of the circuit are described elsewhere [5].

The behavior of the rat was manually recorded on the recording paper as

grooming, moving, or eating after cisternal injection.

Observation of changes in the circulation after ganglion blocker administration (I.V.). Fourteen rats, which were verified to raise blood pressure with L-arginine injection more than 4 h before, were used for observation of circulatory changes by intravenous injection of chlorisondamine chloride. In this experiment, the probes for determination of flow were implanted in 3 rats around the terminal aorta (hindquarter), 5 rats around the superior mesenteric artery, and 6 rats around the renal artery.

Deduction of L-arginine distribution with dye. After the observation was completed, 10 μ l of 5% trypan blue solution was injected intracisternally. Ten minutes after injection, the rat was anesthetized with pentobarbital (30 mg/kg, I.V.), the flow probe was removed and the brain was perfused with enough saline through the left ventricle to wash out the blood from the brain followed by 4% formalin-saline solution. Distribution of dye was sketched for the removed brain.

Data analysis. Data are presented as mean $\pm$ SD. To assess the changes of each parameter before and after intracisternal injection, data were analyzed using paired *t*-test. The difference between the values with L-arginine and those with ACSF 1 and 5 min after injection was analyzed by Mann-Whitney *U*-test. Behavioral changes were analyzed with Wilcoxon's signed-rank test. The changes of each parameter in intracisternal injection of L-arginine with and without intravenous injection of ganglion blockade were analyzed by paired *t*-test.

RESULTS

Observation of blood pressure, flow, heart rate, and behaviors in intracisternal L-arginine injection

Figure 1(a) gives one example of simultaneous recording of heart rate, blood pressure, and hindquarter flow in a rat which was injected intracisternally with 5 μ mol of L-arginine. In this example, heart rate decreased after injection. Figure 1(b) is a recording after injection of ACSF (control). The mean values of these parameters obtained from 7 or 8 rats before and 1, 5, 10, and 30 min after injection are summarized in Fig. 2. Hindquarter flow increased significantly with a concomitant increase in blood pressure after intracisternal injection of L-arginine. When compared with hindquarter flow in ACSF control, there were no significant differences both 1 and 5 min after injection, although a highly significant level of $p < 0.003$ was observed in blood pressure 5 min after injection.

An example for superior mesenteric flow and other parameters is shown in Fig. 3. Time courses of superior mesenteric flow calculated from 7 or 6 rats showed significant decrease for both values before injection and in ACSF control in the pressor state by L-arginine (Fig. 4).

Renal flow, as shown in Figs. 5 and 6, decreased significantly when compared with that before injection but not significantly when compared with that in ACSF control.

Fig. 1. Simultaneous recordings of heart rate (HR), arterial blood pressure (BP), and hindquarter flow (HQP) in conscious rats. Cisternal injection of (a) L-arginine and (b) artificial cerebrospinal fluid at the arrow. For the periods represented by closed rectangles at the bottom, the rat was moving; by open rectangles, grooming.

The time course of the percent change of regional peripheral resistance is presented in Fig. 7. The resistance increased significantly in the superior mesenteric and renal areas in comparison to ACSF control 5 min after injection of L-arginine. Hindquarter resistance changed almost parallel with ACSF control.

The average heart rate was not significantly different between the values of pre- and post-injection as shown in Figs. 2, 4, and 6. As shown in Figs. 1, 3, and 5, however, heart rate in each case increased or decreased 5 min after L-arginine injection. Nine examples in L-arginine injection showed a decrease in heart rate from 408 ± 35 to 366 ± 40 beats/min, when arterial blood pressure increased from

Fig. 2. Time courses of heart rate, arterial pressure, and hindquarter flow in conscious rats. At the arrow, $5\mu\text{mol}$ of L-arginine or artificial cerebrospinal fluid was intracisternally injected. The solid line shows the effect by L-arginine. The dashed line shows the effect by artificial cerebrospinal fluid. The data are presented as mean $\pm$ SD. Statistical significance from the pre-injection value is indicated by * $p < 0.05$, ** $p < 0.01$, *** $p < 0.002$ (paired *t*-test). Italic letters indicate significant level between the result with arginine and that with artificial cerebrospinal fluid, 1 and 5 min after injection, by Mann-Whitney *U*-test.

110 ± 8.6 to 127 ± 12 mmHg. Fifteen rats showed an increase in heart rate from 385 ± 46 to 442 ± 51 beats/min, when the pressure increased from 109 ± 6.8 to 141 ± 9.7 mmHg.

After intracisternal injection of L-arginine, 20 rats showed behavioral changes as summarized in Table 1. Within 10 min after injection of the amino acid and ACSF, grooming, moving, and eating were observed in the corresponding rats. The total time to assume such behaviors after L-arginine injection was significantly longer than that after ACSF injection. Especially grooming behavior was excessive (an average of 3.59 ± 2.68 min in 14 grooming rats), but 6 rats tested in L-arginine did not groom at all. Namely, intracisternal L-arginine injection did not always cause grooming behavior together with hypertension. Examples of recordings are

Fig. 3. Simultaneous recording of heart rate, arterial pressure, and superior mesenteric flow (SMF) in conscious rats. Cisternal injection of L-arginine at the arrow. See Fig. 1 for symbols.

Fig. 4. Time courses of heart rate, arterial pressure, and superior mesenteric flow in conscious rats. See Fig. 2 for details of symbols.

Fig. 5. Simultaneous recording of heart rate, arterial pressure, and renal flow (RF) in conscious rats. Cisternal injection of L-arginine at the arrow. See Fig. 1 for symbols.

Fig. 6. Time courses of heart rate, arterial pressure, and renal flow in conscious rats. See Fig. 2 for details of symbols.

Table 1. Behavioral changes within 10 min after intracisternal injection of 5 μ mol L-arginine or 10 μ l of artificial cerebrospinal fluid.

	Grooming (min)	Moving (min)	Eating (min)
L-Arginine	2.52 $\pm$ 2.85	0.84 $\pm$ 0.82	0.56 $\pm$ 0.61
ACSF	0.21 $\pm$ 0.48	0.23 $\pm$ 0.27	0
Significant level	$p < 0.005$	$p < 0.005$	$p < 0.005$

Values are mean $\pm$ SD. The number is 20. Significant difference between values with L-arginine and those with ACSF (artificial cerebrospinal fluid) was analyzed using Wilcoxon's signed-rank test.

Fig. 7. Percent changes in peripheral resistances of hindquarter area (HQR), superior mesenteric artery (SMR), and renal artery (RR) in conscious rats. See Fig. 2 for details of symbols.

shown in Figs. 1, 3, and 5. The rat was vigorously grooming during the periods shown as open rectangles at the bottom, while it moved for a short time shown as closed rectangles. During grooming, hindquarter flow increased with increase in blood pressure slightly later (Fig. 1). In the pressor state by L-arginine injection,

Table 2. Circulatory changes 5 min after intracisternal injection of 5 μ mol of L-arginine in the rats intravenously injected with chlorisondamine chloride.

	Number	Change (baseline)		Significant level
		Intact	Ganglion blocker*	
Blood pressure (mmHg)	14	33.1 $\pm$ 7.4 (105 $\pm$ 5.2)	10.6 $\pm$ 12.0 (77 $\pm$ 6.6)	$p < 0.01$ ($p < 0.01$)
Heart rate (beats/min)	14	33.8 $\pm$ 44.4 (375 $\pm$ 42)	13.7 $\pm$ 19.4 (322 $\pm$ 32)	n.s. ($p < 0.01$)
Tachycardia	10	55.1 $\pm$ 31.8 (379 $\pm$ 47)	15.9 $\pm$ 20.5 (317 $\pm$ 29)	$p < 0.01$ ($p < 0.01$)
Resistance (mmHg $\cdot$ min $\cdot$ 100 g/ml)				
Hindquarter	3	-1.6 $\pm$ 5.2 (26.3 $\pm$ 8.1)	-0.03 $\pm$ 2.0 (21.4 $\pm$ 7.0)	n.d. (n.d.)
Superior mesenteric artery	5	17.6 $\pm$ 10.0 (20.3 $\pm$ 3.7)	5.2 $\pm$ 2.5 (22.6 $\pm$ 1.3)	$p < 0.05$ (n.s.)
Renal artery	6	48.2 $\pm$ 24.2 (58.0 $\pm$ 15.0)	10.7 $\pm$ 8.8 (49.3 $\pm$ 9.4)	$p < 0.05$ (n.s.)

Values are mean $\pm$ SD. *Chlorisondamine chloride was intravenously injected as a bolus (2.5 mg/kg) or continuously (5.0 mg/kg). Significant difference was analyzed using paired *t*-test. n.s., not significant; n.d., not done.

superior mesenteric flow decreased, but heart rate increased synchronously with grooming (Fig. 3). No grooming was noted in the state of hypertension by L-arginine in Fig. 5. Circulatory events and behaviors did not always synchronize as shown in these examples.

Observation of changes in the circulation with intracisternal L-arginine injection after intravenous ganglion blocker administration

When L-arginine was repetitively injected into the cisterna magna, there was qualitative reproducibility in the circulation as well as in behaviors. It was often noticed that there was a tendency to reinforce the effects with L-arginine in its repetitive injection, when interval was short.

Circulatory changes with central L-arginine injection in 14 ganglion blocker administered rats were summarized in Table 2. When L-arginine was intracisternally injected 10 min after intravenous administration of chlorisondamine chloride as a bolus (2.5 mg/kg) or continuously (5.0 mg/kg), average increase in arterial pressure 5 min after injection of L-arginine was significantly decreased versus that without ganglion blockade. No difference between two ways of ganglion blocker injection in responses for blood pressure and heart rate was observed, probably due to enough dose of it. As for heart rate, there was tachycardia in 10 rats before ganglion blocker injection. Ganglion blocker attenuated an increase in heart rate of the rats caused tachycardia by L-arginine injection

Fig. 8. Dye distribution in the rat brain. (a) Dorsal side after removal of the cerebellum; (b) ventral side; (c) lower side of the cerebellum.

in intact. Increases in the superior mesenteric and renal resistance were also decreased after ganglion blocker injection.

Deduction of L-arginine distribution with dye

One of the sketches of dye distribution in the removed brain is presented in Fig. 8. Dye spread over the surface of the medulla and the caudal cerebellum close to the site of injection, but distribution in detail was not constant. Trypan blue was rarely distributed in the fourth ventricle, the space between the cerebellum and the rostral and dorsal medulla.

DISCUSSION

It was observed in the present study that when intracisternal injection of L-arginine caused an increase in blood pressure in conscious rats, superior mesenteric and renal flow decreased, but hindquarter or terminal aortic flow increased. In general, an increase in arterial pressure causes an increase in regional flow when the resistance in the corresponding vessel does not change. In the high blood pressure caused by L-arginine injection, however, our data from flow determinations gave an opposite result in superior mesenteric and renal arteries, while an exact result in the hindquarter artery was gained. However, the difference was not always significant between flows in L-arginine injection and in ACSF injection. Flow determinations in the different blood pressure like the hypertension after L-arginine injection and the normotension after ACSF injection give less objective information, because the vascular condition and the blood pressure are closely related. Therefore, we compared vascular changes as resistances in the time course (Fig. 7). It is more

clear that vascular resistances in superior mesenteric and renal arteries increased but hindquarter resistance did not change. Moreover, because intravenous injection of chlorisondamine chloride, a ganglion blocker, reduced the hypertensive effect by L-arginine (Table 2), L-arginine is assumed to affect the regional sympathetic tone through the central nervous system. These data indicate that sympathetic vasoconstriction in the superior mesenteric and renal areas contributed to elevate blood pressure in intracisternal injection of L-arginine.

Ganglion blocker significantly decreased increases not only in arterial pressure but also in the superior mesenteric and renal resistances in intracisternal L-arginine injection. However, there remained slight increases in both. This small increment may be caused by just fluctuation related to behaviors after ganglion blockade or by release of vasopressin in L-arginine injection, but these contribution to hypertension caused by L-arginine is not supposed to be major one.

Statistically, behavioral changes after injection of L-arginine were increased in time for grooming, moving, and eating, when compared with those in ACSF. All the three behaviors showed significant change in L-arginine injection. However, moving is very usual behavior and the time to use for eating was not so long compared to that for grooming. Although 6 rats did not groom at all in L-arginine hypertensive state, grooming time in the other rats was especially prolonged by L-arginine injection. There are many peptides which centrally induce excessive grooming, including ACTH, bombesin, ceruletide, beta-endorphin, substance P, prolactin, oxytocin, and vasopressin [6]. Because many of these substances can affect that behavior, a neural circuit to elicit grooming behavior seems to reside in the central nervous system (CNS). L-Arginine or its metabolite may stimulate the circuit of grooming within the CNS.

Certain behaviors such as spontaneous grooming [7], spontaneous walk [5], and after transposition to a novel circumstance [8] in conscious rats show intrinsic patterns in circulation. Namely, during such behaviors, shift of blood flow or redistribution of cardiac output is made to compensate for the flow decrease in certain areas, which results in slight increase of arterial pressure or in almost no change. Thus, regional blood flow with certain behaviors is differentially controlled. As one of the possibilities, we support the presence of a fixed action pattern by a suitable central command for circulation coupled with the behavior [5]. Therefore, it is interesting to determine whether central injection of some substance makes regional differences in peripheral resistance in conscious, freely moving rats. The present study showed intracisternal injection of L-arginine produced vasoconstriction in the viscera but not in the hindquarters. This means that L-arginine in the brain can make regional differences in peripheral resistance. However, while L-arginine centrally induced excessive grooming behavior, the pattern in hemodynamics caused by the same amino acid at the same time was different from that observed in spontaneous grooming [7]. That is, a decrease in hindquarter flow for switching to the carotid area in the spontaneous grooming was not observed in the excessive grooming caused by intracisternal injection of L-arginine. Moreover, 6

rats did not groom at all, when hypertension was caused by intracisternal injection of L-arginine. This paradox that although central L-arginine injection induced excessive grooming, it caused different hemodynamic changes in the spontaneous grooming, and also the result that they were not always induced at the same time may indicate separate active principles and/or sites of L-arginine.

As for heart rate, we have often experienced the exact instances for the pattern in spontaneous grooming, as shown in Fig. 3, although hindquarter flow did not show the pattern at all. Heart rate increased during grooming. However, the case of bradycardia in excessive grooming was also true (Fig. 1). When chlorisondamine chloride as ganglion blocker was intravenously infused to the rats, which showed tachycardia with hypertension in L-arginine injection, an average increase caused by intracisternal injection of L-arginine decreased significantly (Table 2). This means that heart rate increased synchronously with the grooming behavior through the autonomic nervous system when L-arginine centrally induced tachycardia. From this result, it seems that the excessive grooming behavior caused by central L-arginine sometimes operates a fixed action command only for heart rate. In addition, distribution of L-arginine solution was not constant. To state these another way, this amino acid may sometimes stimulate parts of the neural circuit causing the fixed action pattern for circulation coupled with a grooming behavior [5].

Hypertension caused by central L-arginine injection was expected to stimulate baroreflex and then to cause bradycardia and vasodilation in the vascular beds, which have originally sympathetic tone. However, an average of 15% increase in blood pressure led to a decrease of just 10% in heart rate in 9 rats, while an average of 29% increase in blood pressure contrary led to tachycardia in 15 rats, and vasodilation was not observed in any of the vascular beds investigated. From these results, it is difficult to interpret that baroreflex soundly operates. Then, these findings may indicate that L-arginine directly or indirectly affects parts of the neurons in the baroreflex circuit.

Dye distribution provides information on the active site of L-arginine to be deduced. Dye spread over the medulla and the lower cerebellum. It rarely retrograded to the fourth ventricle. L-Arginine has recently been considered to be a substrate for nitric oxide (NO) in blood vessels [9–11]. Peripherally, inhibition of NO production causes an increase in blood pressure in anesthetized guinea pigs [12]. Moreover, regarding the CNS, NO synthase used as a marker enzyme to indirectly prove the NO distribution in the tissue was immunohistochemically found in the cerebellum using antisera to the purified enzyme [13]. Also, in the histochemical technique using nitroblue tetrazolium as a substrate, Vincent and Kimura [14] reported the presence of NO synthase in the medulla, which is thought to be the cardiovascular control center and then to be a candidate as the active site for circulatory effects by L-arginine from dye distribution. We could not ascertain the active principle in hypertension caused by central injection of L-arginine from the present study. These circumstances suggest that L-arginine may

have separate active sites including those within the medulla and/or the cerebellum and also different active principles such as L-arginine per se and NO in the CNS.

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