ORIGINAL ARTICLE

Effects of Intravenous Arginine Administration on Cerebral Circulation in the Rat

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Abstract. The present study was designed to determine the effects of intravenous infusion of L-arginine (ARG), an endogenous precursor of nitric oxide (NO), on the cerebral circulation in the rat. Systemic arterial blood pressure (ABP) was continuously recorded, and local cerebral blood flow (LCBF) was measured by the iodo[¹⁴C]antipyrine method at a point of time as follows: In Saline group (n = 12); at 3 min after an intravenous injection of saline. In N^G-monomethyl-L-arginine (L-NMMA) group (n = 7); at 3 min after an injection of L-NMMA (30 mg/kg/30 sec). In L-NMMA + L-ARG group (n = 7); at 3 min after an infusion of L-ARG free base (300 mg/kg/1 min) 3 min after prior injection of L-NMMA (30 mg/kg/30 sec). In L-ARG-1 group (n = 7); during an infusion of L-ARG free base (300 mg/kg/1 min). In L-ARG-2 group (n = 8); at 3 min after an administration of L-ARG free base (300 mg/kg/1 min). In D-ARG group (n = 5); at 3 min after an infusion of D-ARG free base (300 mg/kg/1 min). (1) The diffuse reduction in LCBF and increase in ABP induced by L-NMMA were almost completely reversed by excess L-ARG. (2) During the administration of L-ARG, a sustained decrease in ABP was observed, but LCBF revealed no significant change. (3) At 3 min after the infusion of L-ARG, LCBF was significantly decreased in several regions, while ABP recovered. (4) The infusion of D-ARG also induced a transient decrease in ABP, but did not alter LCBF. The effect of L-ARG on ABP may not be entirely due to an increase in substrate availability for NO synthase, since the D-enantiomer also had some vasodilatory property. Regarding the therapeutic application of L-ARG, further investigations on its actions should be performed under various conditions. (Keio J Med 44 (1): 19–29, March 1995)

Key words: nitric oxide, arginine, cerebral blood flow, rat, autoradiography

Introduction

Nitric oxide (NO)¹ and other closely related compounds such as S-nitrosothiols² were originally identified as endothelium-derived relaxing factors (EDRFs).³ We have previously reported that intravenous administration of N^G-monomethyl-L-arginine (L-NMMA), a specific inhibitor of NO synthesis,⁴ induces a significant regionally homogenous reduction in local cerebral blood flow (LCBF).⁵ This indicates that endogenous NO formation regulates the basal arterial tone in the cerebral cortex as well as deep brain structures, so that cerebrovascular homeostasis is maintained. Recently, it has also been demonstrated that NO synthase (NOS)-containing nerve fibers from some peripheral ganglia innervate the anterior portion of the circle of Willis and its branches,^{6,7} and

neural processes of NOS-containing parenchymal neurons may be also associated with the cerebral microvessels.⁸ These findings suggest that NO may play crucial roles in the cerebral circulation, not only as one of the EDRFs but also as a neurotransmitter of the perivascular vasodilator nerve fibers or the parenchymal neurons.

The physiological precursor of NO is considered to be a dibasic amino acid, L-arginine (ARG),⁹ or ARG-containing peptides.¹⁰ Although the vasoconstrictor effect of NOS inhibitors has been consistent in a number of reported studies, it is still controversial whether or not exogenously administered L-ARG can contribute to the regulation of the systemic and cerebral circulation. Several papers reported that enzymatic NO synthesis must be saturated with endogenous L-ARG, and substrate availability may not be rate limiting in NO synthesis of

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endothelial cells under resting conditions.^{11,12} In contrast, hypotension¹³ and LCBF increase^{14,15} induced by L-ARG were reported, although the mechanisms involved remain unclear. Moreover, Morikawa *et al*^{15,16} have recently demonstrated that pre- and post-treatment with L-ARG, but not D-ARG, decreased infarct size in the rat brain, and postulated the possibility of preventive treatment with L-ARG in cerebral infarction. The purpose of the present study was to determine the following: (1) whether or not the administration of excess L-ARG reverses the LCBF reduction induced by L-NMMA, (2) the influence of intravenous infusion of L-ARG on systemic arterial blood pressure (ABP) and LCBF.

Materials and Methods

Forty-six Wistar rats weighing 200–300 g were anesthetized with pentobarbital sodium (25 mg/kg, i.p.), and polyethylene catheters were inserted into the femoral arteries and vein. ABP was recorded continuously by a transducer connected to the catheter placed in the femoral artery.

At 4 hours after the surgery, when the animals had recovered to the alert state, arterial blood gases and pH were measured. Then the following procedures (Fig 1) were performed to determine LCBF using the modified N-methyl-iodo[¹⁴C]antipyrine ([¹⁴C]IAP) autoradiographic method^{17,18} whose details were previously described¹⁹: (1) In Saline group (n = 12); at 3 min after an intravenous injection of 1 mL of isotonic saline over 1 min, an infusion of 40 μ Ci [¹⁴C]IAP (spec. act. 58.3 mCi/mmol; NEN/Du Pont, Wilmington, DE, USA) dissolved in 1 mL of saline was started at a constant speed via the venous catheter for a period of 1 min. (2) In L-NMMA group (n = 7); at 3 min after an intravenous injection of N^G-monomethyl-L-arginine acetate (30 mg/kg/30 sec, Calbiochem, La Jolla, CA, USA) which was freshly dissolved in isotonic saline (5 mg/mL) immediately before use, an infusion of [¹⁴C]IAP was started. (3) In L-NMMA + L-ARG group (n = 7); L-NMMA (30 mg/kg) was administered over 30 sec, and 3 min later L-ARG free base (Wako Pure Chemical Industries Ltd., Osaka, Japan) dissolved in isotonic saline (100 mg/mL, pH 10.8), was infused for 1 min at a constant speed (300 mg/kg/min) by means of an infusion pump. Then at 3 min after the completion of the L-ARG infusion, [¹⁴C]IAP was administered. (4) In L-ARG-1 group (n = 7); simultaneous intravenous infusion of L-ARG free base (300 mg/kg) and [¹⁴C]IAP was performed over 1 min in order to measure LCBF during the administration of L-ARG. (5) In L-ARG-2 group (n = 8); at 3 min after an administration of L-ARG (300 mg/kg/1 min), [¹⁴C]IAP was infused. (6) In D-ARG group (n = 5); at 3 min after an infusion of D-ARG free base (300 mg/kg/1 min, Sigma Chemical Co., St. Louis, MO, USA) dissolved in saline (100 mg/

Fig 1 Timing of the infusion of [¹⁴C]IAP for LCBF measurement was as follows: (1) Saline group (n = 12); at 3 min after an intravenous injection of 1 mL of saline. (2) L-NMMA group (n = 7); at 3 min after an intravenous injection of L-NMMA (30 mg/kg) dissolved in saline (5 mg/mL) over 30 sec. (3) L-NMMA + L-ARG group (n = 7); at 3 min after an intravenous infusion of L-ARG free base (300 mg/kg) dissolved in saline (100 mg/mL) over 1 min, which was performed 3 min after the prior injection of L-NMMA (30 mg/kg i.v.). (4) L-ARG-1 group (n = 7); during an intravenous infusion of L-ARG free base (300 mg/kg) over 1 min. (5) L-ARG-2 group (n = 8); at 3 min after an intravenous infusion of L-ARG (300 mg/kg) over 1 min. (6) D-ARG group (n = 5); at 3 min after an infusion of D-ARG free base (300 mg/kg) dissolved in saline (100 mg/mL) over 1 min. At the end of the [¹⁴C]IAP infusion period, the animals were decapitated.

mL, pH 10.9), [¹⁴C]IAP was infused.

At the end of the 1 min infusion of [¹⁴C]IAP, the animals were decapitated, and the brains were quickly removed and frozen. Each timed arterial blood sample (10 μ L), taken during the [¹⁴C]IAP infusion, was placed on a filter paper and dried. It was then eluted for 24 h in a mixture of 1 mL of water and 9 mL of liquid scintillation cocktail (ACS II, Amersham, Arlington Heights, IL, USA). The blood radioactivity was determined employing a liquid scintillation counter (LS 9800, Beckman Instruments, Fullerton, CA, USA). The frozen brain was cut into 20- μ m-thick sections in a cryostat. The sections were mounted on glass cover slips and placed on an X-ray film (SB-5, Eastman Kodak, Rochester, NY, USA) with calibrated ¹⁴C-embedded standards (Amersham, Arlington Heights, IL, USA) for 10 days. Quantitative densitometric analysis of the developed film was performed with a computerized digital image processing system to calculate LCBF.

In another group of Wistar rats, 1 mL of sodium carbonate (Na₂CO₃) solution (pH 11.0) (n = 3), L-ARG hydrochloride (HCl) (300 mg/kg) dissolved in saline (100 mg/mL, pH 5.7) (n = 3) or D-ARG HCl (300 mg/kg) dissolved in saline (100 mg/mL, pH 5.7) (n = 4) was

administered for 1 min, and ABP was continuously monitored in order to determine the effect of pH of the drugs on ABP.

Student's non-paired t-test, analysis of variance (ANOVA) and Bonferroni's modified t-test for multiple comparisons were employed for statistical analysis.

The experimental protocol of the present study was approved as meeting the Animal Experimental Guidelines of School of Medicine, Keio University.

Results

Physiological parameters

Table 1 shows the control values of arterial blood gases and pH in each group. No significant difference in each physiological parameter was noted among these groups.

Mean arterial blood pressure

Each timed MABP in these 6 groups is summarized in Table 2. In the Saline group, MABP did not show any change. Intravenous injection of L-NMMA rapidly increased MABP by approximately 25% compared to the

control level (Fig 2(a))⁵. In the L-NMMA + L-ARG group, when L-ARG was infused following L-NMMA administration, MABP decreased immediately below the control level and recovered gradually (Fig 2(b)). In the L-ARG-1 and L-ARG-2 groups, immediately after the initiation of L-ARG infusion, MABP decreased by approximately 35% compared to the control level. In most of these animals, this decrease was sustained during the infusion, but some animals initially showed a peak-shaped decrease with a gradual recovery. In the L-ARG-2 group, immediately after the infusion of L-ARG was discontinued, MABP began to recover. When LCBF was measured, MABP did not show any significant difference from the control value or from the saline group (Fig 2(c)). The D-ARG group also showed a depression in MABP during its infusion. However, its magnitude was significantly smaller than that in the L-ARG-2 group, and after 2 min following the completion of the infusion MABP slightly overshoot above the control level (Fig 2(d)).

Local cerebral blood flow

Table 3 summarizes the mean values of LCBF in each group. The L-NMMA group exhibited a 25–30% re-

Table 1 Arterial Blood Gases and pH under Control Condition

Group	PaO ₂ (mmHg)	PaCO ₂ (mmHg)	pH
(1) Saline	95 ± 5	42 ± 2	7.32 ± 0.03
(2) L-NMMA	93 ± 10	38 ± 1	7.35 ± 0.05
(3) L-NMMA + L-ARG	95 ± 7	40 ± 4	7.33 ± 0.02
(4) L-ARG-1	94 ± 6	42 ± 4	7.33 ± 0.02
(5) L-ARG-2	95 ± 7	40 ± 4	7.31 ± 0.04
(6) D-ARG	109 ± 18	42 ± 4	7.36 ± 0.05

Values are mean ± SD.

Table 2 Mean Arterial Blood Pressure (mmHg)

Group	Control	Just before	Minimum during Infusion	Postinfusion Interval			
				Just after	1 min	2 min	3 min
(1) Saline	118 ± 14	—	119 ± 14	119 ± 14	118 ± 14	116 ± 12	116 ± 11
(2) L-NMMA	117 ± 9	—	—	122 ± 12##	142 ± 15***##	145 ± 17***##	144 ± 15***##
(3) L-NMMA + L-ARG	116 ± 7	138 ± 8## (after L-NMMA)	90 ± 9***##§†	90 ± ***##§	124 ± 6##§†	126 ± 5##§†	125 ± 6##§†
(4) L-ARG-1	105 ± 20	—	68 ± 16***##	—	—	—	—
(5) L-ARG-2	113 ± 8	—	69 ± 10***##	77 ± 16***##	98 ± 17***##	99 ± 19*	103 ± 18
(6) D-ARG	119 ± 13	—	94 ± 24*##†	106 ± 14##†	122 ± 13†	125 ± 13##†	126 ± 13##†

Values are mean ± SD. Significant difference from Saline group: * p<0.05, **p<0.01. Significant difference from Control value: # p<0.05, ## p<0.01. Significant difference from "Just before" (after L-NMMA) value (in L-NMMA + L-ARG group): § p<0.01. Significant difference from L-ARG-2 group: † p<0.05, ‡ p<0.01.

Fig 2 Recordings of ABP in each group. (a) L-NMMA group; ABP increased rapidly after L-NMMA administration. (b) L-NMMA + L-ARG group; when L-ARG was infused following the L-NMMA, ABP decreased immediately below the control level. After the end of L-ARG infusion, MABP recovered gradually to the control level. (c) L-ARG-2 group; when L-ARG was administered, ABP immediately decreased, and this decrease was sustained during the infusion. Immediately after the infusion was discontinued, ABP began to recover. At 3 min later, no significant difference from the control level was noted. (d) D-ARG group; ABP was depressed immediately after the start of D-ARG infusion and then recovered gradually even during the infusion. Slight overshoot of MABP was noted after the infusion.

duction in LCBF as compared with the saline group in all regions.⁵ On the other hand, in the L-NMMA + L-ARG group, LCBF was not significantly different from that in the Saline group in any regions except the frontal cortex. In the L-ARG-1 group, LCBF in each region revealed no significant change, although MABP was definitely reduced. In the L-ARG-2 group, LCBF was decreased significantly in several forebrain regions such as the frontal cortex, caudate-putamen ($p < 0.01$), motor cortex and accumbens nucleus ($p < 0.05$). In the D-ARG group, LCBF in each region was not significantly different compared with the Saline group. Figure 3 shows representative autoradiograms at the level of the caudate-putamen (a) and the thalamus (b) in these groups.

Effect of pH of infusion agents on ABP

The Na_2CO_3 solution (pH 11.0) showed no influence on ABP ($n = 3/3$) (Fig 4(a)). Some animals subjected to the administration of L-ARG HCl (pH 5.7) ($n = 2/3$) or D-ARG HCl (pH 5.7) ($n = 2/4$) showed a transient decrease in ABP during its infusion (Fig 4(b), (c)).

Discussion

Effects of excess L-ARG following L-NMMA on LCBF and ABP

We previously demonstrated that intravenous injection of L-NMMA induced a marked diffuse reduction in

Table 3 Local Cerebral Blood Flow (mL/100 g/min)

Region	(1) Saline	(2) L-NMMA	(3) L-NMMA + L-ARG	(4) L-ARG-1	(5) L-ARG-2	(6) D-ARG
Frontal Cortex	159.3 ± 24.1	112.9 ± 47.0 *	134.1 ± 25.3 *	147.6 ± 39.4	104.6 ± 53.2 **	147.2 ± 41.1
Frontoparietal Cortex (motor area)	167.5 ± 27.1	110.9 ± 25.3 **	160.4 ± 52.2 #	156.2 ± 35.0	120.4 ± 58.1 *	146.3 ± 15.9
Frontoparietal Cortex (somatosensory area)	164.3 ± 35.3	111.8 ± 24.2 **	154.1 ± 48.9	158.1 ± 33.7	125.9 ± 55.9	159.3 ± 30.8
Caudate-putamen	154.1 ± 27.5	112.6 ± 23.4 **	158.1 ± 38.2 #†	142.4 ± 43.6	111.1 ± 33.0 **	135.8 ± 21.5
Accumbens Nucleus	147.1 ± 26.7	104.3 ± 28.2 **	154.9 ± 44.2 #	127.4 ± 45.9	107.8 ± 47.9 *	143.5 ± 36.2
Globus Pallidus	128.2 ± 25.1	88.6 ± 20.8 **	135.0 ± 59.4	107.4 ± 17.0	91.9 ± 50.0 *	132.1 ± 31.8
Temporal Cortex (auditory area)	172.5 ± 28.7	116.6 ± 29.9 **	175.6 ± 48.3 #	167.5 ± 31.5	134.7 ± 67.3	160.8 ± 34.3
Hippocampus (CA1 region)	104.8 ± 34.6	65.3 ± 16.5 *	92.6 ± 30.5	106.0 ± 16.7	87.1 ± 38.1	112.7 ± 15.4
Hippocampus (CA3 region)	120.3 ± 33.5	74.6 ± 20.8 **	109.6 ± 31.1 #	109.8 ± 20.1	96.7 ± 47.9	131.0 ± 19.9
Dentate Gyrus	125.0 ± 40.4	81.5 ± 18.5 *	110.7 ± 38.2	116.9 ± 20.0	110.3 ± 41.7	142.2 ± 23.8
Thalamus (medial nuclei)	168.2 ± 37.3	107.2 ± 23.8 **	172.1 ± 48.8 ##	160.4 ± 44.4	134.9 ± 65.6	166.3 ± 8.1
Thalamus (lateral nuclei)	164.0 ± 36.6	103.6 ± 25.0 **	161.2 ± 48.4 #	162.4 ± 44.9	132.8 ± 69.4	167.2 ± 17.2
Substantia Nigra	125.4 ± 28.1	90.8 ± 14.2 *	147.9 ± 55.6	125.8 ± 41.6	111.0 ± 75.8	143.7 ± 30.6
Hypothalamus	130.3 ± 21.6	80.6 ± 20.9 **	130.1 ± 45.8 #	126.4 ± 52.5	118.8 ± 55.0	160.2 ± 39.7
Amygdala	124.8 ± 29.4	75.1 ± 17.2 **	122.7 ± 45.2 #	115.9 ± 30.4	96.0 ± 49.4	148.0 ± 22.6
Striate Cortex	158.5 ± 34.8	116.8 ± 19.9 *	171.8 ± 66.5	167.1 ± 59.5	117.1 ± 71.7	148.5 ± 9.5
Cerebellum	147.8 ± 55.9	91.5 ± 14.5 *	160.1 ± 62.1 #	120.0 ± 36.5	107.8 ± 57.8	145.3 ± 38.4

Values are mean ± SD. Significant difference from Saline group: * $p < 0.05$, ** $p < 0.01$. Significant difference from L-NMMA group (in L-NMMA + L-ARG group): # $p < 0.05$, ## $p < 0.01$. Significant difference from L-ARG-2 group: † $p < 0.05$.

LCBF, which was accompanied by an increase in MABP.⁵ In the present study, its vasoconstrictor effect was rapidly and almost completely reversed by the administration of excess L-ARG. These findings are consistent with earlier *in vivo* investigations of systemic blood pressure,^{11,20} blood flow in various vascular beds^{21,22} and the caliber of the basilar artery²³ and pial arteries.^{24,25} In addition, the enantiomeric specificity of the reverse effect by excess L-ARG has been already confirmed.^{4,20,21} Therefore, our data strongly suggest that continuous formation of NO from L-ARG may contribute to the regulation of the basal tone of the cerebral vessels not only in the superficial structures, but also in the deep structures of the brain.

Kováč *et al*²⁶ reported that LCBF reduction elicited by N^G-nitro-L-arginine (L-NA) was pronounced in the cerebellum and hypothalamus, which are rich in consti-

tutive NOS, whereas there was no decrease in LCBF in the cerebral cortex and white matter. In our study, however, the LCBF reduction by L-NMMA was regionally homogenous and did not seem to have any relationship with the localization of neuronal NOS²⁷ or the distribution of perivascular postganglionic NOS-containing fibers. Consequently, our findings also suggest that under the resting condition, the acute vasoconstrictor effect of L-NMMA may be mainly caused by inhibition of the basal release of EDRFs, rather than suppression of ganglionic or parenchymal neural functions.

Change in LCBF during L-ARG infusion

Our data showed that during the infusion of L-ARG free base (300 mg/kg/1 min), LCBF revealed no significant change with a marked decrease in MABP. This

Fig 3(a) LCBF autoradiograms at the levels of caudate-putamen in each group. In comparison with the Saline group (top left), L-NMMA group (top right) exhibited a marked diffuse reduction in LCBF. On the other hand, in L-NMMA + L-ARG group (middle left), LCBF was not significantly different from that in the Saline group. In L-ARG-1 group (middle right), LCBF in each region revealed no significant change, but in L-ARG-2 group (bottom left), LCBF decreased significantly in the caudate-putamen, frontoparietal cortex (motor area) and accumbens nucleus. In D-ARG group (bottom right), LCBF was not significantly different from that in the Saline group.

Fig 3(b) LCBF autoradiograms at the levels of thalamus in each group. In comparison with the Saline group (top left), L-NMMA group (top right) exhibited a marked diffuse reduction in LCBF. On the other hand, in L-NMMA + L-ARG group (middle left), LCBF was not significantly different from that in the Saline group. In L-ARG-1 group (middle right), L-ARG-2 group (bottom left), or D-ARG group (bottom right), LCBF was not significantly different from that in the Saline group at this level.

Fig 4 Recordings of ABP in animals subjected to intravenous administration of Na₂CO₃ and L/D-ARG HCl. (a) Infusion of 1 mL of Na₂CO₃ solution (pH 11.0) over 1 min induced no change in ABP. (b) Infusion of L-ARG HCl (300 mg/kg) dissolved in saline (100 mg/mL, pH 5.7) over 1 min caused a decrease in ABP. (c) The same dose of D-ARG HCl solution (pH 5.7) also elicited a similar decrease in ABP.

finding indicates that both systemic and cerebral resistance vessels were dilated during the infusion, but the dilation of the latter may have been within the range of the autoregulatory response.

The effects of exogenously administered L-ARG on blood flow are still under debate. In some earlier reports, intravenous administration of L-ARG did not affect ABP and peripheral blood flow in the guinea-pig,¹¹ rat,²⁸ rabbit²⁹ and cat.²⁶ Faraci *et al*²³ reported that topical application of L-ARG (100 μ M) had also no effect on the diameter of the rat basilar artery. In contrast, incubated rings that had been depleted of L-ARG, showed relaxation in response to added L-ARG, whereas fresh vascular rings did not relax.^{30–32} The intracellular concentration of L-ARG in freshly isolated endothelial cells was estimated to be 2–4 mmol/L,^{33,34} compared to 0.1–1.0 mmol/L in cultured endothelial cells.^{35–38} On the other

hand, the K_m value of constitutive NOS purified from bovine endothelium³⁹ and rat cerebellum⁴⁰ were reported to be 2.9 and 2 μ mol/L, respectively. Based on these data, supply of exogenous L-ARG seems to be unlikely to influence NO production, except for under conditions in which L-ARG stores in the endothelium have been depleted.

Contrary to the above findings, Nakaki *et al*¹³ reported that intravenous infusion of L-ARG HCl (500 mg/kg/30 min), which increased the plasma concentration of L-ARG to 7 mM, induced sustained hypotension during infusion in humans. This L-ARG-induced hypotension was also observed by Petros *et al*⁴¹ and our group.⁴² Furthermore, the hypotension was accompanied by increases in plasma c-GMP and urinary nitrite/nitrate, indicating that this effect may be due to additional formation of NO.⁴³ In animal studies, Zambetis *et al*⁴⁴

found that intravenous injection of L-ARG (300 mg/kg/30 sec), which is almost the same as our protocol, induced a decrease in ABP in the rat. Transient dose-dependent reduction in ABP following injection of L-ARG^{45,46} and sustained hypotension during intravenous infusion of L-ARG⁴⁶ in rats were also reported. In addition, pial arteries were dilated by intravenous administration^{24,47} and topical application of L-ARG.⁴⁸ These reports are consistent with the decrease in MABP and maintained LCBF during L-ARG infusion observed in the present study.

Change in LCBF at 3 min after L-ARG infusion

At 3 min after infusion of L-ARG, LCBF was significantly decreased in several regions, while MABP recovered to the control level. This finding suggests that the infusion of L-ARG may not always dilate, but rather constrict the cerebral vessels at 3 min after the infusion. In contrast, Morikawa *et al*¹⁵ have recently reported that intravenous infusion of L-ARG HCl (30–300 mg/kg/10 min) caused sustained increases in pial arteriolar diameter and LCBF which was measured by laser-Doppler flowmetry in normal anesthetized rats. They concluded that within component cells of the brain, NOS remains unsaturated with substrate even under the resting condition.^{14,15} Due to the limitation of the autoradiographic technique, we could not measure LCBF continuously. In our study in humans, we observed that infusion of L-ARG HCl (30 g/30 min) induced an increase in LCBF measured by Xe-enhanced CT method at the end of the 30-min infusion period.⁴² Taken together, the decrease in LCBF observed at 3 min after the infusion might be transient and a counter-regulatory phenomenon following the acute vasodilatation induced by L-ARG. Speculations for the mechanisms underlying this reduction in LCBF are described in the following section.

Transient effect of L-ARG on ABP

In the present study, plasma L-ARG level was 2.7 mmol/L at 3 min after the infusion of L-ARG (300 mg/kg/1 min) in one of the animals. However, we always observed an immediate ABP recovery and paradoxical LCBF decrease at 3 min after completion of the infusion. Cernadas *et al*⁴⁶ speculated that exogenous L-ARG may act on a rapidly exhausted pathway, or compensatory mechanisms such as the release of catecholamines may be immediately triggered. According to Hishikawa *et al*,⁴⁹ however, the changes in noradrenaline and adrenaline plasma concentrations after L-ARG infusion were too small to affect ABP. On the other hand, it is known that some endogenous metabolites from L-ARG such as methylated ARGs⁵⁰ and L-arginosuccinate¹² inhibit the vasodilatory effects of L-ARG. Furthermore,

methylated ARGs and L-ornithine, another dibasic amino acid produced from L-ARG, are reported to compete with L-ARG on its active transporter, system Y⁺ in the endothelial membrane.⁵¹ Under high local concentrations of such endogenous inhibitory substances, the availability of L-ARG would become rate limiting and a constant exogenous supply of substrate might be necessary for continuous formation of NO.

Effects of D-ARG on LCBF

Many earlier studies demonstrated that D-ARG did not affect vascular tone.^{15,31,35,46} In the present study, however, the infusion of D-ARG free base (300 mg/kg/1 min) also induced a definite transient decrease in ABP, but it did not alter LCBF at 3 min later. This indicates that L-ARG-induced hypotension may be less enantiomerically specific than the vasoconstrictor effect of L-ARG analogs and the reversal by excess L-ARG, so that some of the vasodilatory effects of L-ARG may not be mediated by *de novo* synthesis of NO. Nevertheless, Rosenblum *et al*²⁴ reported that the dilatation of pial arterioles caused by D-ARG HCl was inconsistent and less marked than that by L-ARG HCl. Similarly, in the present study, the D-ARG-induced hypotension was milder and shorter in duration than that induced by L-ARG. This difference might be explained by the increase in substrate availability for NOS when L-ARG was administered. On the contrary, Calver *et al*⁵² reported that the infusion of a high dose of both of these enantiomers increased the superficial hand vein diameter and the forearm blood flow in man, and there was no difference between these responses. Some authors considered that a high concentration (millimolar order) of ARG could dilate the vessels in a non-enantiomerically specific manner.^{53,54}

A plausible explanation for the hypotension induced by D-ARG is that ARG *per se* may act as a secretagogue of preformed EDRFs.⁵⁵ It has been already reported that polymeric basic amino acids, even D-enantiomer, induced endothelium-dependent relaxation presumably by interaction with anionic sites on the endothelium and subsequent release of EDRFs.^{56,57} High concentrations of dibasic amino acids such as L/D-ARG could trigger a transient secretion of preformed S-nitrosothiols, which would easily decompose and liberate NO in alkaline extracellular milieu.⁵⁸ However, Schmidt *et al*⁵⁴ reported that the vasodilatation elicited by monomeric L-ARG may not be mediated by this mechanism, but due to a direct interaction with the vascular smooth muscle. Anyway, the cationic effects of ARG, which is a dibasic amino acid, and its possibility as a secretagogue of nitrosothiols must be investigated.

The conflicting results about the L/D-ARG actions on vessels in earlier reports seem to be mainly due to

differences in the dose and the rate of administration. In addition, they might be attributed partly to the pH of the drugs,⁵⁹ the species,⁴⁵ the vessels examined⁵⁰ and the consciousness of the animals.⁴⁵

Regarding pH effects, Mitchell *et al*⁵⁹ reported that Na₂CO₃ alkaline buffer and solutions of L/D-ARG free base but not their HCl salts release EDRF from bovine cultured endothelial cells. In our preliminary study, after the administration of the same dose of L-ARG free base (300 mg/kg/1 min, Sigma Chemical Co., St. Louis, MO, USA), blood pH was slightly increased from 7.36 ± 0.06 to 7.43 ± 0.10 ($n = 3$), and after D-ARG free base, from 7.30 ± 0.09 to 7.52 ± 0.17 ($n = 4$). However, the infusion of Na₂CO₃ alkaline buffer (pH 11.0) did not elicit any change in ABP, so that the pH of the infusate cannot fully explain the vasodilatory effects of ARG *in vivo*. However, as previously suggested by Calver *et al*,⁵² the increase in blood pH after the administration of L/D-ARG free base may account for the greater and more consistent response of ABP than that with their HCl salts observed in the present study.

Conclusion

Although the precise mechanism of the vasoaction of exogenous ARG remains to be resolved, intravenous infusion of L- and D-ARG elicited a transient decrease in ABP, and LCBF increase was not observed by L-ARG during or at 3 min after completion of the infusion of L-ARG. For therapeutic application of L-ARG, further investigations on its action should be performed under various conditions.

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