**Effects of Intravenous Administration of L-arginine
on Autonomic Nervous Activities
Analysis of Heart Rate Variability**

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SUMMARY

We studied the differences between isosorbide dinitrate (ISDN) and L-arginine (L-Arg), the precursor of nitric oxide, regarding autonomic nervous function by assessing changes in heart rate variability and blood levels of various vasoactive substances.

Healthy volunteers received infused L-arginine (L-Arg group) or isosorbide dinitrate (ISDN group). ECGs were monitored and analyzed. Blood levels of catecholamines, endothelin, renin activity, angiotensin, human atrial natriuretic peptide, aldosterone, arginine, and citrulline were examined. Time-domain analyses of heart rate variability showed no significant changes in occurrence of R-R variability or coefficient of R-R variability, indicators of parasympathetic activity, after administration of ISDN. By contrast, both measures showed significantly more variation following administration of L-Arg. Frequency-domain analyses showed that high frequency power, an indicator of parasympathetic activity, was significantly elevated in the L-Arg group, whereas there was no significant change in the ISDN group. The blood levels of the measured vasoactive substances in L-Arg and ISDN groups were significantly different.

The differences in autonomic nervous function and various kinds of vasoactive substances between the L-Arg and ISDN groups suggest that L-Arg had other actions on humoral factors in addition to vasodilation. Thus, arginine/nitric oxide may act as a neurotransmitter, altering parasympathetic nervous tension. (*Jpn Heart J* 1998; 39: 331–338)

Key words: L-arginine, Nitric oxide, Autonomic nervous activity, Heart rate variability

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NITRIC oxide (NO) has a wide variety of actions on circulatory regulation. NO not only plays a role in regulation of vascular tone as an endothelium-derived relaxation factor, it also relaxes vascular smooth muscle as a non-adrenaline and non-cholinergic mediator in the central and peripheral nervous systems, and it acts on nerve cells as a neurotransmitter via cGMP.^{1,2)} Recently, NO has been shown to have effects on sympathetic and parasympathetic nervous functions.^{3,4)}

Autonomic nervous function may be evaluated using heart rate variability. This has allowed non-invasive analysis of the time course of autonomic nervous function.⁵⁻⁸⁾ To clarify the differences between the effects of isosorbide dinitrate (ISDN), a donor of NO, and L-arginine (L-Arg), the precursor of NO, on autonomic nervous function, we assessed changes in blood pressure, heart rate variability and blood levels of various vasoactive substances.

SUBJECTS AND METHODS

Eight healthy volunteers (mean age 43 ± 4 years) received drip infusions of 300 ml of fluid containing 30 g of L-Arg (L-Arg group). Six volunteers (mean age 40 ± 5 years) received drip infusions of 300 ml of fluid containing 5 mg of ISDN (ISDN group). These subjects had no history of cardiopulmonary disease, systolic pressures of < 140 mmHg, diastolic pressures of < 90 mmHg, and their standard ECGs showed no signs of abnormalities. The present study was performed in accordance with protocols approved by the Committee on Human Rights in Research of the University of Tokushima and informed consent for participation in this study was obtained from all patients.

To avoid any confounding effects of inserting an infusion needle on the autonomic nervous system, a drip infusion of 100 ml of physiological saline was administered via the median cubital vein over 30 minutes, and then L-Arg or ISDN was administered over a period of 30 minutes. The Holter ECGs were recorded in the supine position, from the time at which the subjects were at rest to the end of intravenous drip infusion. The Holter ECGs were reproduced and analyzed using a work station for evaluation of ECG recordings (Holter Workstation, DMW-9000H, Fukuda Denshi, Tokyo). R-R interval data on ECGs was transferred to a personal computer (NEC PC9801) via a communication cable (RS-232C). Time- and frequency-domain heart rate variabilities were analyzed using a software program (Fukuda Denshi, Tokyo) for evaluating autonomic nervous function. Extra and missing beats were excluded from analysis of the R-R intervals.

For time-domain analysis, the R-R interval coefficient of variation (CV_{RR} ; standard deviation of the R-R interval per unit time/mean R-R interval $\times 100\%$)

and RR_{50} (number of times a 50 msec or greater difference in the R-R interval occurred with two serial heartbeats per hour) were analyzed. For power spectral analysis, data regarding the R-R intervals of 512 heartbeats were analyzed. The power spectrum of the heart rate showed one peak in the low-frequency range (0.04–0.15 Hz) and another in the high-frequency range (0.15–0.40 Hz). These were designated the low frequency power (LF power) and high frequency power (HF power), respectively. In the present study, the LF power, HF power and the ratio of LF power to HF power (LF/HF) were assessed.

Blood levels of catecholamines (i.e., noradrenaline, adrenaline, and dopamine), endothelin, renin activity, angiotensin, human atrial natriuretic peptide (hanp), aldosterone, arginine, and citrulline were examined while the subjects rested quietly, immediately and 15 min after the end of intravenous administration of L-Arg or ISDN (30 min and 45 min after the start of intravenous administration, respectively). Before collecting blood samples, a venous needle was inserted into the median vein of the arm opposite to the one used for intravenous administration of L-Arg or ISDN, in order to assess any effects on the autonomic nervous system, caused by pain during insertion of the needle.

Values are expressed as mean $\pm$ standard errors of mean (mean $\pm$ SEM), and they were statistically analyzed using Student's paired *t*-test. A value of $p < 0.05$ was regarded as statistically significant.

RESULTS

Blood pressure and heart rate variability before and after administration of L-Arg and ISDN

Blood pressure and heart rate. Table I shows blood pressure and heart rates of the subjects before, and 30 and 45 min after intravenous administration of L-Arg or ISDN. Systolic blood pressures decreased significantly 30 min after intravenous administration of either L-Arg or ISDN ($p < 0.05$). Diastolic blood pressure also dropped 30 min after administration of ISDN, but this change was not statistically significant. There was no significant change in pulse rate in either group.

Table I. Blood Pressure and Heart Rate before and after Intravenous Administration of L-Arg or ISDN

	L-Arg			ISDN		
	pre	30 min	45 min	pre	30 min	45 min
SBP (mmHg)	122.7 $\pm$ 4.7	116.9 $\pm$ 3.1*	123.6 $\pm$ 3.9	122.9 $\pm$ 4.7	114.7 $\pm$ 3.2*	117.9 $\pm$ 4.7
DBP (mmHg)	72.7 $\pm$ 2.7	70.3 $\pm$ 4.7	73.8 $\pm$ 5.9	74.2 $\pm$ 5.0	70.2 $\pm$ 4.4*	73.8 $\pm$ 5.0
HR (/min)	68.7 $\pm$ 3.4	68.7 $\pm$ 3.6	66.4 $\pm$ 4.1	68.3 $\pm$ 3.2	67.6 $\pm$ 2.4	66.7 $\pm$ 4.4

Values are mean $\pm$ SEM. L-Arg = L-arginine; ISDN = isosorbide dinitrate; SBP = systolic blood pressure; DBP = diastolic blood pressure; HR = heart rate. * $p < 0.05$ pre-injection vs 30 min and 45 min after injection.

Figure 1. Time course of heart rate variability obtained by time-domain analysis following administration of ISDN (A) or L-Arg (B).

Time-domain analysis of heart rate variability. Figure 1 shows the time course of heart rate variability obtained by time-domain analysis following administration of ISDN or L-Arg. No significant changes were observed in either RR₅₀ or CV_{RR}, indicators of parasympathetic activity, after administration of ISDN. By contrast, both RR₅₀ and CV_{RR} showed significant variations following administration of L-Arg ($p < 0.05$). RR₅₀ was 17.8 ± 7.5 before administration; 29.9 ± 8.8 , 10 min after administration ($p < 0.05$); 37.3 ± 10.7 , 15 min after administration ($p < 0.05$); and 37.0 ± 8.1 , 20 min after administration ($p < 0.05$). CV_{RR} was $4.2 \pm 0.39\%$ before administration; $5.3 \pm 0.6\%$, 15 min after administration ($p < 0.05$); and $6.2 \pm 0.8\%$, 20 min after administration ($p < 0.05$).

Frequency-domain analysis of heart rate variability. Figure 2 shows LF power, HF power, and the LF/HF ratio before and after administration of ISDN or L-Arg. LF power was significantly elevated in both the ISDN and L-Arg groups ($p < 0.05$). HF power, an indicator of parasympathetic activity, did not show any significant change in the ISDN group; whereas it was significantly elevated in the L-Arg group, as early as 5 minutes after administration ($p < 0.05$). The LF/HF ratio, an indicator of sympathetic activity, increased significantly in both the ISDN and L-Arg groups ($p < 0.05$).

Changes in vasoactive substances after L-Arg or ISDN administration

Table II shows the changes in blood levels of catecholamines (noradrenaline, adrenaline, and dopamine), endothelin, renin activity, angiotensin, hanp, aldosterone, arginine, and citrulline before and after administration of L-Arg and

Figure 2. Time course of heart rate variability obtained by frequency-domain analysis following administration of ISDN (A) or L-Arg (B).

Table II. Serum Levels of Vasoactive Substances before and after Intravenous Administration of L-Arg or ISDN

	L-Arg			ISDN		
	pre	30 min	45 min	pre	30 min	45 min
Noradrenaline (pg/ml)	58.7 ± 14.0	58.5 ± 11.4	50.0 ± 9.9	96.9 ± 25.5	129.1 ± 32.2*	101.1 ± 19.5
Adrenaline (pg/ml)	16.8 ± 4.7	16.3 ± 4.7	14.8 ± 4.1	21.0 ± 4.5	16.4 ± 3.3	17.0 ± 3.4
Dopamine (pg/ml)	6.0 ± 1.0	5.2 ± 0.9	6.0 ± 0.9	6.5 ± 0.8	6.0 ± 0.5	6.6 ± 0.8
Endothelin (pg/ml)	2.04 ± 0.22	1.73 ± 0.14*	1.90 ± 0.14	2.03 ± 0.20	1.83 ± 0.20	1.62 ± 0.04*
Renin (ng/ml)	1.38 ± 0.65	1.76 ± 0.79	1.42 ± 0.63	0.94 ± 0.28	1.44 ± 0.44*	1.60 ± 0.56*
Angiotensin (pg/ml)	5.25 ± 0.75	6.40 ± 1.00	4.20 ± 0.74	5.17 ± 0.70	7.86 ± 1.26*	6.14 ± 1.26
Hanp (pg/ml)	13.0 ± 1.8	21.7 ± 4.2*	19.5 ± 3.7*	13.4 ± 2.3	12.4 ± 1.7	12.5 ± 1.5
Aldosterone (ng/dl)	58.7 ± 14.0	58.5 ± 11.4	50.0 ± 9.9	59.0 ± 9.2	56.7 ± 5.4	63.1 ± 13.8
Arginine (μM/dl)	106 ± 7	6326 ± 498**	4240 ± 366**	94.5 ± 4.9	82.2 ± 4.6**	82.5 ± 4.4**
Citrulline (μM/dl)	28.3 ± 2.4	40.0 ± 2.8**	43.1 ± 2.9**	31.4 ± 3.4	29.9 ± 3.1*	28.4 ± 2.7*

Values are mean ± SEM. L-Arg = L-arginine; ISDN = isosorbide dinitrate; hanp = human atrial natriuretic peptide. * $p < 0.05$, ** $p < 0.01$ pre-injection vs 30 min and 45 min after injection.

ISDN.

Blood noradrenaline levels were significantly elevated 30 min after administration of ISDN ($p < 0.05$), but not significantly after administration of L-Arg. Blood adrenaline and dopamine levels showed no significant variation at 30 or at 45 min after administration of ISDN or L-Arg.

The administration of ISDN lead to significant increases ($p < 0.05$) in angio-

tensin and renin activities. However, no significant variations in these parameters were observed following the administration of L-Arg. Blood endothelin levels declined significantly 45 min after administration of ISDN and 30 min after administration of L-Arg ($p < 0.05$). Blood hamp levels increased significantly both 30 min and 45 min after administration of L-Arg ($p < 0.05$); whereas following administration of ISDN, no significant change occurred.

Analysis of blood amino acid levels revealed that both blood citrulline and arginine levels declined significantly following administration of ISDN ($p < 0.05$). However, with administration of L-Arg, elevated blood citrulline levels and markedly elevated blood arginine levels were noted ($p < 0.001$).

DISCUSSION

NO is an unstable low-molecular-weight gas which plays an important role as a signalling molecule in the nervous, vascular, and immune systems, because of its high cell membrane permeability and high chemical reactivity.⁹⁻¹¹⁾

In this study, the elevated blood citrulline levels following administration of L-Arg suggested that NO was produced from L-Arg. This finding agrees with Palmer's report that NO is produced when L-Arg is converted to L-citrulline by NO synthase.¹⁾ However, the changes seen in circulatory humoral factors following administration of L-Arg differed from those seen following administration of ISDN. The time course of autonomic nervous system activation during administration of L-Arg, provided by analysis of heart rate variability, showed that parasympathetic nervous activity increased. This suggests the possibility that L-Arg itself affects the autonomic nervous system independent of NO production.

We observed that after administration of ISDN, LF power and the LF/HF ratio increased. These increases were considered to be caused by enhanced sympathetic nervous activity resulting from vasodilatation due to administration of ISDN. The findings are in agreement with a report by Malliani *et al.*⁶⁾ which showed that following administration of L-Arg, both LF power and the LF/HF ratio increased, as they did following administration of ISDN. In contrast to the findings with ISDN, L-Arg increased HF power, indicating increased parasympathetic nervous activity. Furthermore, the time-domain analysis of heart rate variability also revealed that CV_{RR} and RR_{50} increased, showing that parasympathetic nervous function was also elevated following administration of L-Arg.

These differences might have been caused by the following changes in humoral factors. Noradrenaline levels were elevated significantly after administration of ISDN, whereas there was no significant variation after administration of L-Arg. In agreement with our own study, Hishikawa *et al.*¹²⁾ also described that blood noradrenaline and adrenaline levels showed little changes despite a marked

fall in blood pressure after administration of L-Arg. Moreover, Sakuma *et al.*¹³⁾ injected L-Arg or its inhibitor directly into the central nervous system and reported that NO inhibited sympathetic nervous activity.

Administration of ISDN led to significant increases in angiotensin and renin activity ($p < 0.05$). However, after administration of L-Arg, no significant increase in blood renin, angiotensin, or aldosterone levels was observed.

Endothelin regulates the secretion of neurotransmitters from the noradrenergic and cholinergic nerve terminals in the peripheral autonomic nervous system.^{14,15)} In the present study, following administration of L-Arg or ISDN, blood endothelin levels declined, despite a fall in blood pressure. We assume that endothelin was subject to positive feedback by an L-Arg/NO system.

Blood hamp levels did not show any significant variation after ISDN administration, but were elevated following L-Arg administration. Although atrial pressure was not measured in this study, we presumed that with administration of ISDN, atrial pressure did not rise because of peripheral vasodilation. With administration of L-Arg, blood hamp levels were elevated, a result which is in agreement with the findings of Hishikawa *et al.*¹⁶⁾

The observed differences in autonomic nervous functions and the vasoactive substances released after the administration of L-Arg versus ISDN suggests that L-Arg has actions on humoral factors in addition to its effects on vasodilation. It is clear that there exist vaso-constrictive sympathetic nerves that utilize noradrenaline as a neurotransmitter. However, the existence of parasympathetic vasodilator nerves has not been recognized. The findings of the present study suggest that L-Arg unlike exogenous NO, may act as a neurotransmitter which increases parasympathetic nervous activity.

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