Effects of L-Arginine and of Its Functional Antagonist N-Nitro-L-Arginine on the Behavior of Rats, with Special Reference to Motor Activity

V. A. Dubynin, S. S. Fedyushina, S. N. Stryukov, K. V. Savel'eva, A. A. Kamenskii, and I. P. Ashmarin

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 120, № 11, pp. 465-468, November, 1995
Original article submitted December 23, 1994

L-arginine and N-nitro-L-arginine were tested for their effects on the motor activity of white rats under conditions of free behavior. After oral administration of L-arginine in doses of 50 to 500 mg/kg, the horizontal and vertical components of motor activity were increased both at minute 10 and at 24 h postadministration. N-nitro-L-arginine, on the contrary, reduced the motor activity of rats (mainly its horizontal component). Oral administration of L-arginine 5 min after an intraperitoneal injection of N-nitro-L-arginine did not prevent the effects of the latter compound. The observed behavioral changes probably resulted from the combined action of two mechanisms, namely a direct influence of nitric oxide on brain cells and its action on peripheral systems of the body.

Key Words: *arginine; arginine analogs; nitric oxide; locomotor activity*

Most mediators of the nervous system are amino acids or their derivatives, and for this reason amino acid conversions frequently give rise to substances capable of exerting important effects on central nervous system functioning and on behavior. However, attempts to influence the production of neurotropic amino acid derivatives by introducing additional quantities of amino acid precursors into the body are hampered by the fact that the entry of most amino acids into the brain is effectively regulated by the blood-brain barrier. Nevertheless, the administration of glutamate, glycine, phenylalanine, or tryptophan in relatively high doses may result in enhanced or inhibited activity of various brain regions [1,4,5,7]. Hence the importance of continued research on physiologically active amino acids and their derivatives.

In recent years, nitric oxide (NO) has been shown to play an important role in the activity of various bodily systems, in particular, by acting as a second messenger in endothelial, immune, and nerve cells. The best studied effects of NO include blood pressure reduction, vasodilation, weakened platelet aggregation, and improved cytotoxic properties of macrophages. NO activates guanylate cyclase and stimulates the generation of cyclic guanosine monophosphate, a substance influencing the release of transmitters from presynaptic terminals [10]. The NO precursor in the body is the basic amino acid L-arginine. NO is formed during the reaction in which the terminal guanidine atom of nitrogen is oxidized and which is regulated by a special enzyme, flavin adenine dinucleotide, containing NADPH- and calmodulin-dependent NO synthase. L-arginine analogs, such as N-monomethyl-L-arginine and N-nitro-L-arginine, are able to block NO synthase activity, producing effects opposite to those of L-arginine itself.

Although the physiological effects of L-arginine and its analogs have been studied in depth,

Moscow State University

0007-4888/95/0011-1087$12.50

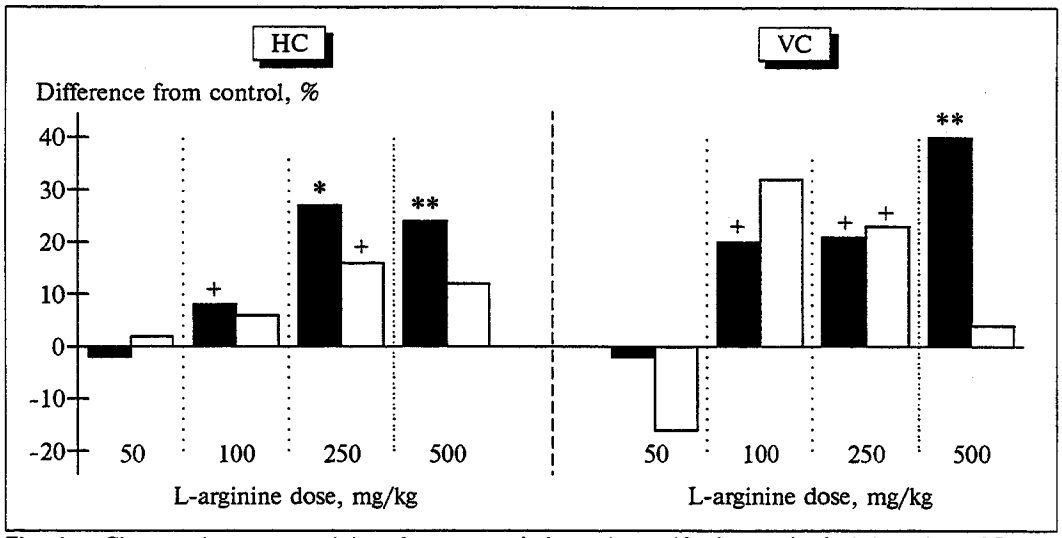

Fig. 1. Changes in motor activity of rats recorded at minute 10 after oral administration of L−arginine in different doses (rats were each tested for 10 min in the Opto−Varimex apparatus). 0 = parameter values in the control group. Black bars: minutes 1−5; white bars: minutes 6−10. Significant differences from the control group: $^{*}p<0.05$, $^{**}p<0.01$ for the sum of values obtained for 5 min of recording; $^{+}p<0.05$ for individual minutes of recording. HC and VC are the horizontal and vertical components of motor activity.

most of the investigations have been carried out with tissue preparations and cultures and virtually nothing is known about the influence of arginine and its derivatives on the behavior of animals. In view of this, we undertook the present study to evaluate the effects of L-arginine on the motor activity of experimental animals and to compare these effects with those of N-nitro-L-arginine.

MATERIALS AND METHODS

A total of 289 random-bred male white rats (body weight 175±25 g) were used. L-arginine was administered intraperitoneally and orally (0.2 and 0.8 ml of aqueous solution, respectively) and N-nitro-L-arginine intraperitoneally in the indicated amount. Control rats received equivalent volumes of distilled water.

Motor activity was recorded in unrestrained rats with an Opto-Varimex apparatus (Columbus Instruments) that permits the horizontal component (HC) of this activity to be registered separately from its vertical component (VC). Motor activity was recorded for 10 min in silence and darkness and expressed in arbitrary units of the apparatus. The results were subjected to statistical analysis by standard methods.

RESULTS

The oral L-arginine dose of 50 mg/kg administered 10 min before testing did not lead to significant changes in the motor activity of rats. The oral dose of 100 mg/kg increased both the HC and VC of activity, the increases being significant during the 3rd and 4th min of recording ($p<0.05$). The 250 mg/kg dose increased the HC by 23% ($p<0.01$) and the VC by 22% ($p<0.05$) over the total (10-min) period of recording, and similar results were obtained after the dose of 500 mg/kg (the HC increased by 20% and the VC by 26%; Table 1). The motor activity of test rats was highest during minutes 1-5, the greatest increases being 28% for the HC and 40% for the VC (Fig. 1). As shown by ANOVA, the positive correlation between the L-arginine dose and motor activity was significant at $p<0.02$.

L-arginine effects were also studied 1 and 2 h after the oral dose of 250 mg/kg, but in these tests the HC and VC of motor activity differed by only 3 to 10% from the control values. This indicates that the initial influence of arginine on motor activity lasted for less than 1 h.

Since NO has been reported to accumulate in the brain [10], we also tested L-arginine for its longer-term effects on motor activity by recording this in rats 24 h after oral administration of the amino acid. In these tests, the HC of motor activity 24 h after the 50 mg/kg dose was 18% above the control level ($p<0.05$) and the VC 20% above it ($p<0.01$), as calculated for the entire recording period. The 100 mg/kg dose did not bring about a significant increase in the HC, but did significantly increase the HC (by 66%; $p<0.05$) during minutes 3-4. After the 250 mg/kg dose, the HC significantly exceeded control values at minutes 1, 2, and 10 ($p<0.05$) and the VC, during minutes 2-4 (by 56%; $p<0.05$); later, however, this

component of motor activity decreased to a greater extent than in the control group, so that the level recorded for minutes 6-8 was only 48% of the control value ($p<0.05$).

In contrast to L-arginine, the NO synthase blocker N-nitro-L-arginine (L-NNA), administered intraperitoneally, inhibited the motor activity of rats (Table 2). A dose of 5 mg/kg decreased the HC of this activity (by 30% over the total period of recording; $p<0.05$), the decreases being most marked during minutes 1-5. The decrease in the VC averaged 20% and was insignificant. The L-NNA dose of 0.5 mg/kg caused an overall decrease of the HC which was significant at minutes 1-5 (18%; $p<0.05$). VC was more or less as in the control. The L-NNA dose of 0.1 mg/kg did not elicit significant changes in motor activity. ANOVA demonstrated a significant negative correlation between the L-NNA dose and the HC of motor activity ($p<0.05$).

In a further series of tests, rats were administered L-arginine orally at 100 mg/kg 10 min after receiving an intraperitoneal injection of L-NNA at 0.5 mg/kg and then tested, 10 min later, for motor activity in the Opto-Varimex apparatus. The tests showed that the arginine did not abolish the effects of L-NNA; in particular, the HC of motor activity was 17% below the control level during minutes 1-5 of recording ($p<0.05$). The results of these tests agreed well with those recorded after L-NNA was administered alone.

At first glance, the behavioral effects of arginine may be attributed to a generalized release of hormones such as insulin, somatostatin, and vasopressin in response to its entry into the body. However, such a release has been shown to occur only 30 to 60 min after arginine administration [3,13], i.e., at times other than those during which behavioral effects of arginine were recorded in the present study. Another possible explanation, namely that L-arginine stimulates motor activity by altering vascular tone and blood pressure, appears untenable, for, although such effects have been reported to occur immediately after arginine administraion, they are very short-lived (around one hour) [10]; moreover, heterodirectional alterations in the cardiovascular system are known to result almost always in decreased, rather than increased, motor activity of animals.

A third possibility is the direct action of NO on the central nervous system. This oxide is a mediator of sympathetic plasticity in the cerebellum - a structure selectively associated with the implementation of motor programs [11]. NO boosts the activity of glutamatergic neurons, and it also promotes enhanced cGMP synthesis by activating guanylate cyclase [2,8,12]. It is therefore likely that systemically administered L-arginine is capable of specifically elevating the motor activity of experimental animals by raising the NO concentration. If this is the case, then the behavioral effects of L-NNA can also be explained in a satisfactory manner. This substance lowers the activity of hippocampal and cerebellar neurons by causing a long-lasting blockade of the brain NO synthase [6,9]. When arginine

TABLE 1. Motor Activity of Rats after Different L-Arginine Doses Administered Orally 10 min or 24 h before Their Testing in the Opto-Varimex Apparatus ($M \pm m$, $n=14$)

Dose (mg/kg) and dosing time	Parameter	Control group		Test group	
		recording time, min			
		1-5	6-10	1-5	6-10
50, 10 min	HC	2499±483	1129±416	2446±517	1149±472
	VC	34.2±7.4	16.3±7.3	33.4±11.5	13.9±5.7
100, 10 min	HC	2058±762	970±378	2223±486	1021±349
	VC	31.6±13.1	14.1±9.1	38.1±13.4	18.6±13.1
250, 10 min	HC	1755±515	1266±224	2254±447*	1470±361
	VC	28.4±9.8	19.9±4.6	34.6±9.3	24.5±14.1
500, 10 min	HC	1730±271	913±321	2140±349**	1020±372
	VC	29.6±11.6	17.2±7.7	41.3±9.2**	17.8±7.4
50, 24 h	HC	1773±627	906±346	1944±438	1122±418
	VC	21.8±9.0	11.1±6.5	26.8±4.9	12.4±6.9
100, 24 h	HC	1773±627	906±346	2006±621	937±441
	VC	21.8±9.0	11.1±6.5	28.8±7.8*	10.2±7.7
250, 24 h	HC	1773±627	906±346	2064±631	820±471
	VC	21.8±9.0	11.1±6.5	28.5±8.6	6.5±7.1

Note. *$p<0.05$, **$p<0.01$ in comparison with the control group. Here and in Table 2, HC and VC are the horizontal and vertical components of motor activity.

TABLE 2. Motor Activity of Rats after Different Doses of N–Nitro–L–Arginine Administered Intraperitoneally ($M \pm m$, $n = 20$)

Dose, mg/kg	Parameter	Control group		Test group	
		recording time, min			
		1-5	6-10	1-5	6-10
0.1	HC	1832±851	1351±680	1747±732	1306±642
	VC	32.1±21.7	26.1±19.6	33.1±20.5	23.7±17.4
0.5	HC	1517±856	780±484	1247±738*	695±512
	VC	24.6±14.5	11.0±8.2	22.3±16.6	10.9±10.9
5.0	HC	2255±888	1024±356	1500±756*	782±377
	VC	35.7±21.2	13.8±9.5	27.9±14.9	11.7±10.4

Note. *$p<0.05$ in comparison with the control group.

is administered after L-NNA, it does not appear to interact with the already deactivated enzyme, NO is not produced in excess, and the excitatory action of the amino acid is not manifested, as was demonstrated in our experiments.

The behavioral changes described above probably resulted from the summation of the effects exerted by NO on brain cells and various peripheral systems of the body. The failure of L-arginine to influence motor activity 1 or 2 h postadministration appears to be due to autonomic effects of NO that develop and neutralize for a time the central action of the amino acid. It is worthy of note that the motor activity of L-arginine-treated rats was significantly higher than that of untreated animals predominantly during minutes 1-5 of recording and later differed little from the control level (being even significantly below it in some cases). Possibly, this was associated with a more active exploration of the surroundings by the test animals, which led to a more rapid extinction of their orienting-exploratory responses as compared to untreated controls.

In general, the results of the present study demonstrate once again the possibility of targeting the action of exogenously administered amino acids on the synthesis of neurotransmitters and neuromodulators.

This study received financial support from the Universities of Russia Program.

REFERENCES

1. M. J. Bakalian and J. D. Fernstrom, *Brain Res.*, **528**, 300 (1990).
2. D. S. Bredt and S. H. Snyder, *Proc. Nat. Acad. Sci. USA*, **86**, 9030 (1989).
3. A. Costa, P. Trainer, M. Besser, and A. Grossman, *Brain Res.*, **605**, 187 (1993).
4. S. I. Deutsch, J. Mastropaolo, B. L. Schwarts, *et al.*, *Clin. Neuropharmacol.*, **12**, 1 (1989).
5. S. I. Deutsch and J. M. Morihisa, *Clin. Neuropharmacol.*, **11**, 18 (1988).
6. M. A. Dwyer, D. S. Bredt, and S. H. Snyder, *Biochem. Biophys. Res. Commun.*, **176**, 1136 (1991).
7. C. M. Epstein, J. F. Trotter, A. Averbook, *et al.*, *Electroencephalogr. Clin. Neurophysiol.*, **72**, 133 (1989).
8. J. Garthwaite, G. Garthwaite, S. Moncada, *et al.*, *Eur. J. Pharmacol.*, **172**, 413 (1989).
9. A. Mizutani, H. Saito, and K. Abe, *Brain Res.*, **605**, 309 (1993).
10. S. Moncada, *Acta Physiol. Scand.*, **145**, 201 (1992).
11. K. Shibuki and D. Okada, *Nature*, **349**, 326 (1991).
12. P. L. Wood, T. S. Rao, J. Cler, *et al.*, *J. Neurochem.*, **55**, 346 (1990).
13. S. Yasin, A. Costa, P. Trainer, *et al.*, *Endocrinology*, **133**, 1466 (1993).