Amino Acids (1992) 2: 225–232

Amino
Acids

Oral N^G-nitro-L-arginine in conscious dogs: 24 hour hypertensive response in relation to plasma levels

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Accepted September 20, 1991

Summary. Haemodynamic changes after oral administration of 30 mg/kg N^G-nitro-L-arginine (L-NNA) were studied in conscious chronically instrumented mongrel dogs throughout a 24 h observation period in order to evaluate the long-term efficacy of L-NNA-induced inhibition of endothelium-dependent relaxation and its relation to plasma L-NNA level. Diastolic blood pressure remained elevated for the entire 24 h observation period, but systolic blood pressure was raised only up to the 6 h value. The hypertensive response was accompanied by bradycardia. The increase in blood pressure and the plasma L-NNA level both reached their maxima at 3 h. The plasma L-NNA level at the end of the observation period was diminished by only 21.7% with respect to the maximum increase, whilst the maximum increase in mean arterial blood pressure was attenuated by 72.2% at 24 h. These data show a dissociation between plasma L-NNA level and the respective blood pressure.

Keywords: Amino acids – N^G-Nitro-L-arginine – High performance liquid chromatography – Endothelium-derived relaxing factor – Blood pressure – Conscious dogs

Introduction

In 1980 Furchgott and Zawadzki reported that in vitro vascular relaxation induced by acetylcholine is mediated through the release of a diffusible relaxing substance, termed endothelium-derived relaxing factor (EDRF) [1].

There have been numerous in vitro investigations suggesting that EDRF generated by acetylcholine in blood vessels and by bradykinin in cultured endothelial cells is nitric oxide (NO) [2, 3]; however, other investigators considered EDRF to be a NO-containing compound which is more active in inducing vasodilatation than NO itself [4,5]. EDRF is derived from the amino acid L-arginine (for review, see [6]). The arginine analogs N^G-monomethyl-L-arginine (L-NMMA) [7, 8], N^G-nitro-L-arginine (L-NNA) [9] and N-

iminoethyl-L-ornithine (L-NIO) [10] were found to block NO synthesis *in vitro* stereospecifically. L-NNA inhibits the endothelium-dependent relaxation induced by acetylcholine more potently than L-NMMA and L-NIO in vitro [9], as well as in conscious rats [10]. *In vivo* blockade of NO biosynthesis by these inhibitors causes hypertension and bradycardia by a stereospecific, L-arginine-reversible mechanism in anesthetized animals, namely the rat [10,11,12], rabbit [13], guinea pig [14] and dog [15], and in the conscious rat [16, 17] and dog [18, 19]. Thus, it is apparent that NO is normally produced by vascular endothelial cells at a significant basal rate and plays an important role in blood pressure homeostasis [12,13].

Since no data are available on the long-term control of blood pressure via L-arginine: NO pathways the relation between the hypertensive response and the plasma level was studied over a 24 h period following oral administration of L-NNA to conscious dogs. The conscious dog was used since autonomic regulation is undesirably influenced by anaesthetics and moreover, the canine autonomic nervous activity is comparable to that of man. Other species, e.g. rats, have the shortcoming of a high sympathetic tone.

Materials and methods

The investigations were performed on 5 conscious mongrel dogs of either sex (17–22 kg). Each dog was free from disease and was maintained on a normal diet (Loyal, Master Foods, Austria; containing 0.4% sodium, 1.1% calcium and 0.9% phosphate) and tap water was provided ad libitum. The dogs were confined to individual cages maintained at 21°C on a 12-hour light-dark cycle. Prior to instrumentation the dogs were trained to stand quietly using a frame constructed in our department.

Surgical procedure

One hour after premedication (1 mg/kg s.c. morphine, Heilmittelwerke, Vienna, Austria), chronic instrumentation was performed under pentobarbital anesthesia (25 mg/kg i.v., Gatt, Vienna, Austria) and artificial respiration (O_2/NO_2 mixture with 1.7% enflurane, Abbott, Vienna, Austria; using an Engstroem Respirator 300, Bromma, Sweden). A skin incision was made in the left carotid area and both the left carotid artery and jugular vein were mobilized. Tygon catheters (Ögussa, Vienna, Austria) were inserted in the descending aorta and in the right atrium under X-ray control, allowing measurement of phasic arterial blood pressure (P_{ART}) and right atrial pressure (P_{RA}), respectively. Both catheters were filled with heparinized saline solution, exteriorized between the scapulae and covered by a jacket (Tubigrip® J, Bständig, Vienna, Austria) worn by the animal. Each dog was given buprenorphin-hydrochloride (0.3 mg s.c., Boehringer-Mannheim, Germany) at 8-hour intervals as required over the 24-hour postoperative period and ampicillin (500 mg s.c., Bayer-Austria, Vienna) in the morning immediately before and on the first 3 days after surgery. The dogs recovered completely within a couple of days. The investigations were commenced 2 weeks after chronic instrumentation.

Measurements

Arterial blood pressure (P_{ART}) and mean atrial blood pressure (P_{RA}) were measured by connecting appropriate catheters to CP-01 pressure transducers (Hugo Sachs Elektronik, March, Germany) and displayed on a recorder (Graphtec-Linearcorder mark VII WR 301, Hugo Sachs Elektronik, March, Germany). In addition, these signals were fed into a 16-channel data acquisition system (Burr Brown corporation, Tucson, Arizona, USA) inter-

faced with a microcomputer (MAT 16 MHz, 80 Mb harddisc, Kolbinger Electronics, Breitenfurt, Austria). This system digitized and averaged data (using a Pascal program written in our department) over a 15 second period and calculated values for heart rate (HR), $P_{AS}/P_{AD}/P_{AM}$ (systolic/diastolic/mean aterial blood pressure) and P_{RA}. All data were stored on disc for subsequent review and analysis. Arterial blood samples were withdrawn from the aorta into heparinized tubes and N^G-nitro-L-arginine (L-NNA; Serva, Heidelberg, Germany) was measured by high performance liquid chromatography (HPLC).

Plasma N^G-nitro-L-arginine concentration

We used a high performance liquid chromatography (HPLC) system consisting of a L-6200 intelligent pump with a 655A-40 autosampler (Merck-Hitachi, Darmstadt, Germany), a 166 programmable detector (Beckmann, San Ramon, CA, USA) and a D-2500 integrator (Merck-Hitachi, Darmstadt, Germany). For chromatographic separation we used a μ- Bondapak C18 * 3.9 mm, 10 μm (Waters, Milford, MA, USA). The mobile phase was 0.01 N phosphoric acid (Merck, Darmstadt, Germany). The flow rate was 1.5 ml/min and the wavelength was 280 nm. HPLC analysis was performed by adding 50 μl 4.4 M perchloric acid (Merck, Darmstadt, Germany) to 500 μl plasma, which was vortex-mixed and centrifuged (16000 g) for 5 minutes. A standard concentration curve was prepared by adding known amounts (2.5– 40 μg/ml) of L-NNA to drug-free plasma.

Experimental design

The night before and throughout the experiment the dogs were fasted, but allowed free access to water. Baseline measurements of HR, P_{ART} and P_{RA} were made after a stabilization period which guaranteed that animals were in a steady state before experimentation started. 30 mg/kg N^G-nitro-L-arginine (L-NNA, Serva, Heidelberg, Germany) was given orally by means of a gelatin capsule. Over the next 3 hours all parameters were continuously recorded. The dogs were then returned to their cages. Measurements were taken again 6, 12 and 24 hours after oral drug administration, following a stabilization period of 30 minutes on each occasion. Blood samples were taken before and 1, 3, 6, 12 and 24 hours after the administration of L-NNA.

All measurements made after oral administration of L-NNA were compared for each animal with the respective pre-drug control. Values are expressed as mean $\pm$ SD. Statistical evaluation was carried out using the analysis of variance and the paired t-test; $p < 0.05$ was considered significant. A regression analysis was undertaken between plasma level of L-NNA and P_{AM}.

Results

Haemodynamic changes

The respective baseline values for heart rate (HR) and arterial blood pressure (P_{ART}) are shown in Table 1. The rise in PART started 15 to 45 min after oral administration of L-NNA (30 mg/kg) and reached its maximum at 3 h, coinciding with the maximum decrease in HR (Table 1 and Fig. 1). Diastolic blood pressure remained significantly increased throughout the 24 h observation period, whilst systolic blood pressure was raised only up to the 6 h value (Fig. 1). Mean arterial blood pressure (P_{AM}; mean $\pm$ SD, not shown in Figure) rose from a mean predrug value of 13.2 $\pm$ 1.0 kPa to a maximum value of 17.4 $\pm$ 2.0 kPa ($p < 0.05$) at 3 hours and remained significantly increased until the 12 h value (15.0 $\pm$ 1.5 kPa, $p < 0.05$). The decrease in heart rate was significant up to 6 h. The loss of significance thereafter is accounted for by the fact

Table 1. Haemodynamic responses to oral N^G-nitro-L-arginine (30 mg/kg) in conscious dogs

Parameter	Absolut value before L-NNA	Delta values after L-NNA					
		1	2	3	6	12	24 h
HR beats/min	126 ± 12	−18 ± 10*	−14 ± 15	−20 ± 10*	−17 ± 9	−15 ± 19	−18 ± 23
P_{AS} kPa	17.3 ± 1.3	3.1 ± 3.3	3.6 ± 2.8*	3.7 ± 2.5*	2.1 ± 1.3*	0.8 ± 1.2	0.9 ± 1.7
P_{AD} kPa	9.2 ± 0.9	3.8 ± 2.9	4.5 ± 2.3*	4.7 ± 1.9**	3.6 ± 1.3**	2.7 ± 1.0**	1.4 ± 1.0*

Mean values ± SD; $n = 5$; *L-NNA* N^G-nitro-L-arginine; *HR* heart rate; P_{AS}/P_{AD} systolic/diastolic blood pressure; $*p < 0.05$, $**p < 0.01$ compared with the value before administration of L-NNA.

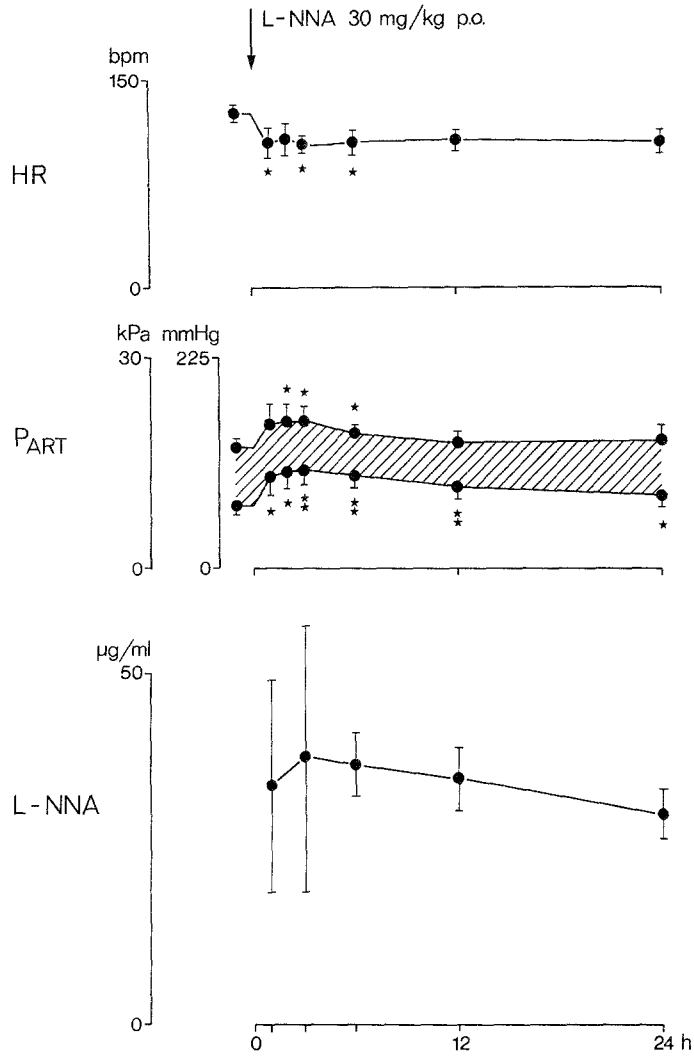

Fig. 1. Line graphs showing changes after oral (*p.o.*) administration of N^G-nitro-L-arginine (L-NNA; 30 mg/kg) in conscious dogs ($n = 5$). *Upper panel*: heart rate, *HR*; *middle panel*: systolic and diastolic arterial blood pressure, P_{ART}; *lower panel*: plasma level of L-NNA measured by high performance liquid chromatography, *L-NNA*; values are mean, bars indicate SD; $*p < 0.05$, $**p < 0.01$ compared with the value before administration of L-NNA

that one dog exhibited an increase in heart rate after 12 and 24 h (+12 and +16 beats/min, respectively), in spite of an elevated blood pressure. No changes occurred in right atrial pressure throughout the entire experiment.

Administration of vehicle (i.e. a gelatin capsule without L-NNA) caused neither haemodynamic nor other effects.

Plasma N^G-nitro-L-arginine concentration in relation to haemodynamic changes

The absence of L-NNA in the plasma prior to oral drug administration was confirmed by HPLC. At the end of the experiment (i.e. after 24 h) plasma L-NNA

level still amounted to 29.9 ± 3.6 μg/ml, which is a decrease of only 21.7% of its maximum value of 38.2 ± 18.8 μg/ml at 3 h (Fig. 1). The increase in P_{AM} also reached its maximum (+4.2 ± 1.0 kPa, $p < 0.05$) at 3 h. However, the maximum increase in P_{AM} then declined to a value of only +1.2 ± 0.6 kPa ($p < 0.05$) at 24 h, representing a diminution of 72.4% from the maximum rise. The initial individual changes of the plasma levels of L-NNA were significantly correlated with the individual changes of P_{AM} ($r = 0.684$, $p < 0.05$) until maximum values were reached at 3 h. In contrast, no correlation was detected between changes in the plasma level of L-NNA and changes in P_{AM} from 3 to 24 h ($r = 0.257$, $p < 0.356$; Fig. 2).

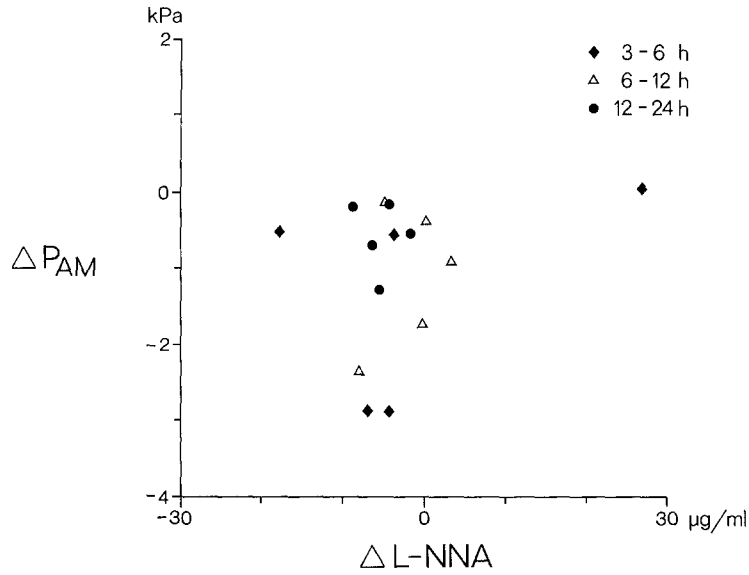

Fig. 2. Scatterplot of individual changes in plasma levels of N^G-nitro-L-arginine (*L-NNA*) and respective individual changes in mean arterial blood pressure (P_{AM}) in conscious dogs ($n = 5$). Changes between: 3 and 6 hours solid diamond (◆) ; 6 and 12 hours open triangle (△) and 12 and 24 solid circle (●); no correlation was found ($r = 0.257$, $p = 0.356$)

No changes in behavior were observed in any dog throughout the entire experiment.

Discussion

This study shows for the first time that *oral* administration of N^G-nitro-L-arginine (L-NNA) exerts a sustained increase in blood pressure in the *conscious dog*. In addition, the time course analyzed was 24 h and data are presented on plasma levels of L-NNA, which have not been determined so far. Our findings of a marked hypertensive response to oral L-NNA associated with bradycardia, are consistent with observations on the cardiovascular response to i.v. N^G-nitro-L-arginine methyl ester (L-NAME) and N^G-monomethyl-L-arginine (L-NMMA) in anaesthetized and conscious rats [10, 16, 17].

The increase in blood pressure is certainly the consequence of a rise in total peripheral resistance due to local vasoconstriction [16, 17]. Circulatory control mechanism involving an L-arginine: NO pathway at CNS sites [20] could take part in the attenuation of the hypertensive response following oral administration of L-NNA. Although this assumption cannot be excluded an efferent control of blood pressure involving the L-arginine: NO pathway seems unlikely since the intracerebroventricular administration of L-NAME failed to exert an increase in blood pressure [21].

Although the maximum plasma L-NNA increase was attenuated by only 21.7% at 24 h, the maximum increase in mean arterial blood pressure was diminished by 72.2% at the correspondening time. The fact that the arterial blood pressure did not correlate with the plasma L-NNA concentration after maximum hypertensive effects were reached, seems to be of special interest. In contrast, Gardiner et al. [22] reported that hypertension and increased vasoconstriction were sustained over the entire observation period (i.e. 9 h) in conscious, vasopressin-deficient rats receiving L-NAME or L-NMMA in their drinking water. This mode of administration, however, allows a more or less continuous uptake of L-NAME and, thus, probably leads to a progressive increase in plasma concentration which could be sufficient to maintain the blood pressure increase. The lack of correlation in our study between the changes in mean aterial blood pressure and the changes in plasma L-NNA levels between 3 and 24 h (Fig. 2) makes it likely that counterregulatory responses could account for the attenuated hypertensive effect.

Hypertension was associated with bradycardia throughout the 24 h observation period. This finding is consistent with the results of recent studies on the effects of i.v. injection of L-NAME or L-NMMA in conscious rats [16, 17] but suggests in contrast to findings in vasopressin-deficient rats [22], that oral L-NNA does not interfere with cardiac baroreflex control in the conscious dog.

Although right atrial blood pressure did not show any consistent changes following a single oral dose of L-NNA, no conclusions can be drawn as to the importance of the L-arginine: NO pathway in the control of venous tone, especially in the presence of an elevated blood pressure caused by an increase in afterload [16].

The findings of our present study provide further evidence for an important physiological role of basal nitric oxide biosynthesis in normal blood pressure homeostasis, but also stress the importance of other homeostatic mechanisms.

Acknowledgement

The authers would like to thank Anton Karel, Karl Koppatz, Peter Krivanek and Eva Kotai and for excellent technical assistence, Gertrude Brugger for skilled graphical work and Dr. L. Kastner for the correction of the English text.

All animals used in this investigation were handled in accordance with the animal welfare regulations of the University of Vienna and the study protocol was approved by the Animals Subjects Comittee of this institution and the Austrian Ministry of Science.

This study was supported by the 'Fonds 600 Jahre Wiener Universität der Kammer der Gewerblichen Wirtschaft, Vienna, Austria' and 'Medizinisch-wissenschaftlicher Fonds des Bürgermeisters der Bundeshauptstadt Wien'.

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Received August 18, 1991