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Arginine NO-dependent and NO-independent effects on hemodynamics

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ABSTRACT

L-arginine administration decreases mean arterial blood pressure (MABP), presumably by excess nitric oxide (NO) synthesis. However, some reports indicate that D-arginine, not a substrate of NO synthase (NOS), also induces hypotension. To clarify this phenomenon, the hemodynamic effects of L- and D-arginine and their modification by NOS inhibition with L-nitroarginine methyl ester (L-NAME) were assessed. MABP, cardiac output, stroke volume, heart rate and systemic vascular resistance were recorded in Sprague-Dawley rats under urethane or ketamine/diazepam anesthesia, with or without blockade of NO synthesis by L-NAME. Both stereoisomers of arginine induced a dose-related drop in MABP of similar magnitude and time course, but recovery from hypotension was slower in L-arginine than in D-arginine. The hypotension induced by both stereoisomers was due to a decrease in systemic vascular resistance (SVR) with increase in cardiac output (CO) and stroke volume (SV). Administration of L-NAME induced a pronounced increase in MABP and SVR, with decreases in CO and heart rate (HR). Infusion of L-arginine after L-NAME significantly decreased MABP and SVR at the highest dose while D-arginine failed to do so. After L-NAME, MABP was significantly lower under L-arginine than under D-arginine at all doses. These experiments suggest a dual mechanism in the hypotensive effect of L-arginine: a NO independent action on vascular resistance shared with D-arginine, and a NO dependent mechanism that becomes evident in the presence of NOS inhibition with L-NAME. Cardiac effects of NO do not appear to play a role in L-arginine hypotension.

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1. Introduction

Activation of nitric oxide synthase (NOS) in the vascular endothelium leads to conversion of L-arginine to L-citrulline with release of NO (Palmer et al., 1987). This gas relaxes vascular smooth muscle through formation of cyclic guanylate phosphate (Katsuki and Murad, 1977; Grueter et al., 1979). Administration of L-nitroarginine methyl ester (L-NAME), and other competitive inhibitors of NOS, induces a pronounced increase of arterial blood pressure (ABP), supporting the hypothesis that a sustained release of NO contributes to establish the level of ABP (Rees et al., 1989b). The potential role of L-arginine dietary supplementation or parenteral administration in the treatment of arterial hypertension rests on the premise that blood and tissue levels of this amino acid are rate limiting, under in vivo conditions, for the production of the vasodilator gas nitric oxide (NO). Given that the Km of NOS for L-arginine has been estimated between 1.5 and 3 μM in brain (Bredt and Snyder, 1990) and peripheral tissues (Jaing et al., 1996) while circulating L-arginine concentrations range from 50 to 200 μM with values even higher within endothelial cells (Hardy and May, 2002) it seems unlikely that the infusion of exogenous L-arginine could induce effects through enhanced production of NO. In spite of this fact, some authors have found that exogenous L-arginine administration decreases ABP in normal and hypertensive human subjects and in experimental animals (Nakaki et al., 1990; Calver et al., 1991), a phenomenon that has been ascribed to a "paradoxical" enhancement of NO production through NOS. This fact remains controversial because others have failed to induce changes in arterial blood pressure or heart rate in anesthetized rats by infusion of L-arginine (Rees et al., 1990). Even for the experimental conditions under which L-arginine hypotension has been documented, it is not clear if the effect is related to NO production (Jun and Wennmalm, 1994).

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The hemodynamic effects of both stereoisomers of arginine and their kinetics have not been fully characterized; nevertheless they could help to understand the mechanism mediating the hypotensive effect in both cases. Experiments in vitro have shown that NO generation by endogenous production or administration of nitrovasodilators decreases myocyte contractility (Brady et al., 1993) and in vivo experiments have indicated attenuation of beta-adrenergic positive inotropic action by NO endogenous generation (Hare and Colucci, 1995). However, the hypothesis that the hypotensive effects of L-arginine might be in part due to a depression of myocardial contractility with the consequent decrease of stroke volume and cardiac output has never been tested.

These experiments were undertaken to establish the kinetics of MABP changes induced by intravenous infusion of L-arginine and D-arginine in anesthetized rats and to test the ability of L-NAME to block the hemodynamic effects of both stereoisomers of arginine. In addition, a dose effect study of both enantiomers of arginine on cardiac output, stroke volume, total vascular resistance, arterial blood pressure and heart rate was undertaken to detect any differential effects on these variables.

2. Materials and methods

2.1. Animals

Female Sprague-Dawley rats, 250–300 g body mass, were used. Animals were anesthesized with 1.5 g/kg urethane or 50 mg/kg ketamine plus 5 mg/kg diazepam administered intraperitoneally. Additional doses were given as necessary to maintain an adequate level of anesthesia as determined by the absence of withdrawal reflex to hindpaw pinch. All procedures were approved by the Bioethics Committee and were carried out in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH Pub. no. 85-23, Revised 1996).

2.2. Drugs

L-arginine, D-arginine and L-NAME were obtained from the Sigma-Aldrich Chemical Co. L-arginine and D-arginine were administered intravenously by continuous infusion at the rates of 0.3, 0.6 or 1.2 mmol/kg/min during 3 min at the fluid volume rate of 0.3 ml/min. L-NAME was dissolved in saline at a concentration of 20 mg/ml and intravenously injected in bolus in a dose of 20 mg/kg.

2.3. Procedures

The femoral artery and vein on one side were cannulated with PE-50 polyethylene and 0.51 mm inner diameter, 0.94 outer diameter silastic catheters respectively. Arterial blood pressure was continuously sampled from the arterial catheter with a transducer and carrier amplifier (World Precision Instruments, Inc., Sarasota, FL). Mean arterial blood pressure (MABP) and heart rate (HR) were calculated on line from the arterial blood pressure recordings by Labchart 7 software (ADInstruments, Inc., Colorado Springs, CO). Rectal temperature (TEMP) was recorded with a T-type thermocouple thermometer (BAT 12 Electronic Thermometer, Physitemp Instruments Inc., Clifton, NJ). Cardiac output (CO) was measured by thermodilution as follows. A silastic catheter was advanced through the external jugular vein into the right atrium. A fast thermocouple probe (IT-1E, Physitemp Instruments Inc., Clifton, NJ) was introduced into the external carotid artery and advanced to the thoracic aorta. The probe was connected to a thermocouple thermometer (BAT 12 Electronic Thermometer, Physitemp Instruments, Clifton, NJ) interfaced through an analog output to the digitizer. A bolus of 100 μL of cold saline (21–22 °C) was injected through the atrial catheter and the temperature change detected by the thoracic aorta probe was recorded and analyzed off line with LabChart 7 software (ADInstruments, Inc.). MABP and HR derived on line from the arterial blood pressure signals were averaged over 1 min at the end of 3 min of intravenous infusions of L- or D-arginine at the rates of 0.3, 0.6 or 1.2 mmol/kg/min. CO was measured during the last 10 s of the 3 min of drug or vehicle infusion. Systemic vascular resistance (SVR) was calculated by dividing MABP by CO and stroke volume (SV) by dividing CO by heart rate.

2.4. Statistical analysis

Results are reported as means ± standard error. A repeated measures analysis of variance with the within factor "dose" (0, 0.3, 0.6 or 1.2 mmol/kg/min) and the between factor "enantiomer" (L or D-arginine) was performed for every variable and if the F-ratio for main effects of drugs was significant, then a multiple comparisons procedure (Dunnett's two-sided multiple-comparison test) was used to assess significance of differences between means of different doses and the corresponding controls with vehicle infusion. A probability level was set at $P<0.05$ to declare a difference as significant. The time courses of MABP for the periods of time during and immediately after infusion of L- or D-arginine were analyzed by non-linear regression with model equations $MABP = A*\exp(-B*t)+C$ during infusions (MABP dropping) and $MABP = 1-(A*\exp(-B*t))+C$ immediately after cessation of infusions (MABP recovering), where A = Initial − Final MABP (during infusions) and A = Final − Initial MABP (immediately after cessation of infusions); B = Rate constant (reciprocal of time) and C = Final MABP at the end of infusion or recovery.

3. Results

In the experiments under urethane anesthesia, infusion of saline at the same volume rate used for drug solutions did not induce changes in any of the variables under study and the values during this period were taken as the control condition. Infusion of both L- and D-arginine induced highly significant dose-related decreases in MABP and SVR ($n=6$ per group). However, ANOVA did not indicate significance for the factor "enantiomer" implying a similar effect on these variables in both cases (Fig. 1 and Table 1). Increases in CO and SV were also significant in the case of L-arginine and showed a similar, although not statistically significant, increase in the case of D-arginine (Fig. 1 and Table 1).

Administration of L-NAME induced a pronounced significant increase in MABP and SVR, with decreases in CO, SV and HR (Fig. 1 and Table 1). Administration of L-arginine after L-NAME significantly decreased MABP and SVR in a dose-dependent manner while D-arginine induced a marginally significant small decrease in SVR and a non-significant decrease in MABP only at the highest dose. After L-NAME administration, MABP was significantly lower under L-arginine than under D-arginine at all doses, while SVR was lower under L-arginine than under D-arginine at the two highest doses (Fig. 1 and Table 1).

The kinetics of the MABP drop induced by both stereoisomers of arginine, and the recovery period after cessation of infusions were analyzed in detail at the dose of 1.2 mmol/kg/min (Figs. 2 and 3). The data from beginning to end of infusions of L- or D-arginine and the period of time immediately following their cessation were fitted to mono-exponential models for each animal. Group averages ($n=6$ per group) for each stereoisomer indicated similar rate constants for MABP drop (L-arginine = 5.01 ± 0.96 min^{-1} and D-arginine = 5.73 ± 0.55 min^{-1}) (Table 2, drop parameter B). However, recovery from

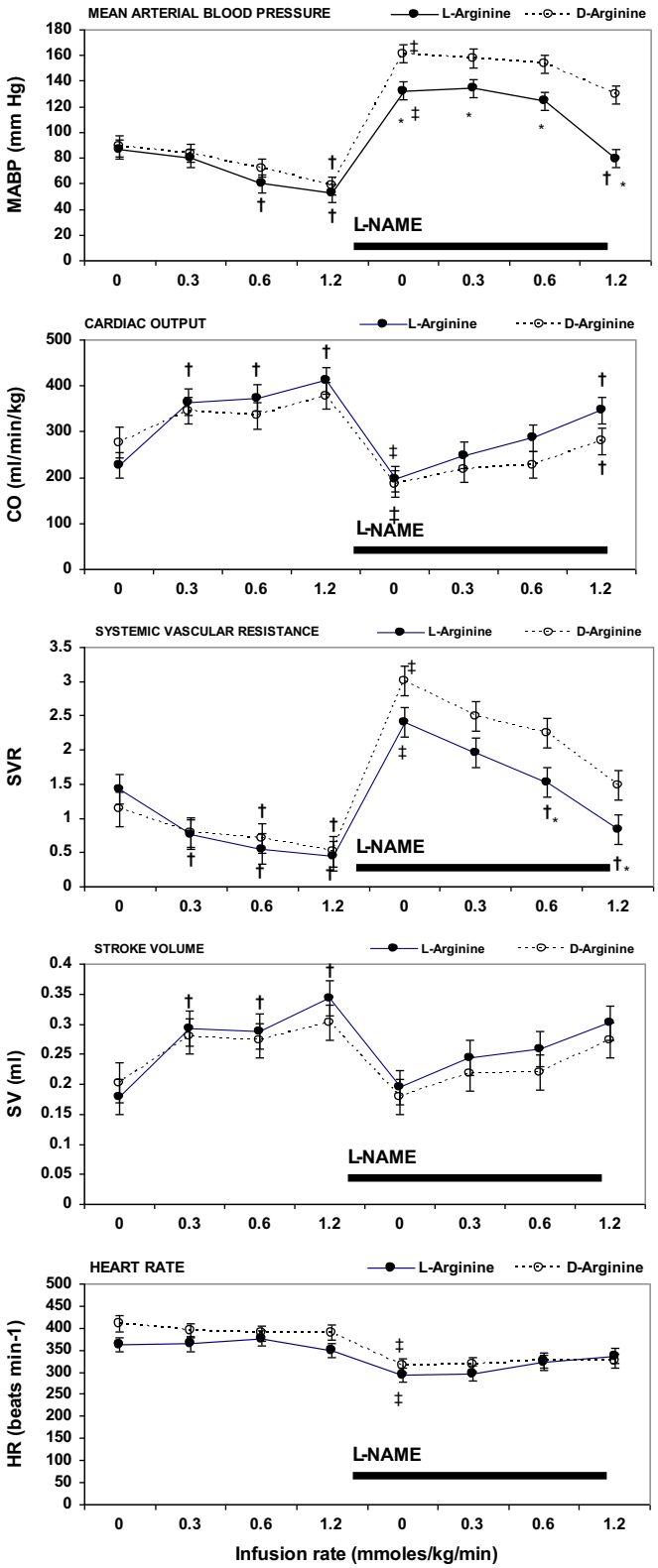

Fig. 1. Mean arterial blood pressure (MABP, mmHg), cardiac output (CO, ml/kg/min), systemic vascular resistance (SVR, mmHg/(ml/kg/min)), stroke volume (SV, ml) and heart rate (HR, beats/min) for the control condition and incremental doses of L- or D-arginine before (left panels) and after (right panels) administration of L-NAME, 20 mg/kg intravenously. Values are means ± S.E.M ($n=6$ per group) over the last minute of a 3 min infusion period. Symbols represent statistically significant differences ($P<0.05$) between L- and D-arginine at the same dose (∗), between a given dose and the control condition (†) or between before and after L-NAME without L- or D-arginine (‡). Experimental results shown were obtained under urethane anesthesia (1.5 g/kg, i.p.).

Table 1
Statistical significance (probability) of ANOVA F-ratios for the factors "enantiomer" (L- or D-arginine) and "dose" (0, 0.3, 0.6 and 1.2 mmol/kg/min).

Variables	Pre-L-NAME			Post-L-NAME		
	Enantiomer factor	Dose factor		Enantiomer factor	Dose factor	
		L-Arg.	D-Arg.		L-Arg.	D-Arg.
MABP	N.S.	0.004	0.003	0.0002	0.003	N.S.
CO	N.S.	0.0008	N.S.	0.002	0.03	N.S.
SV	N.S.	0.002	N.S.	0.04	N.S.	N.S.
SVR	N.S.	< 0.00-01	0.007	< 0.0001	0.009	0.05
HR	0.04	N.S.	N.S.	0.038	0.004	N.S.

MABP: arterial blood pressure, CO: cardiac output, SV: stroke volume, SVR: systemic vascular resistance, HR: heart rate, N.S.: no significance, and $N=6$ per group.

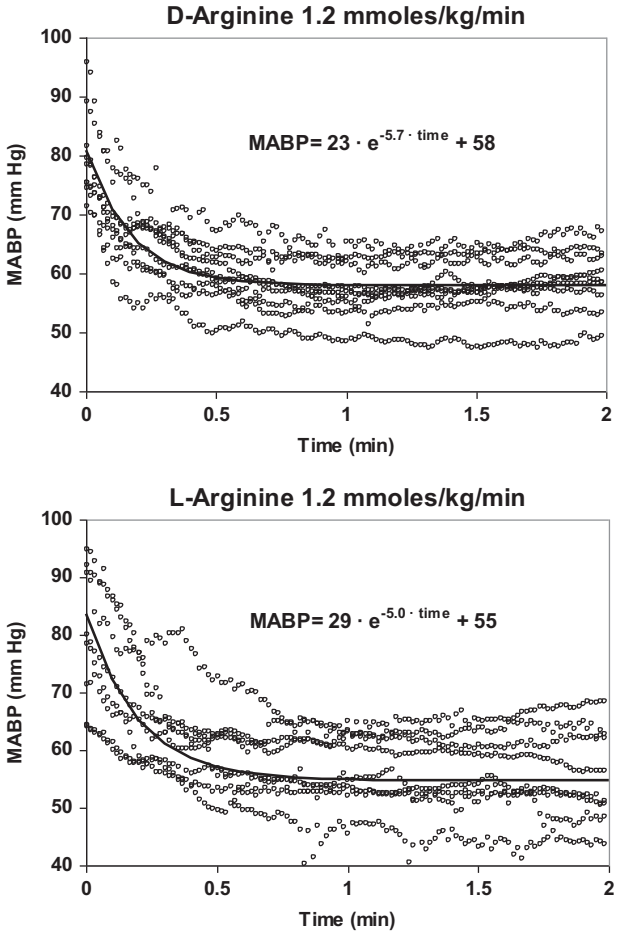

Fig. 2. Time course of MABP during infusion of D-arginine (top panel) and L-arginine (bottom panel). The continuous line represents the best fit of all data to the equation $MABP=A*\exp(-B*t)+C$. All individual experiments are shown as dot lines. None of the parameters of the curve fits differed significantly between L- and D-arginine. Experimental results shown were obtained under urethane anesthesia (1.5 g/kg, i.p.). Number of animals was 6 per group.

hypotension as quantified by the rate constants (Table 2, recovery parameter B) was slower for L-arginine (0.51 ± 0.11 min^{-1}) than for D-arginine (1.47 ± 0.23 min^{-1}), $P=0.003$.

In order to determine if the results described above were dependent on the use of urethane as an anesthetic, another series of experiments was undertaken in which animals were anesthetized with ketamine/diazepam. L-arginine or D-arginine were infused during 3 min at the rate of 1.2 mmol/kg/min, $n=5$ per group. The

hypotensive effect of both isomers was present, under ketamine/diazepam, at approximately the same magnitude as observed before under urethane (Fig. 4, bottom panels). Heart rate was not changed by L-arginine infusion (Fig. 4, top panels). However, D-arginine infusion induced a significant tachycardia that was not altered by the administration of L-NAME (Fig. 4, top panels).

Following the first period of L- or D-arginine infusions, L-NAME was injected at the dose of 20 mg/kg. This arginine analog induced a pronounced increase in MABP in both groups (first data point in panels B of Fig. 4). After MABP reached a stable level a second period of L- or D-arginine infusion was started in order to test the ability of these isomers to antagonize the hypertensive effect of L-NAME. L-arginine induced a progressive decrease in MABP that by the end of the infusion period completely reversed the effect of L-NAME (Fig. 4B). On the other hand, infusion of D-arginine under L-NAME induced a significant but small decrease in MABP that did not change as infusion progressed in time (Fig. 4B).

After a second dose of L-NAME, L-arginine infusion still induced a significant decrease in MABP (Fig. 4C) while no effect was observed by D-arginine infusion. A third dose of L-NAME completely prevented the hypotensive effect of L- and D-arginine (Fig. 4D).

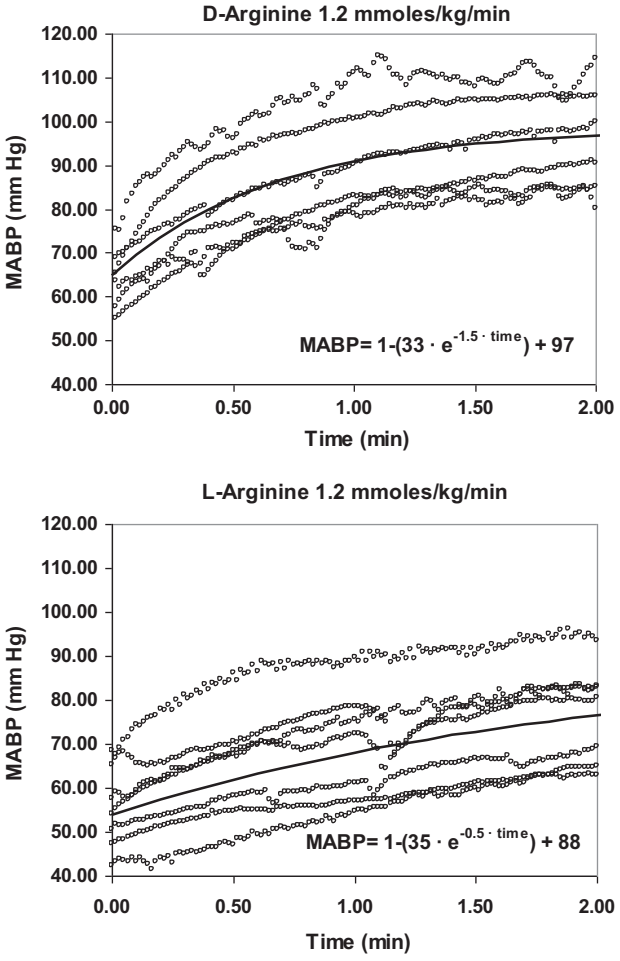

Fig. 3. Time course of MABP immediately after cessation of D-arginine (top panel) and L-arginine (bottom panel) infusions. The continuous line represents the best fit of all data to the equation $MABP = 1-(A^*\exp(-B^*t))+C$. All individual experiments are shown as dot lines. Parameter B (rate constant) was significantly lower in L-arginine than in D-arginine ($P=0.0034$). Experimental results shown were obtained under urethane anesthesia (1.5 g/kg, i.p.). Number of animals was 6 per group.

4. Discussion

Previous accounts on the effects of L- or D-arginine on arterial blood pressure in normotensive experimental animals are fragmentary and often contradictory. Murakami et al. reported lack of hypotension on administration of L-arginine at a low infusion rate in dogs but found an increase in renal blood flow. They did not test for a D-arginine effect (Murakami et al., 1991). Fineman et al. reported no effect of L-arginine on arterial blood pressure but found enhancement of pulmonary blood flow with no effect of D-arginine in this vascular bed (Fineman et al., 1991).

Jun and Wennmalm showed that both L- and D-arginine intravenous infusions elicited hypotension and concluded that there was no intervention of NOS activity on L-arginine hypotension due to a similar effect of D-arginine (Jun and Wennmalm, 1994). We have confirmed the general results of that pioneering work and expanded it by measuring CO and SVR and the kinetics of L- and D-arginine hypotension and its recovery that indicates some differences in the hemodynamic effects of both isomers. Rees et al. found that the hypertensive effect of the NOS inhibitor N^G-monomethyl-L-arginine (L-NMMA), was reversed by L- but not D-arginine (Rees et al., 1989a), an effect also shown for L-NAME (Rees et al., 1990). They also mentioned that L-arginine did not induce hypotensive effects in the absence of NOS inhibitors although no data was shown. Cernadas et al. showed a transient and very marked hypotensive effect of an intravenous bolus of L-arginine but provided no measurements of CO, SVR or kinetics. They mentioned that D-arginine did not induce hypotension but provided no data for this lack of effect (Cernadas et al., 1992). Regarding local effects on blood vessels, it has been reported that the vasodilatation induced by local infusion of L-arginine in the brachial artery of human volunteers can also be observed with infusion of D-arginine, which is not a substrate of NOS (Calver et al., 1991) suggesting again that the local dilator effect of an excess of L-arginine may not be related to NO production.

Our present work indicates that in the absence of L-NAME treatment, both stereoisomers of arginine induce a dose related drop of MABP. These results are in agreement with a previous report claiming lack of evidence for the intervention of NO synthesis in the L-arginine induced hypotension under similar experimental conditions (Jun and Wennmalm, 1994). Moreover, by measuring CO, we have demonstrated that the hypotensive effect of both isomers of arginine is associated with a decrease in SVR

Table 2
Parameters of monoexponential models[a] for drop and recovery of MABP during and after 1.2 mmol/kg/min D- or L-arginine infusion (Mean ± S.E.).

	MABP recovery parameters				MABP drop parameters			
	A	*B*	*C*	*n*	*A*	*B*	*C*	*n*
D-arginine	33.62 ± 2.31	1.47 ± 0.23	97.51 ± 3.99	7	22.89 ± 1.13	5.73 ± 0.55	57.93 ± 1.68	6
L-arginine	35.32 ± 5.04	0.51 ± 0.11	88.18 ± 7.07	9	28.98 ± 3.93	5.01 ± 0.96	54.71 ± 2.55	6
Probability	0.77	0.003	0.28		0.12	0.50	0.29	

[a] Models are described under methods. Parameters are A=Final MABP − Initial MABP (recovery) and Initial MABP − Final MABP (drop); B=Rate constant (reciprocal of time); C=Final MABP. Statistical significance of differences between parameters for D- and L-arginine (probability) was calculated by two-sample homoscedastic t-tests.

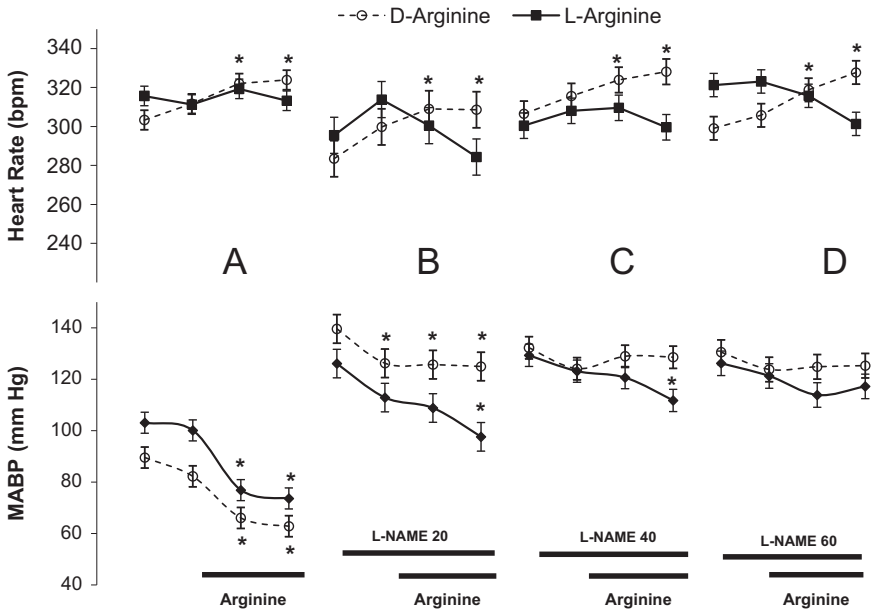

Fig. 4. Heart rate (HR, beats/min, top panels) and mean arterial blood pressure (MABP, mmHg, bottom panels), averaged over the preceding minute period and every minute during a continuous 3 min infusion of L-arginine (continuous lines) or D-arginine (dashed lines) at a rate of 1.2 mmol/kg/min before (A) and after (B–D) incremental cumulative doses of L-NAME. Horizontal bars represent the time during which L- or D-arginine were infused. L-NAME was injected as a bolus before the 2nd (B), 3rd (C) and 4th (D) period of L- or D-arginine infusions. (*) Statistical significance ($P < 0.05$) between means of arginine infusion and the preceding minute before infusion started. Experimental results shown were obtained under ketamine (50 mg/kg) and diazepam (5/mg/kg). Number of animals was 6 per group.

and increases in SV and CO, pointing to a common mechanism of action independent of NO generation, given the stereospecificity of this phenomenon

However, the kinetics of L- and D-arginine action indicated a lower recovery rate from the hypotension induced by L-arginine than that induced by D-arginine. Moreover, the hypertensive effect of L-NAME was lower in rats previously treated with L-arginine and dose escalation of this isomer could antagonize the L-NAME hypertension while D-arginine could not. It thus appears that although both stereoisomers might share a common mechanism of action that reduces SVR independently of NOS activity, a contribution of enhanced NO synthesis to the hypotensive effect of L-arginine might explain its ability to antagonize the stereospecific hypertensive effect of L-NAME and the slower recovery rate from the arterial hypotension it elicits in the absence of L-NAME.The experiments under ketamine/diazepam confirmed the differential effects of L-and D-arginine on MABP after administration of L-NAME. It is possible that the NO-dependent and NO-independent vasodilatory actions of L-arginine (shared with D-arginine) might reside in different vascular beds. Dallinger et al. have reported an increase in renal plasma flow measured with para-aminohippurate clearance with L-arginine but not D-arginine, but a similar increase induced by both isomers in mean flow velocity of the ophthalmic artery measured by ultrasonography (Dallinger et al., 2003). It is also possible that a general NO-independent mechanism of arginine on MABP may exist in the circulation of all organs while a NO-dependent mechanism may reside in a few of them. Such a NO-independent general mechanism of arginine has been suggested by Walter et al. These authors reported that both L- and D-arginine at high concentrations, as used in all experiments showing hypotensive effects of L- or D-arginine, decrease low-shear blood viscosity in vitro (Walter et al., 2000). If this phenomenon occurs in vivo, such an effect would decrease systemic vascular resistance independently of NO effects.

The hypertension induced by L-NAME and other NOS inhibitors is generally ascribed to a decrease in the release of NO in the environment of peripheral resistance vessels. However, there is a possibility that the L-NAME induced hypertension might be due to enhanced sympathetic tone, the inhibition of which by NO might in part explain its hypotensive effect (Zanzinger et al., 1994; Biancardi et al., 2007). However, recent evidence has indicated lack of sympathetic overactivity during prolonged L-NAME treatment (Dos Santos et al., 2010) casting doubt on that idea.

We have used female rats because we are encouraged by Funding Agencies and Institutions to use females in study populations to limit the currently predominant use of males. Sex hormones and gender have important peripheral and central effects on modulation of cardiovascular responses to stress (Regitz-Zagrosek et al., 2013), the function of the renin–angiotensin system (Xue et al., 2013) and other levels of cardiovascular function (Maranon and Reckelhoff, 2013). However, there is no particular reason to favor males in cardiovascular studies that are not directed to the analysis of interactions between sex hormones and cardiovascular function, except for a possible variation in cardiovascular variables between phases of the estrous cycle. In this regard, studies in rats have not convincingly shown an influence of the normal estrous cycle on baseline arterial blood pressure values. The study by Takezawa et al. (1994), for instance, showed average variations of less than 2 mmHg between phases of the estrous cycle in conscious, behaving animals instrumented by telemetry. Such minimal variations were correlated with and probably caused by activity. It is unlikely that any differences would be found in anesthetized animals.

The tachycardia induced by D-arginine infusion in animals anesthetized with ketamine/diazepam, most likely a baroreceptor mediated response, was in contrast to the lack of such phenomenon under L-arginine infusion. Tachycardia in response to hypotension is a well documented component of the baroreceptor reflex. It has been shown that inhibition of NO synthase by inhibiting NO production increases the gain of the baroreceptor heart rate response and that L-arginine, but not D-arginine, can antagonize this effect (Liu et al., 1996). This might explain why tachycardia is observed with D-arginine induced hypotension but not with L-arginine hypotension. The fact that tachycardia is not observed with urethane anesthesia may relate to the depression of such response in this type of anesthesia (Stornetta et al., 1987) in

contrast to preservation of baroreflexes with ketamine (Hoka et al., 1988).

The results of our experiments provided no indication of a difference in cardiac output between the two arginine isomers, giving no support to the hypothesis that at least part of the hypotensive effect of L-arginine could be due to a depression of cardiac contractility by NO that other authors have found in different preparations (Brady et al., 1993; Hare and Colucci, 1995).

In conclusion, our results suggest a dual mechanism in the hypotensive effect of L-arginine: a NO independent action on vascular resistance shared with D-arginine, and a NO dependent mechanism that becomes evident in the presence on NOS inhibition with L-NAME. The cardiac effects of NO do not appear to play a role in L-arginine hypotension.

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