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Effects of L-arginine on endothelial and cardiac function in rats with heart failure

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Abstract

We examined the effects of chronic oral L-arginine treatment on endothelial and cardiovascular function in rats with heart failure induced by coronary artery ligation. Both heart failure and sham-operated rats were treated with either L-arginine in drinking water (12.5 or 50 g/l) or water placebo for 8 weeks following surgery. Plasma L-arginine levels in heart failure rats (153 ± 11 μM) were lower than sham rats (201 ± 13 μM, $P < 0.05$). The lower dose L-arginine treatment improved endothelium-dependent relaxation of isolated aortic rings of heart failure rats, while the higher dose of L-arginine treatment did not. Neither low nor high dose of L-arginine treatment improved hemodynamic parameters in heart failure rats. Thus, chronic oral L-arginine treatment at a dose of 12.5 g/l in drinking water improves endothelium-dependent relaxation, but fails to improve in vivo cardiac function in rats with heart failure.

Keywords: Heart failure; L-Arginine; Nitric oxide (NO); Endothelial function; Cardiovascular function

1. Introduction

Nitric oxide (NO) is a potent endogenous vasodilator synthesized from L-arginine by NO synthase and is released in response to many vascular stimuli, including acetylcholine (Palmer et al., 1988). NO release from endothelium plays an important role in the control of normal vascular function. In heart failure, however, endothelium-dependent relaxation to acetylcholine is impaired (Drexler et al., 1992; Teerlink et al., 1994; Feng et al., 1996c), and may contribute to increased vascular resistance in heart failure (Kubo et al., 1991; Ontkean et al., 1991; Drexler and Lu, 1992). This decreased endothelium-dependent relaxation in heart failure is associated with a decreased activity and mRNA expression of endothelial NO synthase (Feng et al., 1996c; Smith et al., 1996).

L-Arginine is a semi-essential amino acid which is involved in multiple biological processes. As the biological precursor of NO, L-arginine availability is a rate-limiting step for NO synthase (Baydoun et al., 1990; Mitchell et al., 1990; Sessa et al., 1990). Decreased L-arginine availability results in decreased NO production (Xia et al., 1996). Supplementation of L-arginine has been shown to normalize endothelium-dependent relaxation in heart failure in acute in vivo studies (Hirooka et al., 1994; Ogilvie and Zborowska-Sluis, 1995). We have demonstrated that an acute L-arginine treatment in organ bath in vitro restores endothelium-dependent relaxation in aortic rings of heart failure rats (Feng et al., 1998). These studies suggest that the decreased endothelium-dependent relaxation in heart failure may be due to decreased L-arginine availability at the vicinity of endothelial NO synthase enzyme (Forstermann et al., 1994) and that supplementation of the NO synthase substrate restores endothelial function. However, whether chronic L-arginine treatment improves cardiac function in heart failure remains to be determined.

Thus, the goal of this study was to evaluate the effectiveness of chronic oral L-arginine as a treatment directed at improving endothelium-dependent vasorelaxation and cardiac function in an animal model of heart failure induced by myocardial infarction.

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2. Methods

2.1. Animals

Animals used in this study were handled in accordance with the guidelines of the Animal Care Committee at the University of Western Ontario, Canada. All experiments were conducted on male Sprague–Dawley rats weighing 200–250 g. All animals were maintained on normal rat chow and given water ad libitum in a 12-h light–dark cycle. Animals were caged individually after surgical operation.

2.2. Heart failure rat model

Rats were randomly selected to undergo coronary artery ligation or sham surgery using the techniques similar to those previously described (Pfeffer et al., 1979). Rats were anaesthetized with sodium pentobarbital (50 mg/kg i.p.), then intubated and artificially ventilated with a respirator (SAR-830, CWE, Ardmore, PA). A left intercostal thoracotomy was performed. The chest was opened through the fourth intercostal space, ribs spread using a chest retractor, and the left side of the heart was exposed. After opening the pericardium, the proximal left coronary artery was ligated between the pulmonary out-flow tract and the left atrium by positioning a suture around the artery together with a small bundle of heart muscle that is transfixed with it (Feng et al., 1990; Feng et al., 1992). The lungs were thereafter hyper-inflated using positive end-expiratory pressures and the thorax was closed with three intercostal sutures. The muscle and skin layers were then closed. After coronary artery ligation approximately 30% of the rats died within several hours, those surviving this initial period had 11% mortality within 8 weeks. Sham-operated rats underwent the same surgery minus the coronary artery ligation. To relieve pain, buprenorphine (0.01 mg/kg, s.c.) was given to all animals right after surgery.

All experiments were performed 8 weeks post-surgery with L-arginine or placebo treatment. At the end of the experiments, the heart was dissected free of adjacent tissues, the ventricles were separated from the atria, and the right ventricular free wall was dissected from the septum. Right and left ventricle and atrial sections were blotted dry and weighed. Left ventricle volume was then measured by the weight of water injected into the left ventricle under atmospheric pressure.

2.3. Measurement of infarct size

The infarct size resulting from coronary artery ligation was evaluated by cutting the left ventricles of ligated hearts into four transverse slices of equal thickness from apex to base. Photographs were taken of these slices and the infarcted area was measured by tracing the endocardial circumferences of the fibrotic and normal areas on the photographs with a distance meter. Infarct size was expressed as a fraction of the total cross-sectional endocardial circumference of the left ventricle (Feng et al., 1992; Feng et al., 1996a). Rats with infarct size $> 30\%$ were included in this study.

2.4. Hemodynamic measurements

Rats were anaesthetized with sodium pentobarbital (50 mg/kg, i.p.) for catheter placements. The tail artery was cannulated with polyethylene tubing (PE-50) for the purposes of measuring mean arterial blood pressure and heart rate. A second PE-50 catheter was inserted into the left ventricle through the right carotid artery for measurement of left ventricle systolic and end-diastolic pressures as well as the maximal rate of pressure development $(+dP/dt)$ and rate of relaxation $(-dP/dt)$ of left ventricle. Pressures and heart rate measurements were taken using a Hewlett-Packard pressure transducer (model 1290A) connected to a pressure monitor and recorded on a Gould recorder (model 2400S).

Measurement of cardiac output (CO) required two separate cannulations. Firstly, a thermodilution probe (2.5F, Baxter, Mississauga, Ontario) was passed into the upper thoracic aorta via the right femoral artery so that the tip was at the aortic arch. Secondly, a PE-50 catheter was inserted into the superior vena cava via the right jugular vein for injection of heparinized saline (0.9% NaCl). Cardiac output measurements were obtained by injecting 0.2 cc of 20–22°C saline into the superior vena cava and detecting changes in aortic blood temperature by a cardiac output computer (American Edwards Laboratories, CA) via the thermodilution probe. Measurements were made in duplicate and normalized by dividing by the body weight of the individual rats. This normalized value of cardiac output is expressed as ml min^{-1} (100 g body weight)$^{-1}$.

All measurements were made both under anaesthesia allowing 30 min of stabilization and in conscious state approximately 2 to 3 h post-surgery at which time the animals were alert and able to execute coordinated movements. Hemodynamic measurements were obtained when the rats were resting quietly.

2.5. Organ bath study

Thoracic aortae were dissected free of fat and connective tissue. Care was taken to avoid endothelial damage. The vessels were cut into transverse ring segments of 4 mm in length, and mounted in a 5 ml organ bath containing aerated Krebs' solution maintained at 37°C. The Krebs' bicarbonate solution had the following composition (mM): NaCl, 118; $NaHCO_3$, 22; Glucose, 11; KCl, 4.7; $MgSO_4 \cdot 7H_2O$, 1.2; KH_2PO_4, 1.2; $CaCl_2$, 2.5. Krebs' in the bath was aerated with 95% O_2–5% CO_2, maintaining a pH of 7.4. Vessels were mounted at a resting tension of 1 g, and allowed to equilibrate for 1 h under these conditions before the experiment was begun.

Cumulative concentration-responses to acetylcholine and sodium nitroprusside were performed; acetylcholine was used to evaluate the endothelium-dependent relaxation of the vessel and sodium nitroprusside to evaluate the endothelium-independent relaxation of the vessel. Vessel rings were preconstricted with 10^{-5} M prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) and challenged with increasing amounts of either acetylcholine from 10^{-9} to 10^{-5} M, or sodium nitroprusside from 10^{-10} to 10^{-6} M. Responses were expressed as percent relaxation to $PGF_{2\alpha}$ constriction for each tissue. Between individual concentration–response curves, vessels were washed repeatedly with fresh aerated Krebs' solution and allowed to return to baseline tension.

2.6. L-Arginine treatment

L-Arginine treatment started immediately after coronary artery ligation or sham operation and continued for 8 weeks. In order to establish the dose of L-arginine being administered to the rats chronically, randomly selected sham and heart failure rats were given a stock mixture of either 12.5 or 50 g/l L-arginine free base as their drinking water and the consumption of said mixture was monitored. The amount of water consumed by both non-treated and L-arginine-treated rat groups was measured by weighing the water bottle and contents. These drinking water measurements were taken twice weekly and the average daily consumption was estimated by dividing the measured change in water present in the water bottle by the number of days since last measurement. L-Arginine consumption (g kg^{-1} day^{-1}) = daily water consumption × L-arginine concentration in drinking water/body weight. This calculation did not include L-arginine intake from the rat chow which contains 1.38% L-arginine. L-Arginine intake for an adult rat is 0.138–0.276 g L-arginine (10–20 g rat chow) per day.

2.7. Measurement of plasma L-arginine levels

At the end of 8-week L-arginine or placebo treatment, rats were anaesthetized with sodium pentobarbital (50 mg/kg i.p.) and the right carotid artery was cannulated with a PE-50 catheter for blood collection. Blood samples were collected in heparinized tubes, centrifuged for 15 min at $2000 \times g$, and plasma collected and stored at -80°C for future use. Samples of 0.2 ml heparinized plasma were deproteinized by addition of 0.3 ml sulfosalicylic acid solution, and centrifuged at $10{,}000 \times g$ for 5 min. The supernatant was filtered through a 0.22 μm filter prior to being analyzed for the presence of L-arginine by ion exchange chromatography in an automated amino acid analyzer.

2.8. Nitrate / nitrite assay

Plasma nitrate/nitrite levels were measured at the end of 8-week L-arginine or placebo treatment with a modification of a previously described assay (Grisham et al., 1995). Sodium nitrate ($NaNO_3$, Sigma) was diluted to yield a standard curve ranging from 5 to 50 μM. A 100 μl aliquot of sample, $NaNO_3$ standard, or water as blank were incubated at 37°C for 3 h in the presence of 0.2 units/ml *Aspergillus* nitrate reductase (Boehringer Mannheim), 50 mM Hepes buffer, pH 8.4 (Sigma), 0.5 μM flavin adenine dinucleotide (FAD, Sigma), and 0.1 mM nicotinamide adenine dinucleotide phosphate (NADPH, Sigma). Following incubation, 5 μl of 1500 U/ml lactic dehydrogenase (LDH, Sigma) and 50 μl 100 mM pyruvic acid (Sigma) were added and the mixture was incubated for 10 min at 37°C. Some 50 μl 10 mg/ml protamine sulphate (Sigma) was then added to each tube to remove heparin. Samples were vortexed, incubated for 5 min at room temperature, and centrifuged for 10 min at $10{,}000 \times g$ to remove the precipitate. A total of 500 μl of the supernatant was transferred to glass culture tubes and 1 ml Griess reagent (equivolumes of 0.2% naphthylethylenediamine and 2% sulfanilamide in 5% phosphoric acid) was added. After 10 min incubation at room temperature, the absorbance at 543 nm was read. All samples were done in duplicate.

2.9. Statistical analysis

Data were expressed as the means ± S.E.M. EC_{50} values were calculated with GraphPad Prism using non-linear regression. Analysis of variance analyses (ANOVA) were performed with Fisher's LSD post hoc test to detect significance between the multiple groups. Differences were considered significant at the level of $P \leq 0.05$.

3. Results

3.1. General characteristics of heart failure

In rats following coronary artery ligation, myocardial infarction was approximately 40% of the left ventricle (Table 1). There was no significant difference in the infarct sizes in any heart failure groups (P = n.s.). Scar tissue, ventricular wall thinning in the infarcted area, cardiac dilatation, as well as hypertrophy of the non-infarcted ventricular muscle characteristic of heart failure were all obvious in the infarcted hearts compared to those of sham.

The heart weights standardized by body weight of heart failure rats were significantly increased compared to sham ($P < 0.01$), this result was uniform throughout the heart as it was seen in atria, and both the right and left ventricles (Table 1). Similarly, the left ventricular volume of heart failure rats was also significantly increased compared to sham ($P < 0.05$). However, neither low dose nor high dose chronic L-arginine treatment affected any of these parameters (P = n.s.).

Table 1
Effects of chronic L-arginine treatment on hemodynamic changes in sham and heart failure rats. LV, left ventricle; CI, cardiac index; LVEDP, left ventricular end-diastolic pressure; LVSP, left ventricle systolic pressure; MAP, mean arterial pressure. Values are expressed as means ± S.E.M.

	Untreated		Low dose L-arginine		High dose L-arginine	
	Sham	Heart failure	Sham	Heart failure	Sham	Heart failure
n	11	11	5	9	5	9
Infarct size (%)	–	40.2 ± 1.3	–	38.2 ± 1.2	–	40.8 ± 1.8
Weights						
Body (g)	509 ± 13	500 ± 13	493 ± 19	481 ± 12	452 ± 8	475 ± 11
Heart/body (mg/g)	2.5 ± 0.1	3.1 ± 0.2[a]	2.6 ± 0.1	3.0 ± 0.1[a]	2.5 ± 0.1	3.1 ± 0.1[a]
Atria/body (mg/g)	0.21 ± 0.01	0.44 ± 0.08[a]	0.22 ± 0.02	0.36 ± 0.02[b]	0.22 ± 0.03	0.38 ± 0.02[b]
RV/body (mg/g)	0.46 ± 0.01	0.76 ± 0.09[a]	0.49 ± 0.02	0.65 ± 0.06[b]	0.47 ± 0.01	0.65 ± 0.06[b]
LV/body (mg/g)	1.80 ± 0.04	1.92 ± 0.05[b]	1.91 ± 0.10	1.99 ± 0.04[a]	1.86 ± 0.04	2.02 ± 0.05[a]
LV volume (μl)	109 ± 10	291 ± 50[a]	94 ± 10	302 ± 30[a]	89 ± 10	354 ± 10[a]
CI (ml min^{-1} (100 g body)$^{-1}$)	21.2 ± 1.0	17.7 ± 1.2[b]	18.5 ± 1.5	15.2 ± 0.9[a]	20.8 ± 1.3	19.0 ± 1.5
LVEDP (mm Hg)	4.2 ± 1	12.1 ± 2.2[a]	4.8 ± 1.6	12.8 ± 2.2[a]	4.6 ± 1.7	10.0 ± 2.0[b]
LVSP (mm Hg)	145 ± 3	124 ± 4[b]	138 ± 5	128 ± 3[b]	145 ± 5	125 ± 4[b]
$+dP/dt$ (mm Hg/sec)	5870 ± 132	4592 ± 238[a]	5902 ± 190	4828 ± 216[a]	5904 ± 336	4836 ± 260[a]
$-dP/dt$ (mm Hg/sec)	6026 ± 242	4732 ± 288[a]	5862 ± 310	4892 ± 226[a]	6136 ± 342	4804 ± 186[a]
MAP (mm Hg)	114 ± 5	107 ± 4	109 ± 8	106 ± 4	115 ± 8	105 ± 4
Heart rate (beats/min)	407 ± 13	400 ± 15	403 ± 23	392 ± 17	406 ± 15	402 ± 9

[a] $P < 0.01$, [b] $P < 0.05$, significantly different from untreated Sham.

3.2. L-Arginine consumption and plasma concentration.

Water consumption plateaued at approximately 50 ml of water per day 3 weeks post surgery. L-Arginine consumption was calculated from the water consumption from each rat per day and L-arginine concentration in the drinking water. The low dose of L-arginine received by heart failure rats (1.3 ± 0.1 g kg^{-1} day^{-1}) in the final week was not different from that received by sham rats (1.5 ± 0.1 g kg^{-1} day^{-1}). The high dose of L-arginine for heart failure rats (4.8 ± 0.2 g kg^{-1} day^{-1}) was also not different from that received by sham rats (5.4 ± 0.2 g kg^{-1} day^{-1}).

Plasma concentrations of L-arginine in heart failure were significantly decreased compared to sham ($P < 0.05$, Fig. 1). With L-arginine treatment, plasma L-arginine levels increased in both heart failure and sham rats in a dose-dependent manner. Low dose L-arginine treatment in heart failure rats restored plasma levels to that of untreated sham rats while the high dose L-arginine treated heart failure rats had significantly higher plasma arginine levels than untreated sham rats ($P < 0.05$). The low dose L-arginine treated sham rats had plasma concentrations that were not different from either untreated sham or low dose L-arginine treated heart failure rats (P = n.s.). High dose treated sham rats displayed significantly higher plasma L-arginine levels than untreated sham rats ($P < 0.05$) but were not different from high dose L-arginine treated heart failure rats (P = n.s.).

3.3. Plasma nitrate / nitrite levels

Plasma nitrate/nitrite levels in untreated heart failure were increased compared to untreated sham rats ($P = 0.05$, Fig. 2). Low dose L-arginine treatment did not significantly alter plasma nitrate/nitrite levels in heart failure or sham rats (P = n.s.). High dose L-arginine treatment did not increase plasma nitrate/nitrite levels in sham rats

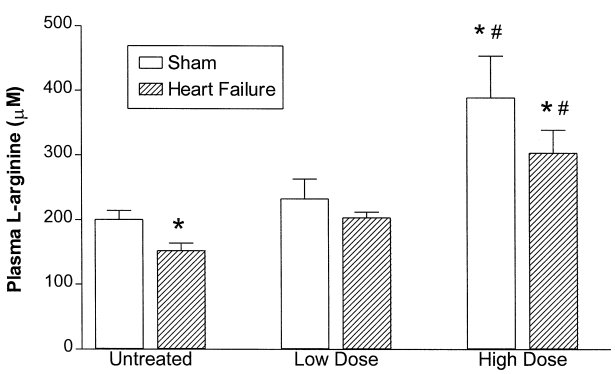

Fig. 1. The plasma L-arginine concentrations in heart failure and sham-operated (Sham) rats. $n = 5$–7 animals per group. $^{*}P < 0.05$ vs. untreated sham; $\#P < 0.05$ vs. respective low dose L-arginine treatment group.

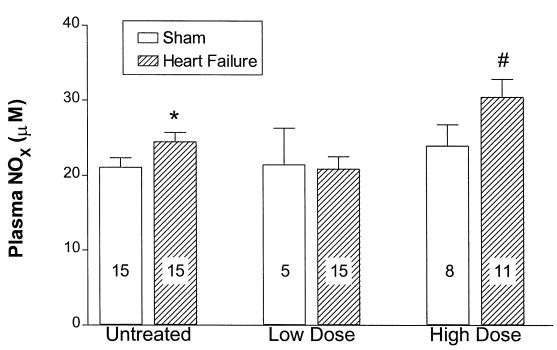

Fig. 2. Plasma nitrate/nitrite (NO_X) levels in heart failure and sham-operated (Sham) rats. $^{*}P < 0.05$ vs. untreated sham; $\#P < 0.05$ high dose L-arginine treated vs. untreated heart failure. Numbers in columns indicate number of animals per group.

(P = n.s.). However, compared to untreated heart failure, plasma nitrate/nitrite levels were significantly increased in heart failure rats controls after high dose L-arginine treatment ($P < 0.05$, Fig. 2).

3.4. Hemodynamic changes

Hemodynamic changes obtained in conscious state are listed in Table 1. Heart failure rats displayed decreased cardiac output compared to sham ($P < 0.05$). As well, the infarcted rats exhibited elevated left ventricular end-diastolic pressures, depressed systolic left ventricular pressure, and reduced rates of pressure development and relaxation ($+dP/dt$ and $-dP/dt$, respectively) ($P < 0.01$). There was no significant change in heart rate or mean arterial pressure. Furthermore, there were no statistical differences in any of these parameters after chronic low or high dose L-arginine treatment (P = n.s.) (Table 1). Sham rats were given L-arginine treatment as controls. There was no sign of infarction in these rats. There was also no difference in any hemodynamic or physical characteristics in these animals after L-arginine treatment (Table 1). Similar hemodynamic changes were obtained in these animals under pentobarbital anaesthesia (data not shown).

Table 2
Comparison of the EC_{50} values for untreated and L-arginine treated heart failure and sham rats in relaxation responses to acetylcholine and sodium nitroprusside. Values are expressed as means ± S.E.M.

	Acetylcholine ($-\log[M]$)	Nitroprusside ($-\log[M]$)
Sham		
Untreated	7.54 ± 0.12	8.47 ± 0.12
Low dose L-arginine	7.74 ± 0.06	8.28 ± 0.15
High dose L-arginine	7.66 ± 0.18	8.20 ± 0.32
Heart failure		
Untreated	7.11 ± 0.09[a]	8.53 ± 0.12
Low dose L-arginine	7.43 ± 0.12[b]	8.48 ± 0.16
High dose L-arginine	7.17 ± 0.09[a]	8.39 ± 0.19

[a] $P < 0.05$ vs. respective groups in Sham.
[b] $P < 0.05$ vs. untreated Heart failure.

3.5. Endothelium-dependent relaxation

Organ bath experiments were conducted to evaluate the endothelium-dependent and -independent relaxation of tho-

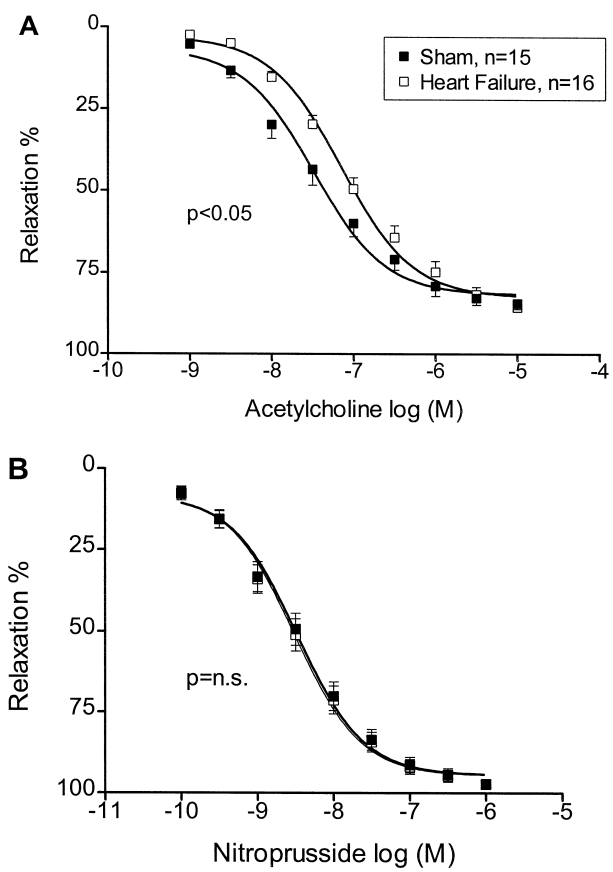

Fig. 3. Cumulative concentration-response to acetylcholine and sodium nitroprusside in aortic rings from heart failure and sham-operated (Sham) rats. Relaxation response to acetylcholine (A) in heart failure rats was significantly shifted to the right compared with sham, $P < 0.05$. There was no significant difference in relaxation to nitroprusside (B) bewteen heart failure and sham rats, P = n.s.

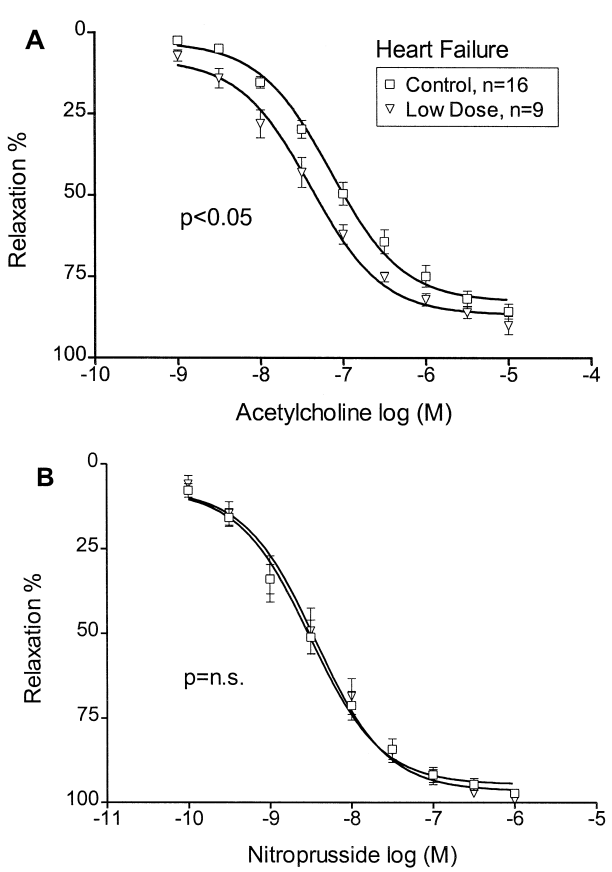

Fig. 4. Cumulative concentration-response to acetylcholine and sodium nitroprusside in heart failure rats after low dose L-arginine treatment. The relaxation response to acetylcholine (A) was significantly improved as shown by a left-shift in the curve compared to untreated heart failure rats, $P < 0.05$. Low dose L-arginine treatment did not alter relaxation response to nitroprusside (B) in heart failure rats, P = n.s.

racic aortae. Relaxation response to acetylcholine was shifted to the right in untreated heart failure compared to untreated sham rats (Fig. 3A). The EC_{50} value of untreated heart failure rats was higher than that of sham ($P < 0.05$, Table 2). There was no significant difference between untreated sham and untreated heart failure in relaxation response to sodium nitroprusside (P = n.s., Fig. 3B). The results indicated a decreased endothelium-dependent relaxation in heart failure.

Low dose L-arginine significantly shifted the relaxation response to acetylcholine to the left in heart failure rats (Fig. 4A). The EC_{50} values of low dose L-arginine treated heart failure rats were similar to those of untreated sham (Table 2), but were significantly decreased from untreated heart failure ($P < 0.05$). There was no significant difference in the endothelium-independent relaxation to nitroprusside after low dose L-arginine treatment in heart failure rats (Fig. 4B).

High dose L-arginine treatment did not alter relaxation response to acetylcholine or nitroprusside in heart failure rats (Fig. 5A; Table 2). Neither low nor high dose L-arginine treatment altered endothelium-dependent or endothelium-independent relaxation in sham rats (P = n.s., Fig. 5B and Table 2).

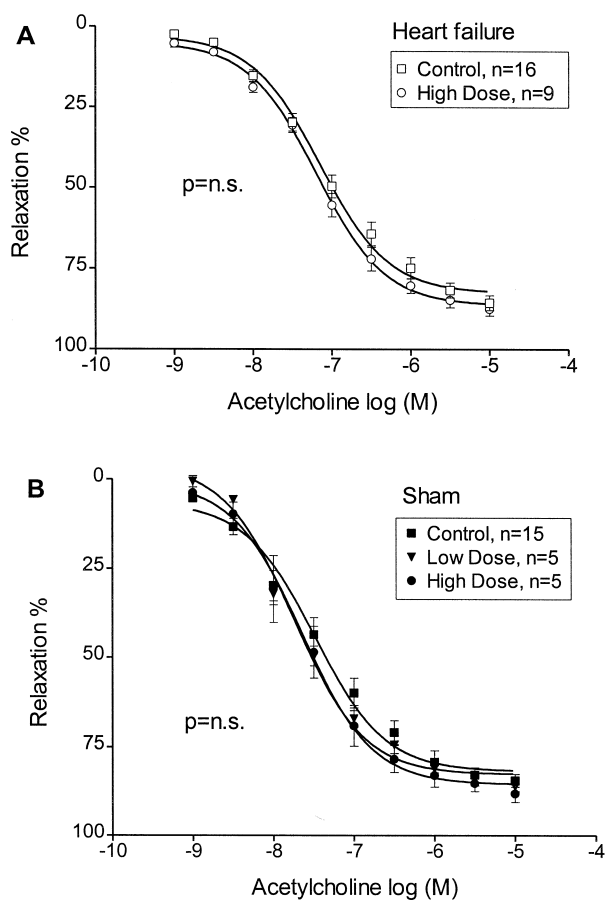

Fig. 5. Cumulative concentration-response to acetylcholine in thoracic aortic rings. High dose L-arginine treatment did not alter relaxation response to acetylcholine in heart failure (A). Neither low nor high dose L-arginine treatment altered relaxation response to acetylcholine in sham rats (B).

4. Discussion

The data in this study demonstrate that a lower dose of L-arginine (1.3 g kg^{-1} day^{-1}) given chronically to heart failure rats, while restoring both plasma L-arginine levels and endothelium-dependent vasorelaxation to the level of sham rats, failed to significantly alter basal hemodymanics. A higher dose L-arginine treatment (4.8 g kg^{-1} day^{-1}) of heart failure rats, on the other hand, produced plasma L-arginine concentrations in excess of those of sham rats but failed to improve either endothelial or cardiac function.

L-Arginine availability has been demonstrated to be a rate-limiting step for NO synthase (Mitchell et al., 1990). Decreased L-arginine availability is not only associated with decreased NO release but also increased production of oxygen free radicals (Xia et al., 1996). In the present study, basal levels of plasma L-arginine in sham rats were about 200 μM which agrees with the values reported by other investigators (Pieper and Peltier, 1995). In rats with heart failure, there was a 24% decrease in basal plasma levels of L-arginine compared to sham rats. Supplementation with L-arginine in the organ bath has been demonstrated to restore endothelium-dependent relaxation in the aorta of heart failure rats (Feng et al., 1998). Since L-arginine treatment in vitro is not likely to cause release of insulin and growth hormones in the aortic rings, the effect observed is mainly due to increased L-arginine availability at the vicinity of endothelial NO synthase enzyme in endothelial cells. Taken together, these results suggest that L-arginine availability to NO synthase may be decreased in rats with heart failure. The mechanism of decreased L-arginine availability in heart failure is currently unknown, but may include factors such as decreased L-arginine absorption, blockade of L-arginine recycling from L-citrulline, or L-arginine depletion by inducible NO synthase over-expression (de Belder et al., 1993; Habib et al., 1996; Haywood et al., 1996).

Chronic oral L-arginine treatment in vivo dose-dependently increased plasma L-arginine levels in both sham and heart failure rats. Low dose L-arginine treatment restored plasma L-arginine to the physiological level of sham rats, while high dose treatment caused a much higher plasma L-arginine levels in both sham and heart failure rats. The normalized plasma L-arginine level in heart failure rats in low dose treatment group was associated with improvement in endothelium-dependent relaxation. Furthermore, low dose L-arginine selectively improved endothelium-dependent relaxation while the endothelium-independent relaxation to nitroprusside was not affected. The results support the notion that L-arginine supplementation at this dose level may increase L-arginine availability and subsequently provide more substrate to NO synthase and im-

prove endothelial function in heart failure. We have previously demonstrated that an endogenous NO synthase inhibitor, asymmetric dimethylarginine is elevated in heart failure rats (Feng et al., 1998). The elevated asymmetric dimethylarginine level may inhibit endothelial NO synthase activity and cause endothelial dysfunction. In this regard, L-arginine supplementation may competitively antagonize the effects of asymmetric dimethylarginine and improve endothelium-dependent relaxation in rats with heart failure (Feng et al., 1998).

In contrast, higher doses of L-arginine failed to exhibit any beneficial effect on endothelium-dependent relaxation in heart failure rats even though plasma concentrations of L-arginine achieved were greater than those with low dose treatment. The reason for this is not clear. Since high dose L-arginine treatment in heart failure caused a significant increase in plasma nitrate/nitrite levels, it is possible that high dose L-arginine treatment increases NO production through inducible NO synthase which is induced in heart failure (Haywood et al., 1996; Fukuchi et al., 1998). The excessive NO production may inhibit vascular endothelial NO synthase activity through a negative feedback mechanism (Buga et al., 1993; Cohen et al., 1996) and may account for the unchanged endothelium-dependent relaxation after high dose L-arginine treatment in heart failure rats. We should point out that in the present study endothelium-dependent response was performed in aorta which is a conduit vessel. We demonstrated that endothelium-dependent response to acetylcholine was also decreased in the mesenteric arteries of heart failure rats (unpublished observation), indicating acetylcholine response in aorta mirrors that in mesenteric arteries, which are resistant vessels. However, effects of chronic L-arginine treatment on endothelium-dependent relaxation in resistance vessels and the microvasculature in heart failure require further investigation.

Acute intravenous administration of L-arginine has been demonstrated to have vasodilatory and negative inotropic effects in heart failure rats (Feng et al., 1996b) while increase cardiac output and decrease pulmonary resistance in a dog model of heart failure (Ogilvie and Zborowska-Sluis, 1995). In patients with pulmonary hypertension due to heart failure, central venous infusion of L-arginine produced significant reductions in pulmonary wedge pressure, pulmonary vascular resistance and systemic vascular resistance (Mehta et al., 1995), and chronic oral treatment with L-arginine improves functional status in patients with heart failure (Rector et al., 1996). The present study demonstrated no significant differences after L-arginine treatment in any morphological or hemodynamic criteria used to characterize the disease state of heart failure. While low dose L-arginine treatment improved endothelium-dependent relaxation in the aorta, it did not improve cardiac output or cardiac contractility, or left ventricular volume. Thus, it appears that chronic oral L-arginine treatment fails to improve cardiac function under basal resting conditions in heart failure rats. The exact mechanism for the dissociation between effects of endothelium-dependent relaxation and hemodynamics after L-arginine treatment is not known. Shear stress, a major stimulus for endothelial NO synthase is decreased in heart failure due to decreased cardiac output (Sessa et al., 1994; Hirai et al., 1995). It is possible that the decreased shear stress may not be able to stimulate enough NO release from endothelium to improve systemic vascular function, even though the endothelium might have that capability after L-arginine treatment. Whether L-arginine, especially low dose L-arginine improves cardiovascular function in heart failure rats under stress conditions, such as exercise requires further investigation.

The supplementation of exogenous L-arginine in sham rats not unexpectedly failed to produce any effects in vitro or in vivo. Under normal physiological conditions, it is believed that the constitutive NO synthase is saturated with excess L-arginine which is likely taken up by the liver and shunted through the urea cycle (Rogers et al., 1972; White and Christensen, 1982).

The lack of effect of the chronic oral L-arginine treatment on basal hemodynamic parameters reveals no obvious benefit of such a treatment in a rat model of heart failure. However, the improved endothelial function elicited by the low dose L-arginine treatment indicates a positive effect that may have consequences not elucidated by the current studies. A possible beneficial effect of L-arginine treatment on cardiovascular function cannot yet be ruled out at different stages of heart failure or under exercise conditions when endothelial-dependent relaxation is stimulated.

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