

Chronic dietary L-arginine down-regulates adenosine receptor and NO synthase expression in rat heart.

Roselyn B. Rose'Meyer¹✉, Glenn J. Harrison¹, Andrew Fenning², Tamsin L. Jenner¹,
and Lindsay Brown³

¹School of Medical Sciences, Griffith University, Gold Coast, Queensland, Australia

²School of Chemical and Biomedical Sciences, Central Queensland University,
Queensland Australia

³School of Biomedical Sciences, The University of Queensland, Queensland,
Australia

✉ Roselyn Rose'Meyer
School of Medical Sciences
Griffith University
PMB50 Gold Coast Mail Centre
Queensland
Australia 9726
Ph 617 5552 8938
Fax 617 5552 8908
Email r.rosemeyer@griffith.edu.au

Abstract:

L-arginine increases myocardial nitric oxide production (NO). NO mediates many of the cardiovascular actions of adenosine and modulates adenosine metabolism. In this study we examined the effect of chronic L-arginine (5%) intake on cardiac NO synthase (NOS) and adenosine receptor expression and cardiac function in rat Langendorff isolated perfused hearts. Our results show that 4 week chronic L-arginine ingestion increases the weight of rat hearts by 17.6% ($P < 0.05$). L-arginine treatment decreased the expression of all the cardiac adenosine receptors, with reductions in adenosine A₁ (20 fold), A_{2A} (7.7 fold), A_{2B} (25.6 fold) and A₃ (76 fold) mRNA ($P < 0.05$). NOS expression was variably affected with no change in the expression of NOS1 and 4.2 fold down-regulation of NOS3 expression with chronic L-arginine treatment ($P < 0.05$). NOS2 was expressed in control tissues, however in L-arginine treated hearts the amount of NOS2 mRNA was reduced to non-detectable levels. Following chronic L-arginine treatment an increase in coronary perfusion pressure (CPP) was observed ($P < 0.05$). Purine efflux was used as an indicator of metabolic efficiency. L-arginine did not alter catecholamine-induced purine efflux ($P > 0.05$), however, noradrenaline-mediated increases in contractility and myocardial oxygen consumption were reduced. Vasodilator responses to 5'-N-ethylcarboxamidoadenosine (NECA) were reduced in hearts from L-arginine treated rats and the NOS synthase inhibitor N ω -Nitro-L-arginine-methyl ester (L-NAME, 3 μ M) did not inhibit responses to NECA. In conclusion, 4 week dietary supplementation of L-arginine reduced the expression of cardiac adenosine receptors and NO synthases with a subsequent decrease in noradrenaline stimulated cardiac function and adenosine receptor mediated coronary vasodilation.

Keywords: L-arginine, nitric oxide synthase, adenosine receptors, L-NAME

Adenosine is released in the heart during the conditions of ischemia or hypoxia [1, 2]. Adenosine production is stimulated by a decrease in the ratio of oxygen supply relative to the demands in the myocytes [3]. Adenosine release is augmented with increased cardiovascular work that occurs during exercise and has also been established in catecholamine-stimulated isolated hearts [4, 5].

Adenosine binds to G-protein coupled receptors of which there are four subtypes. In the cardiovascular system, adenosine A₁ receptor subtype mediates negative chronotropic, dromotropic, inotropic responses in the heart [6, 7], whereas adenosine A₂ and A₃ receptors are primarily involved in stimulating vasodilation and modifying cardiac contractility [8, 9]. Many actions of cardiac adenosine receptors occur via NO-cGMP pathways [10, 11, 12, 13, 14, 15].

Three NOS isoenzymes have been identified: the constitutively expressed, Ca²⁺ sensitive, NOS1 (neuronal or nNOS); NOS2 formed following stimulation by cytokines; and NOS3 previously known as endothelial or eNOS [16].

The supplementation of L-arginine to the diet has been proven to increase serum levels of arginine and oxidation products of NO [17]. The addition of L-arginine to the diet has been demonstrated to reduce cardiac remodeling associated with hypertension [18, 19] and heart failure [20]. Chronic L-arginine treatment does not change basal cardiac levels of cGMP [19, 21] or reduce responses to isoprenaline [21]. However, NO affects purine uptake mechanisms [22, 23] reduces adenosine production [24] and can also regulate the expression of various genes [25] including up-regulating sarcoplasmic/endoplasmic reticulum Ca²⁺-ATPase expression (SERCA, 26).

As NO mediates many of the actions of adenosine in the heart and can alter adenosine metabolism, reductions in cardiac remodeling associated with hypertension or heart failure following L-arginine dietary supplementation may be due to changes in adenosine receptor expression, function or adenosine metabolism. The effect of chronic L-arginine intake in normotensive rats on adenosine receptors and efflux was evaluated in Langendorff isolated perfused hearts.

Materials and Methods

Animals

The hearts from male Wistar rats from two different groups were used during this study and involved rats treated with L-arginine and aged matched control animals. The rats were purchased from the Central Animal House of the University of Queensland in Brisbane. They were housed in controlled conditions whereby the temperature was maintained at $23 \pm 2^{\circ}\text{C}$ with a 12 hour light-dark cycle. In addition, the rats were allowed free access to water and food at all times. L-arginine was administered as a 5% mixture in powdered rat food for 4 weeks prior to experiments. This was the equivalent of 3.6 ± 0.1 g/kg body weight/day [20]. Furthermore, all protocols were conducted according to the Guidelines for Animal Experimentation determined by the National Health and Medical Research Council of Australia, and approved by the University of Queensland and Griffith University Animal Experimentation Ethics Committees.

Langendorff isolated perfused heart preparation

Hearts from each age group were perfused in the non-recirculating Langendorff mode based on a method described by Rose'Meyer et al. [26]. Briefly, the hearts were rapidly excised and immersed into ice-cold Krebs-Henseleit solution containing (in mM): NaCl 118, KCl 4.7, CaCl_2 1.75, MgSO_4 1.2, glucose 11, EDTA 0.5 and NaHCO_3 25. The aorta was cannulated and heart perfused at 15-18 ml/min (to give a final coronary flow rate of 9.0–10.0 ml/min/gtissue) with Krebs-Henseleit solution gassed with 95% O_2 and 5% CO_2 and kept at 37°C . Ventricular fluid accumulation was prevented by inserting a small piece of polyethylene tubing through the apex of the left ventricle to drain the cavity and ascertain aortic valve patency. A water-filled latex balloon was then introduced into the left ventricle and connected to a pressure

transducer (Gould P23-ID, Oxnard Ca, USA). End-diastolic pressure (EDP) was adjusted to 4-8 mmHg by inflating the balloon. Left ventricular developed pressure (LVDP) was monitored continuously using a MacLab data acquisition system (ADInstruments, Castle Hill, Australia). Coronary perfusion pressure (CPP) was measured using a pressure transducer connected to a water filled probe inserted into the side arm of the aortic cannula and was recorded using the Maclab data acquisition system. The pulmonary artery was cannulated for the collection of coronary venous effluent to determine venous PO₂ using Chiron blood gas analyser, and for the subsequent determination of venous purine levels by reverse-phase HPLC as previously described [27]. Rate pressure product (RPP) was calculated by multiplying heart rate (HR) by LVDP. The LVDP signal was electronically differentiated to measure +dP/dt and -dP/dt as time derivatives of pressure.

Myocardial oxygen consumption (MVO₂, μl O₂min⁻¹ g⁻¹) was calculated as:

$$(PO_{2p} - PO_{2v}) \times \text{coronary flow} \times (c/760)$$

where PO_{2p} and PO_{2v} refer to coronary perfusate and venous effluent PO₂ values (mmHg), respectively, and c = 22.7 (Bunsen solubility coefficient of O₂ perfusate at 37⁰C, μl O₂ atm⁻¹ml⁻¹). After 30 mins equilibration, concentration-response curves to noradrenaline (10⁻⁹ – 10⁻⁶ M) were performed. Noradrenaline was used in these experiments as it preferentially binds to β₁ and α₁ -adrenoceptors compared to β₂-adrenoceptors [28]. β₂-adrenoceptors induce coronary vasodilation and also have positive inotropic actions in the rat heart [29, 30]. Rat coronary resistance vessels do not exhibit α₁-adrenoceptor mediated vasoconstriction. In our laboratory phenylephrine (10⁻⁹ – 10⁻⁵ M) does not induce vasoconstriction in this model (data not shown). At the end of the experiment, hearts were frozen in liquid nitrogen and stored at -70⁰C.

5'-N-ethylcarboxamidoadenosine (NECA) concentration-response curves were obtained in control tissues and hearts from L-arginine treated rats. Following the first concentration-response curve, L-NAME (3 μ M) was infused for 30 mins then a second NECA concentration-response curve was obtained in the presence of L-NAME.

RNA extraction and cDNA synthesis

Whole hearts were homogenized using a rotar stator homogenizer in Trizol (50% guanidium thiocyanate and 30% phenol). Total cellular RNA was isolated using the RNeasy Midi RNA isolation Kit. During this process on-column DNase I (54.5Kunits) treatment and cleansing of the lysate using 70% ethanol was performed according to the RNeasy[®] Midi/Maxi Handbook (June 2001), and the eluted RNA stored at -70°C . The total RNA samples were then precipitated, resuspended and converted into cDNA using reverse transcriptase MMLV *RNaseH*-(Superscript[™]) according to the method described by Rose'Meyer *et al.* [31].

Real time PCR

Real time quantitative PCR analysis was performed on the cDNA samples using the iQ iCycler[™] real time PCR system (Bio-Rad) to allow the measurement of gene expression of the adenosine receptor A_1 , A_{2A} , A_{2B} and A_3 mRNA, and 18S rRNA. The quantitative method has previously been described by Rose'Meyer *et al.* [31] where SYBR Green I was utilized to detect the increase in product as the PCR amplification progressed. The amplification reaction was previously optimised for MgCl_2 with the respective primer sequences [31]. Four samples from each age group were analysed in quadruplicate for each adenosine receptor gene. Comparative analysis involved

correcting the threshold cycle values using 18S rRNA as the internal controls, followed by calculating a fold determination of the change in adenosine receptor expression with L-arginine treatment.

NOS expression in cDNA samples was determined using the Bio-Rad iQ™ SYBR® green supermix. The primers used in the real time PCR are as follows: NOS1 forward 5'-AGACCCTGTGTGAGATCTTCAA-3' reverse 5'-GTCATACTCCTCCATGGACATT-3' (67 base pairs); NOS2 forward 5'-GAGAGATCCGGTTCACAGTCT-3' and reverse 5'-GCTTCCGACTTTCCTGTCTCA-3' (169 base pairs); NOS3 5'-CTGGCAGCCCTAAGACCTAT-3' and reverse 5'-CGCAGACAAACATGTGTCCTT-3' (107 base pairs). Real time PCR was performed using a thermal cycler (Bio-Rad gene cycler™) with the following parameters: denaturation 94°C for 30 seconds, annealing at 59°C for 45 seconds, extension at 72°C for 1 min at 45 cycles.

Statistical Analysis

Unless stated otherwise, all purine efflux and functional values shown are mean \pm S.E.M. Gene expression data is presented as relative fold expression ratio using 18S rRNA as the internal standard [31]. For fold data analysis between control and treated groups an unpaired Students t-test was implemented. Functional and efflux data at each stimulation rate was analysed using a 2-way repeated measures ANOVA (Statistica, StatSoft Inc., Tulsa, USA). To test individual comparisons the Newman-Keuls post-hoc test was employed. Comparisons for basal periods were made using unpaired Student's t-tests. Concentration-response curves to the noradrenaline and NECA were analysed by nonlinear regression using the program GraphPad Prism Software Inc., San Diego, USA. Differences were considered significant at $P < 0.05$.

Chemicals and supplies

The RT buffer, DTT, Trizol[®] reagent and Superscript[™] reverse transcriptase were acquired from Invitrogen Life Technologies (Carlsbad, California, USA). Noradrenaline (Arterenol bitartrate), N ω -Nitro-L-arginine-methyl ester (L-NAME), 5'-N-ethylcarboxamidoadenosine (NECA) and SYBR green I 10000x were purchased from Sigma Chemical Co. (St Louis, Missouri, USA). Rox[™] Fluorescein Calibration Dye and iQ[™] SYBR[®] green supermix were obtained from Bio-Rad (Deven Massachusetts, USA). Taq DNA polymerase was purchased from Amersham-Pharmacia Biotech (Little Chalfont, Buckinghamshire, England). Primers and random decamers were acquired from Geneworks (Adelaide, South Australia, Australia). The RNase free DNase set and RNeasy Midi RNA Isolation Kits were obtained from Qiagen (Germantown, Maryland, USA). RNasin[®] RNase inhibitor and deoxyribonucleotides were purchased from Promega (Madison, Wisconsin, USA). 96 well IQ iCycler plates were obtained from Astral Scientific (Gyemea, NSW, Australia).

Results

Comparative analysis of adenosine receptors and NOS with respect to 18S rRNA

Figure 1A shows change in fold expression of cardiac adenosine in hearts from chronic L-arginine treated rats when compared to control. Data analysis found a significant down regulation of expression all of the cardiac adenosine receptors studied with L-arginine treatment, ($P < 0.05$). While moderate down-regulation was observed for adenosine A_{2A} receptors (7.4 fold), a greater reduction was observed for adenosine A₁ (20.0 fold) and A₃ (25.6 fold) receptor mRNA, with the largest decrease in adenosine A_{2B} (76 fold) receptor expression.

The effect of L-arginine on NOS expression was also determined in cardiac tissue (see figure 1B). Results showed no change in the expression of NOS1 mRNA and a 4.2 fold down-regulation of NOS3 expression with chronic L-arginine treatment ($P < 0.05$). NOS2 was expressed in control tissues, however in L-arginine treated hearts the expression of NOS2 was reduced to non detectable levels.

Purine efflux in control and treated hearts

Efflux of purines (adenosine, inosine, hypoxanthine and xanthine) during basal conditions is presented in figure 2. Both adenosine, inosine and purine efflux during equilibration was the same for control and L-arginine treated hearts ($P > 0.05$). The perfusate efflux of adenosine rose with increased concentrations of noradrenaline in control and L-arginine treated hearts with a significant increase in adenosine and adenosine metabolite efflux induced by noradrenaline at 100 and 300 nM ($P < 0.05$). The release of adenosine in L-arginine treated hearts during the noradrenaline concentration-response curve was no different to control values ($P > 0.05$). The venous release of the adenosine metabolites demonstrated similar trends to adenosine efflux

with increasing catecholamine concentration in L-arginine treated tissues. Chronic L-arginine treatment did not alter noradrenaline induced purine efflux from isolated perfused hearts.

Basal functional parameters in control and L-arginine treated hearts

The body weights of L-arginine treated rats were not different to aged-matched control rats ($P>0.05$). Heart wet weight corrected for body weight data indicated that hearts from L-arginine treated rats were heavier than age-matched control hearts (see table 1). The functional hemodynamic variables during basal conditions are presented in table 1. All of the parameters studied except the $+dP/dt$, MVO_2 and CPP were comparable in control and L-arginine treated hearts. The $+dP/dt$ and MVO_2 were lower in L-arginine treated preparations during equilibration while CPP values were increased in L-arginine treated hearts when compared to control tissues ($P<0.05$). Chronic L-arginine treatment reduced cardiac contractility and myocardial oxygen consumption and increased coronary resistance in isolated rat hearts.

Effect of noradrenaline on heart function

Noradrenaline (1-300 nM) induced concentration-dependent increases in heart rate (HR), left ventricular developed pressure (LVDP) and rate pressure product (RPP) in control and L-arginine-treated hearts ($P<0.05$, figure 3). Chronic L-arginine treatment did not affect noradrenaline concentration-responses curves on HR. Analysis of RPP and LVDP data indicated that chronic L-arginine treatment did not alter the sensitivity to noradrenaline with pEC_{50} values of 8.25 ± 0.50 (control hearts, $n=8$) versus 8.02 ± 0.63 (L-arginine treated hearts, $n=8$, $P>0.05$). Maximal responses to noradrenaline were reduced with LVDP values of 226 ± 16 mmHg ($n=8$) in control hearts compared to 190 ± 14 mmHg ($n=8$) in tissues following chronic L-arginine treatment ($P<0.05$).

Noradrenaline (1-1000 nM) induced concentration-dependent increases in +dP/dt and -dP/dt in control and L-arginine-treated hearts ($P < 0.05$, figure 4a and b). While pEC_{50} values were no different between control and treated hearts, a significant attenuation of contractile response (+dP/dt) to noradrenaline was observed in L-arginine treated hearts ($P < 0.05$) with no change in -dP/dt responses ($P > 0.05$). In L-arginine treated hearts, elevated CPP values were observed at lower noradrenaline concentrations (1-10 nM). At higher noradrenaline levels (30-300 nM), the CPP decreased significantly from 91 mmHg to 61 mmHg constituting a decrease of 30 mmHg in CPP compared to a 13 mmHg decline in coronary resistance in control hearts. Myocardial oxygen consumption was reduced throughout the noradrenaline concentration-response curve in hearts from L-arginine treated rats ($P < 0.05$).

Effect of NECA on coronary vasodilation

NECA induced a concentration-dependent decrease in coronary perfusion pressure (CPP) in hearts from control and L-arginine treated rats (see figure 5). Chronic L-arginine treatment caused a rightward shift in the concentration response curve to NECA with the pEC_{50} value in control tissues 9.3 ± 0.4 (n=8) compared to 8.6 ± 0.2 (n=7) in L-arginine treated hearts ($P < 0.05$). The efficacy of NECA was reduced with L-arginine treatment with maximal decreases in CPP 16 ± 3 mmHg (n=7) compared to 34 ± 2 mmHg (n=7) in control hearts ($P < 0.05$). The NOS inhibitor L-NAME (3 μ M) caused a rightward shift in the NECA concentration-response curves in control hearts (pEC_{50} value 8.5 ± 0.1 , $P < 0.05$), however had no effect on responses to NECA in tissues from L-arginine treated rats (pEC_{50} value 8.4 ± 0.1 , $P > 0.05$).

Discussion

Currently there are four adenosine receptor subtypes that have been identified, A₁, A_{2A}, A_{2B} and A₃ receptors. All are found in cardiac tissue and have cardioprotective effects as they reduce heart rate, contractility and induce coronary vasodilation [31]. Adenosine receptors are clearly involved in the regulation of NO in the heart and many actions of adenosine occur via NO-cGMP pathways. L-arginine supplementation has been explored in the management of cardiovascular diseases arising from diabetes, hypertension, heart failure and ischemic heart disease [32]. In our study, dietary supplementation with L-arginine increased heart weight 14% compared to control hearts. In other animal studies, L-arginine supplementation has been demonstrated to reduce cardiac hypertrophy in SHR [19] and DOCA-salt hypertensive rats [20]. While dietary L-arginine may protect against pathological changes, it appears to increase heart weight when used under normal physiological conditions. The supplementation of L-arginine to the diet has been proven to increase serum levels of arginine (261%) and oxidation products of NO (70%, 17).

This study found that chronic L-arginine treatment caused down-regulation of all four adenosine receptor subtypes as well as NOS2 and NOS3 in the rat heart. NO has been reported to alter the expression of other genes such as transcription factors and SERCA [25, 26]. Also in eNOS *-/-* mice, the loss of NO results in upregulation of expression of atrial natriuretic peptide (ANP), a physiological stimulator of cardiac guanylyl cyclase activity [33].

Experiments investigating the effect of the adenosine receptor analogue NECA on coronary resistance vessels showed a reduction in vasodilator response in hearts from L-arginine supplemented rats. NECA induces endothelial dependent vasodilation via adenosine A_{2B} receptors in rat coronary vessels [34]. Furthermore the

NOS inhibitor L-NAME inhibited NECA induced vasodilation in control hearts but had no effect in hearts from L-arginine treated rats. Changes in functional responses to NECA with dietary L-arginine indicate possible reductions in the protein levels of NOS3 or adenosine A_{2B} receptor.

In our experiments, the efflux of adenosine and its metabolites during noradrenaline-mediated β -adrenoceptor stimulation from L-arginine treated hearts was no different from control tissues. Adenosine efflux during episodes of myocardial hypoxia/ischemia is considered cardioprotective, partially by attenuating the actions of catecholamines on the heart [35] and reducing myocardial noradrenaline release [36]. Chronic L-arginine treatment attenuates inotropic responses and myocardial oxygen consumption in isolated hearts without affecting the chronotropic actions of noradrenaline. A reduction in cardiac responses cannot be attributed to changes in adenosine efflux. Although, starting from lower baseline values of +dP/dt with L-arginine treatment may account for reductions in maximal contractile responses to noradrenaline. Given that adenosine efflux is also governed by myocardial oxygen uptake and that chronic L-arginine intake reduced cardiac contractility and myocardial oxygen consumption with β -adrenoceptor mediated stimulation, a reduction in adenosine efflux could be expected but was not observed. NO plays a role in the metabolism and uptake systems for adenosine. Adenosine transport into endothelial and vascular smooth muscle cells is mediated by NO [22, 23] while a decrease in NO synthesis increases adenosine formation due to protein kinase C-mediated activation of ecto-5' nucleotidase [24].

In our study, chronic L-arginine treatment did not alter diastolic relaxation during basal conditions or with noradrenaline-mediated stimulation. However, L-arginine treatment increased basal CPP and reduced adenosine receptor mediated

vasodilation of the coronary vessels. This research suggests that short term L-arginine supplementation in normotensive rats reduces cardiac metabolic efficiency and decreases the physiological actions of the metabolic mediator adenosine.

In conclusion, chronic L-arginine treatment decreases the expression of cardiac adenosine receptors, NOS2 and NOS3. It also reduces myocardial contractility without altering purine efflux during noradrenaline mediated stimulation. Increased coronary resistance observed with L-arginine supplementation may be due to a loss of adenosine receptor mediated vasodilation and/or deficit of endothelial NO production.

References

1. Olsson RA. Adenosine receptors in the cardiovascular system. *Drug Dev Res* 1996; 39: 301-307.
2. Mubagwe K, Flameng W. Adenosine, adenosine receptors and myocardial protection: an updated overview. *Cardiovasc Res* 2001; 52: 25-39.
3. Berne RM. Cardiac nucleotides in hypoxia: possible role in regulation of coronary blood flow. *Am J Physiol* 1963; 204: 317-322.
4. Headrick JP, Matherne GP, Berr SS, Han DC, Berne RM. Metabolic correlates of adenosine formation in stimulated guinea-pig heart. *Am J Physiol (Heart Circ Physiol 29)* 1991; 260: H165-H172.
5. Ning X-H, He M-X, Gorman MW, Romig GD, Sparks HV. Adenosine formation and energy status in isolated guinea-pig hearts perfused with erythrocytes. *Am J Physiol (Heart Circ Physiol 31)* 1992; 262: H1075-H1080.
6. Auchampach JA, R Bolli. Adenosine receptor subtypes in the heart: therapeutic opportunities and challenges. *Am J Physiol (Heart Circ Physiol)* 1999; 276: H1113-H1116.
7. Shryock JC, Belardinelli L. Adenosine and adenosine receptors in the cardiovascular system: biochemistry, physiology, and pharmacology. *Am J Cardiol* 1997; 79 (12A): 2-10.
8. Tabrizchi R, Bedi S. Pharmacology of adenosine receptors in the vasculature. *Pharmacol Ther* 2001; 91: 133-147.
9. Xu H, Stein B, Liang B. Characterization of a stimulatory adenosine A_{2a} receptor in adult rat ventricular myocyte. *Am J Physiol (Heart Circ Physiol)* 1996; 270: H1655-H1661.

10. Ikeda U, Kurosaki K, Shimpo M, Okada K, Saito T, Shimada K. Adenosine stimulates nitric oxide synthesis in rat cardiac myocytes. *Am J Physiol (Heart Circ Physiol)* 1997; 273: H59-H65.
11. Feoktistov I, Biaggioni I. Adenosine A_{2B} receptors. *Pharm Rev* 1997; 49: 381-402.
12. Olanrewaju H, Mustafa SJ. Adenosine A_{2A} and A_{2B} receptors mediated nitric oxide production on coronary artery endothelial cells. *Gen Pharm* 2000; 35: 171-177.
13. Sterin-Borda L, Gómez RM, Borda E. Role of nitric oxide/cyclic GMP in myocardial adenosine A₁ receptor-inotropic response. *Br J Pharmacol* 2002; 135: 444-450.
14. Wyatt AW, Steinert JR, Wheeler-Jones PD, Morgan AJ, Sugden D, Pearson JD et al. Early activation of the p42/p44MAPK pathway mediates adenosine-induced nitric oxide production in human endothelial cells: novel calcium-insensitive mechanism. *FASEB J* 2002; 16: 1584-1594.
15. Zhao TC, Kukreja RC. Late preconditioning elicited by activation of adenosine A₃ receptor in heart: Role of NF- κ B, iNOS and mitochondrial K_{ATP} channel. *J Mol Cell Cardiol* 2002; 34: 263-277.
16. Leopoldo R. Nitric oxide in the pathogenesis of cardiac disease. *J Clin Hypertens* 2006; 8(12 Suppl 4): 30-39.
17. Fu WJ, Haynes TE, Kohli R, Hu R, Shi W, Spencer TE et al. Dietary L-arginine supplementation reduces fat mass in Zucker diabetic fatty rats. *J Nutrit* 2005; 135: 714-721.

18. Susic D, Francischetti A, Frohlich ED. Prolonged L-arginine on cardiovascular mass and myocardial hemodynamics and collagen in aged spontaneously hypertensive rats and normal rats. *Hypertension* 1999; 33 (part II): 451-455.
19. Matsuoka H, Nakata M, Kohno K, Koga Y, Nomura G, Toshima H et al. Chronic L-arginine administration attenuates cardiac hypertrophy in spontaneously hypertensive rats. *Hypertension* 1996; 27: 14-18.
20. Fenning A, Hoey A, Harrison G, Rose-Meyer R, Brown L. L-arginine attenuates cardiovascular impairment in DOCA-salt hypertensive rats. *Am J Physiol* 2005; 289: 1408-1416.
21. Bartunek J, Dempsey S, Weinberg E, Ito N, Tajima M, Rohbach S, BH Lorell. Chronic L-arginine treatment increases cardiac cyclic guanosine 5'-monophosphate in rats with aortic stenosis: effects on left ventricular mass and beta-adrenergic contractile reserve. *JACC* 1998; 32: 528-535.
22. Montecinos VP, Guayo C, Flores C, Wyatt AW, Pearson JD, Mann GE et al. Regulation of adenosine transport by D-glucose in human fetal endothelial cells: involvement of nitric oxide, protein kinase C and mitogen-activated protein kinase. *J Physiol* 2000; 529: 777-790.
23. Aguayo C, L Sobrevia. Nitric oxide, cGMP and cAMP modulate nitrobenzylthioinosine-sensitive adenosine transport in human umbilical artery smooth muscle cells from subjects with gestational diabetes. *Exp Physiol* 2000; 85(4): 399-409.
24. Minamino T, Kitakaze M, Node K. Inhibition of nitric oxide synthesis increases adenosine production via an extracellular pathway through activation of protein kinase C. *Circulation* 1997; 96: 1586-1592.

25. Bogdan C. Nitric oxide and the regulation of gene expression. *Trends in Cell Biol* 2001; 11: 66-73.
26. Malyshev IY, Aymasheva NP, Malenyuk EB, Manukhina EB, Khaspekov GL, Mikoyan VD et al. Nitric oxide increases gene expression of Ca²⁺-ATPase in myocardial and skeletal muscle sarcoplasmic reticulum: physiological implications. *Lab Invest* 2000; 6: 480-485.
27. Rose'Meyer RB, Harrison GJ, Headrick JP. Enhanced coronary response to endogenous adenosine in reserpinized rat heart. *Naunyn-Schmeideberg's Arch Pharmacol* 2003; 367: 266-73.
28. Piercy V. Method for assessing the activity of drugs at β 1- and β 2-adrenoceptors in the same animal. *J Pharmacol Meth* 1988; 20: 125-133.
29. Bartel S, Krause E-G, Wallukat G, P Karczewski. New insights into β 2-adrenoceptor signaling in the adult rat heart. *Cardiovas Res* 2003; 57: 694-703.
30. Rybin VO, Pak E, Alcott S, Steinberg SF. Developmental changes in β 2-adrenergic receptor signaling in ventricular myocytes: role of Gi proteins and caveolae microdomains. *Mol Pharmacol* 2003; 63: 1338-1348.
31. Rose'Meyer RB, Mellick A, Garnham BG, Harrison G, Massa HM, Griffiths LR. The measurement of adenosine and estrogen receptor expression in rat brains following ovariectomy using quantitative PCR analysis. *Brain Res Protocols* 2003; 11: 9-18.
32. Cheng JWM, Balwin SN. L-arginine in the management of cardiovascular diseases. *Ann Pharmacother* 2001; 35: 755-764.
33. Gyurko R, Kuhlencordt P, Fishman MC, Huang PL. Modulation of mouse cardiac function in vivo by eNOS and ANP. *Am J Physiol (Heart Circ Physiol)* 2000; 278: H971-H981.

34. Rose' Meyer RB, Harden FA, Varela JI, Harrison GJ, Willis RJ. Age-related changes to adenosine in rat coronary resistance vessels. *Gen Pharmacol* 1999; 32: 35-40.
35. Song Y, Belardinelli L. Electrophysiological and functional effects of adenosine on ventricular myocytes of various mammalian species. *Am J Physiol (Cell Physiol)* 1996; 271: C1233-1243.
36. Burgdorf C, Richardt D, Kurz T, Adler S, Notzold A, Kraatz EG et al. Adenosine inhibits norepinephrine release in the postischemic rat heart: the mechanism of neuronal stunning. *Cardiovasc Res* 2001; 49: 713-720.

Table 1. Isovolumetric parameters in control and L-arginine treated rat hearts during basal conditions.

Treatment	Control	L-arginine
Heart wet weight (g)	1.7 ± 0.1	2.0 ± 0.1*
Body weight (g)	415.9 ± 9.9	440.6 ± 10.0
Hrtwtg/Bodywtg (g/Kg)	4.021 ± 0.18	4.592 ± 0.11*
HR (bpm)	260.0 ± 8.7	249.8 ± 13.5
LVDP (mmHg)	111 ± 10	95 ± 6
+dP/dt (mmHgsec ⁻¹)	2968 ± 154	2390 ± 141*
-dP/dt (mmHgsec ⁻¹)	-1824 ± 154	-1707 ± 130
MVO ₂ (μl min ⁻¹ g ⁻¹)	100.2 ± 6.7	78.3 ± 4.9*
RPP (mmHg min ⁻¹)	29020 ± 3018	22450 ± 2076
CPP (mmHg)	66.6 ± 8.7	92.9 ± 5.2*

All values are means ± S.E.M. (n= 8 per group). HR, heart rate; LVDP, left ventricular developed pressure; dP/dt are time derivatives of left ventricular pressure; MVO₂, myocardial oxygen consumption; RPP, rate pressure product; CPP, coronary perfusion pressure. *, different from control values (P<0.05)

Figure 1 Panel A represents gene expression differences in adenosine receptors while Panel B shows fold changes in NOS in hearts from L-arginine treated rats when compared to control expression. *P<0.05 vs control values.

Figure 2. The effect of chronic L-arginine treatment on adenosine (panel a), inosine (panel b) and purine (panel c) efflux during noradrenaline concentration-response curves in rat isolated perfused heart. Each data point represents mean \pm SEM. † indicates a significant difference from basal values, n= 4-8 per group, P<0.05.

Figure 3. The effect of chronic L-arginine treatment on noradrenaline concentration-response curves in rat isolated perfused heart. Heart rate (HR, panel a) left ventricular developed pressure (LVDP, panel b) and rate pressure product (RPP, panel c) Each data point represents mean \pm SEM. *denotes a significant change from untreated control values, n= 8 per group, P<0.05.

Figure 4. The effect of chronic L-arginine treatment on noradrenaline concentration-response curves in rat isolated perfused heart. Speed of contraction (+dP/dt, panel a), speed of relaxation (-dP/dt, panel b), coronary perfusion pressure (CPP, panel c) and myocardial oxygen consumption (MVO₂, panel d). Each data point represents mean \pm SEM. * indicates a significant difference from untreated control values while, n= 8 per group, P<0.05.

Figure 5. The effect of L-NAME (3 μ M) on NECA concentration-response curves in rat isolated perfused heart in control (panel A) and chronic L-arginine (panel B) treated rats. ● represents control values while ■ indicates data following L-NAME treatment. Each data point represents mean \pm SEM.

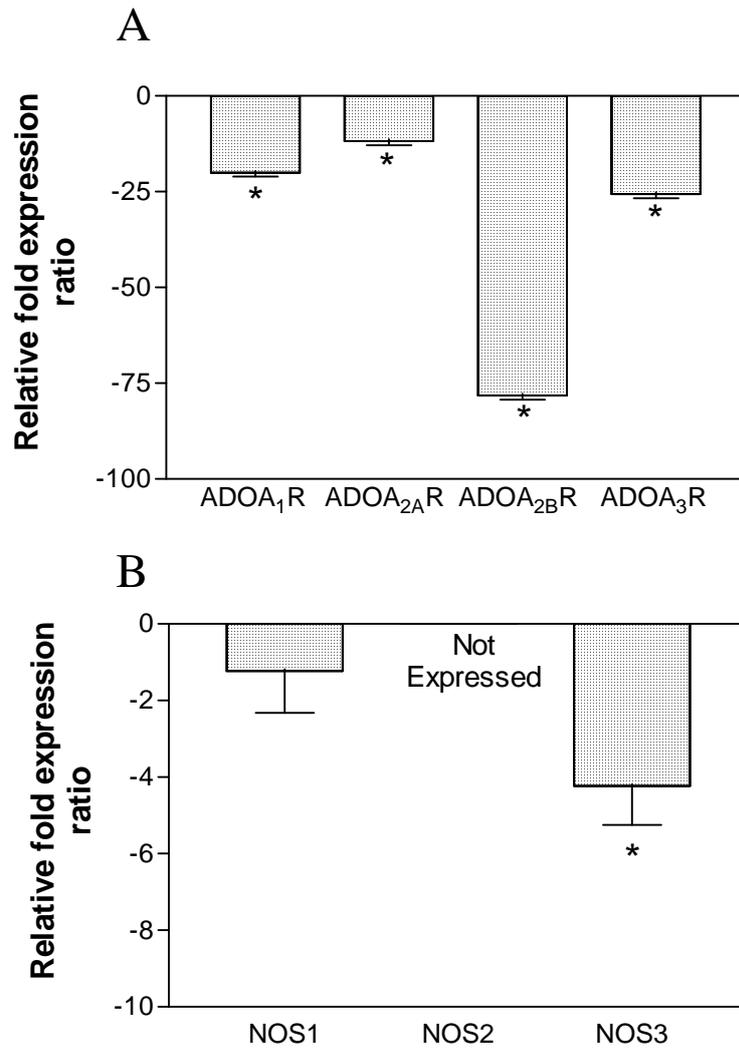


Figure 1

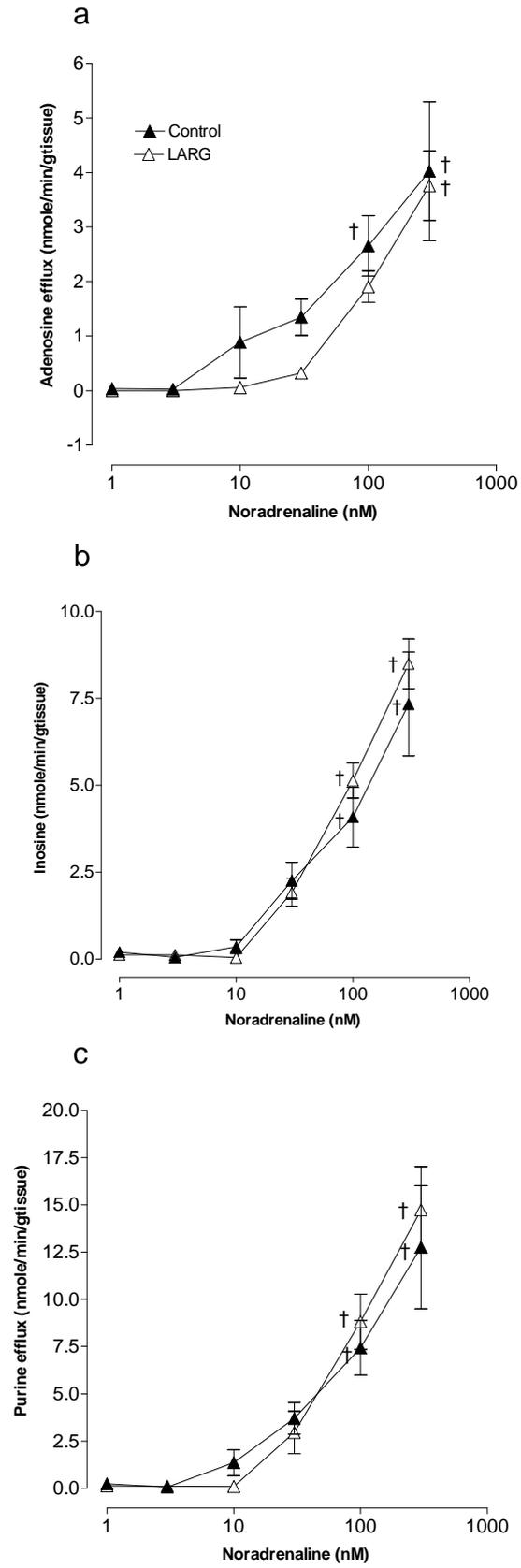


Figure 2

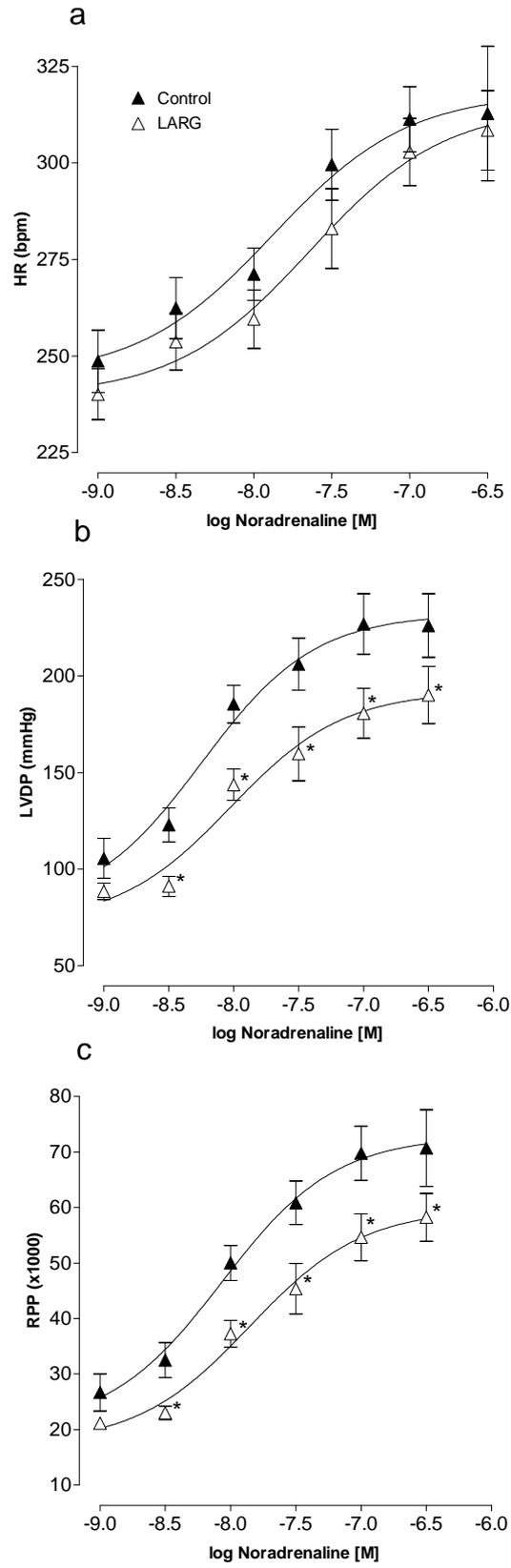


Figure 3

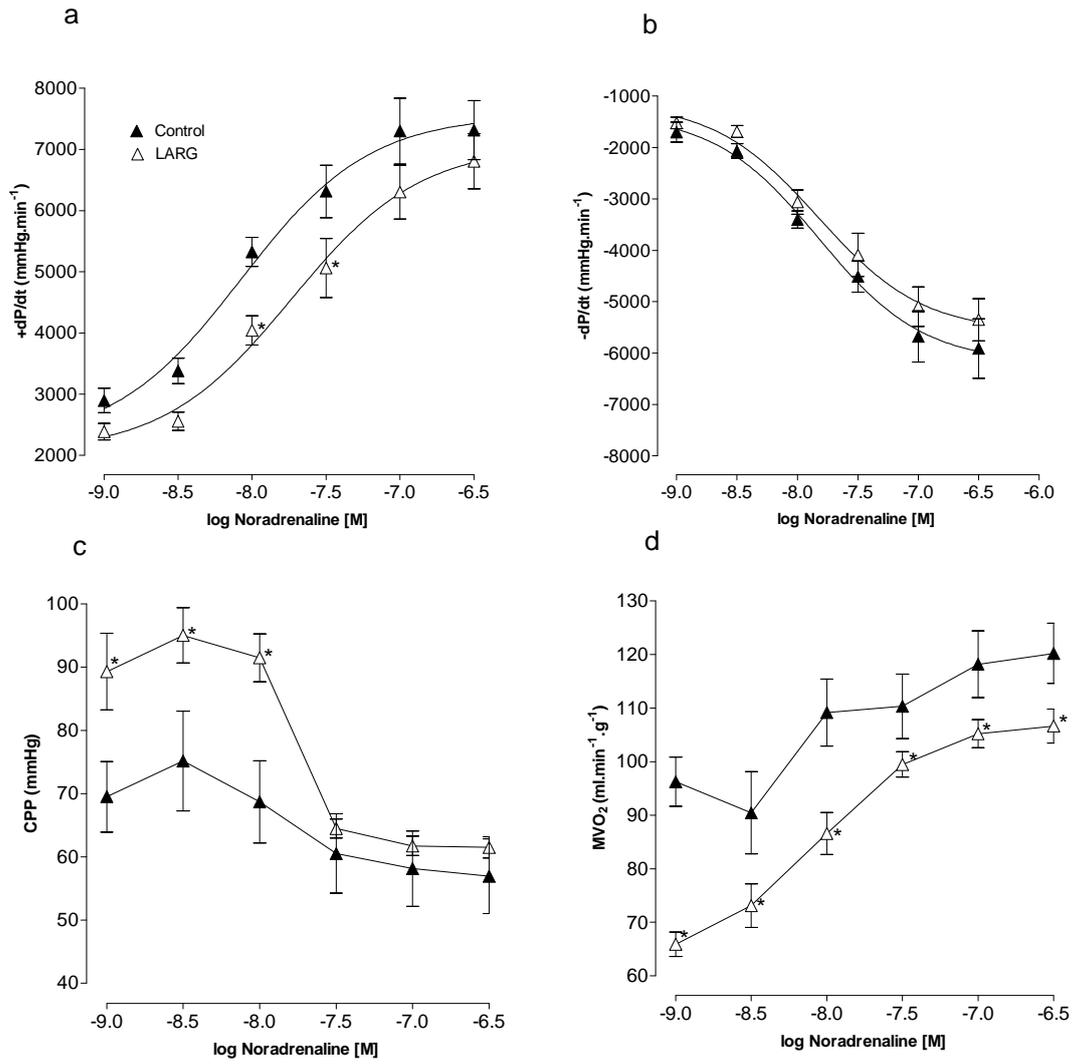


Figure 4

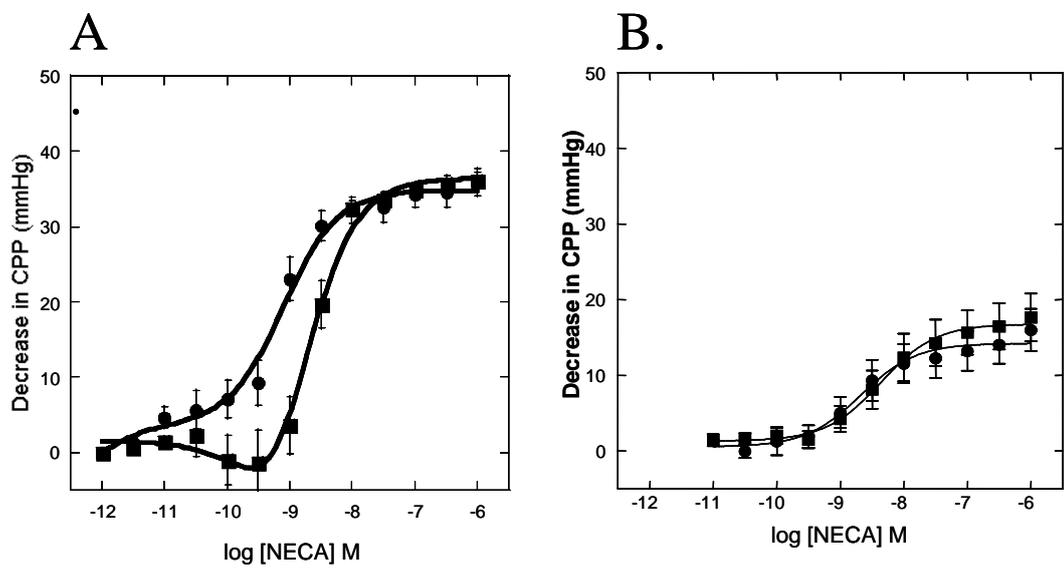


Figure 5.