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IMPROVED CARDIOVASCULAR FUNCTION WITH AMINOGUANIDINE IN DOCA-SALT HYPERTENSIVE RATS

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Short title:
Aminoguanidine & remodelling in hypertensive rats

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SUMMARY

1. The ability of aminoguanidine, an inhibitor of collagen cross-linking, to prevent changes in cardiac and vascular structure and function has been determined in the DOCA-salt hypertensive rat as a model of the cardiovascular remodelling observed in chronic human hypertension.

2. Uninephrectomised rats (UNX) administered DOCA (25mg every 4th day sc) and 1% NaCl in drinking water for 28 days developed cardiovascular remodelling shown as systolic hypertension, left ventricular hypertrophy, increased thoracic aortic and left ventricular wall thickness, increased left ventricular inflammatory cell infiltration together with increased interstitial collagen and increased passive diastolic stiffness, impaired contractility, prolongation of the action potential duration and vascular dysfunction.

3. Treatment with aminoguanidine (0.05 – 0.1% in drinking water; average 182±17 mg kg^{-1} day^{-1} in DOCA-salt rats) decreased blood pressure (DOCA-salt 176±4; + aminoguanidine 144±5*mmHg; *P<0.05 vs DOCA-salt), decreased left ventricular wet weights (DOCA-salt 3.17±0.07; + aminoguanidine 2.66±0.08*mg g^{-1} body wt), reduced diastolic stiffness constant (DOCA-salt 30.1±1.2; + aminoguanidine 24.3±1.2* (dimensionless)), improved cardiac contractility (DOCA-salt 1610±130; + aminoguanidine 2370±100*mmHg s^{-1}) and vascular reactivity (3.4-fold* increase in maximal contractile response to noradrenaline, 3.2-fold* increase in maximal relaxation response to acetylcholine, 2-fold* increase in maximal relaxation response to sodium nitroprusside) and prolonged the action potential duration at 50% repolarization without altering collagen content or inflammatory cell infiltration.

4. Thus, cardiovascular function in DOCA-salt hypertensive rats can be improved by aminoguanidine independent of changes in collagen content. This suggests that collagen cross-linking is an important cause of cardiovascular dysfunction during cardiovascular remodelling in hypertension.

KEYWORDS: aminoguanidine; collagen cross-linking; DOCA-salt rats; hypertension; remodelling

ABBREVIATIONS: AGEs, advanced glycation end-products; $APD_{20,50,90}$, action potential duration at 20, 50 or 90% of repolarisation; DOCA, deoxycorticosterone acetate; UNX, uninephrectomied

INTRODUCTION

Cardiovascular remodelling in chronic hypertension involves ventricular hypertrophy, interstitial and perivascular fibrosis, electrical remodelling in the heart and endothelial dysfunction. The importance of the increased collagen deposition in cardiovascular dysfunction has long been recognised (Weber *et al*., 1993) with recent studies investigating the role of relevant mediators such as angiotensin II (Sun *et al*., 2004). Collagen cross-linking may be a more important determinant of the increased ventricular stiffness in hypertensive rats than collagen content (Norton *et al*., 1997); collagen cross-linking was increased in the DOCA-salt hypertensive rat (Ooshima & Midorikawa, 1977). An increased collagen cross-linking has been proposed as the major cause of the increased arterial wall stiffness and decreased myocardial compliance during ageing (Lakatta, 1993; Li *et al*., 1996; Cantini *et al*., 2001). The formation of cross-links on long-lived matrix components follows non-enzymic reactions between glucose and proteins to form advanced glycation end-products (AGEs) (Brownlee, 1995). These products are markedly increased in ageing and diabetic rats

(Brownlee, 1995). Increased AGE formation has also been shown in the aorta of stroke-prone Spontaneously Hypertensive Rats (SHRs) (Mizutani *et al*., 2002). Further, methylglyoxal (the highly reactive dicarbonyl precursor of AGEs), AGEs, oxidised glutathione and oxidative stress were significantly higher in vascular smooth muscle cells from 12 week old SHR compared with age-matched normotensive Wistar-Kyoto rats (Wu & Juurlink, 2002). In 5 week old stroke-prone SHR, OPB-9195, an inhibitor of AGE formation, lowered systolic blood pressure and glycated albumin concentrations (Mizutani *et al*., 2002).

The nucleophilic hydrazine, aminoguanidine, is an effective inhibitor of AGE formation thus slowing or preventing age-related aortic stiffening and cardiac hypertrophy in rats (Li *et al*., 1996; Corman *et al*., 1998). Further, aminoguanidine is a selective inhibitor of inducible NO synthase (Nilsson, 1999) and may quench hydroxyl radicals and inhibit free radical formation, lipid peroxidation and oxidant induced apoptosis (Giardino *et al*., 1998). In rats, aminoguanidine ameliorated the neuropathic, ocular and cardiac complications of diabetes (Miyauchi *et al*., 1996; Norton *et al*., 1996; Swamy-Mruthinti *et al*., 1996), atherogenesis (Panagiotopoulos *et al*., 1998) and renal dysfunction in ageing rats (Reckelhoff *et al*., 1999). Aminoguanidine (pimegedine) has also been tested for the treatment of diabetic nephropathy in humans (Abdel-Rahman & Bolton, 2002).

The aim of the present study was to determine whether administration of aminoguanidine attenuates the cardiac and vascular dysfunction in the DOCA-salt hypertensive rat, independent of changes in collagen content. This study has used a prevention protocol with oral administration of aminoguanidine following initiation of cardiovascular remodelling in DOCA-salt hypertensive rats. We have used echocardiography to define cardiac structure *in vivo* and the isolated Langendorff heart to define function *ex vivo*. Histological methods were

used to define infiltration of inflammatory cells and collagen deposition. Isolated thoracic aortic rings were used to examine endothelial dysfunction. Microelectrode studies on isolated left ventricular papillary muscles were used to determine changes in cardiac action potentials.

MATERIALS AND METHODS

DOCA-salt hypertensive rats

Male Wistar rats aged approximately 8 weeks old were obtained from the Central Animal Breeding House of The University of Queensland. All experimental protocols were approved by the Animal Experimentation Ethics Committee of The University of Queensland, under the guidelines of the National Health and Medical Research Council of Australia which conform to the NIH Guidelines. Uninephrectomy was performed on all rats. Rats were anaesthetized with intraperitoneal injections of Zoletil® (tiletamine 25mg kg^{-1} and zolazepam 25mg kg^{-1}) and xylazine (10mg kg^{-1}), a lateral abdominal incision was used to access the left kidney, the left renal vessels and ureter were ligated and the kidney removed. The incision site was surgically sutured. Uninephrectomized rats were given either no further treatment (UNX rats) or 1% NaCl in the drinking water with subcutaneous injections of deoxycorticosterone acetate (DOCA; 25mg in 0.4ml of N,N-dimethyl formamide (DMF)) every fourth day (DOCA-salt rats). Rats were subsequently assigned into one of four groups: (i) uninephrectomized controls (UNX)(n=21); (ii) uninephrectomized controls receiving 0.1% aminoguanidine in drinking water for 4 weeks (UNX+AG)(n=24); (iii) DOCA-salt hypertensive rats (DOCA)(n=29); and (iv) DOCA-salt hypertensive rats receiving 0.1% aminoguanidine in drinking water for 1 week, then 0.05% aminoguanidine in drinking water for 3 weeks (DOCA+AG)(n=31). All experiments were performed 4 weeks after uninephrectomy.

Assessment of physiological parameters

All rats for this study were housed separately. Food and water intake and body weights were measured daily for all rats. Daily aminoguanidine consumption was calculated from the daily water intake. Systolic blood pressure was measured in selected rats lightly sedated with intraperitoneal Zoletil® (tiletamine 15mg kg^{-1} with zolazepam 15mg kg^{-1}). A tail pulse transducer (MLT1010) and an inflatable tail cuff were used, connected via a Capto SP844 physiological pressure transducer (MLT844/D) to a PowerLab data acquisition unit (ADInstruments, Sydney, Australia).

Echocardiographic Studies

Rats were anaesthetized with intraperitoneal injections of Zoletil® (tiletamine 25mg kg^{-1} and zolazepam 25mg kg^{-1}) and xylazine (10mg kg^{-1}) to produce anaesthesia with minimal cardiovascular depression. Serial, non-invasive, *in vivo* echocardiographic images were obtained using the Hewlett Packard Sonos 5500 echocardiography machine (12 MHz neonatal transducer) with an image depth of 3 cm and using 2 focal zones (Brown *et al*., 2002). Left ventricular M-mode measurements at the level of the papillary muscles were used to obtain wall thicknesses. Suprasternal long axis views were used to obtain the internal diameters of the ascending aortic arch (Brown *et al*., 2002).

Isolated Heart Preparations

The diastolic stiffness constant was determined using a non-recirculating isolated Langendorff heart preparation (Brown *et al*., 1999). Briefly, rats were anaesthetized with sodium pentobarbitone (60mg intraperitoneal) and heparin (200 $IU.kg^{-1}$) was administered via the femoral vein. After two minutes, the heart was rapidly excised and stunned in ice-cold

crystalloid perfusate (modified Krebs-Henseleit bicarbonate buffer (KHB) containing [in mM]: NaCl 119.1; KCl 4.75; $MgSO_4$ 1.19; KH_2PO_4 1.19; $NaHCO_3$ 25.0; glucose 11.0 and $CaCl_2$ 2.16). The aorta was isolated and cannulated via the dorsal root. Retrograde perfusion was initiated at 100cm of constant pressure with KHB bubbled with carbogen (95% O_2/5% CO_2), giving a pH of 7.4 and the temperature maintained at 35±0.5ºC. A water-filled latex balloon catheter was inserted in the left ventricle via the mitral orifice for measurement of left ventricular developed pressures. The catheter was connected via a three-way tap to a micrometer syringe and to a disposable pressure transducer (MLT844, ADInstruments) via a disposable clip-on dome (MLA844, ADInstruments) all connected to a MacLab system. The hearts were electrically paced at 250 beats min^{-1} by touching two electrodes to the surface of the right atrium. End-diastolic pressures were measured from 0 to 30mmHg. Myocardial diastolic stiffness was calculated as the diastolic stiffness constant (κ, dimensionless), that is the slope of the linear relation between stress (σ, dyne cm^{-2}) and tangent elastic modulus (E, dyne cm^{-2}) (Brown *et al*., 1999). To assess contractile function, maximum +dP/dT (rate of contraction) and –dP/dT (rate of relaxation) were calculated at a diastolic pressure of 10mmHg.

Histological studies of left ventricular collagen distribution, inflammatory cell infiltration and thoracic aortic wall thickness

Left ventricles (at mid-papillary level) and thoracic aortas were cut transversely, treated with Telly's fixative and Bouin's solution and stained with picrosirius red as previously described (Allan *et al*., 2005; Fenning *et al*., 2005). Thoracic aortic wall thicknesses were also measured using NIH-image software (National Institute of Health, USA). For left ventricular inflammatory cell infiltration measurements, sections of 5 μm thickness were stained with hematoxylin and eosin. Inflammatory cells were identified by cell morphology and analysed via a blinded experimenter protocol using a Meopta binocular light microscope. Sections

were examined for pathology as shown by the presence of inflammatory cells (for example, polymorphonuclear neutrophils (PMNs)), and graded on a scale of 0-4. For grading, a 45X lens was used while a 100X lens was used to check cell/nucleus morphology. Sections were given a grade of 1 if no abnormalities were detected. A grade of 1 indicated the appearance of low numbers of PMNs in the interstitial spaces. A grade of 2 indicated the presence of PMNs in higher numbers and moderate infiltration to other areas of the interstitium. A grade of 3 was given to indicate a more prominent presence of PMNs in the interstitium and significant infiltration to other areas of the myocardium. A maximal grade of 4 indicated severe scarring of the myocardium and high levels of PMNs in the interstitium especially accumulating within the scar tissues and throughout the myocardium.

Microelectrode studies

Isolated left ventricular papillary muscles were prepared for microelectrode studies as previously described (Fenning *et al*., 2005). After equilibration, action potential durations at 20%, 50% and 90% of repolarization were recorded from the papillary muscle over a 30-minute period, while continually being perfused with a drug-free Tyrode solution. Data were acquired, derived and analysed using Chart software (ADInstruments).

Isolated Thoracic Aortic rings

Thoracic aortic rings (approximately 4 mm in length) were suspended in an organ bath chamber with a resting tension of 10 mN and bathed in a modified Tyrode solution containing [in mM]: NaCl 136.9; KCl 5.4; $MgCl_2$ 1.05; $CaCl_2$ 1.8; $NaHCO_3$ 22.6; NaH_2PO_4 0.42; glucose 5.5; ascorbic acid 0.28 and sodium ethylene diamine tetra-acetic acid (EDTA) 0.1. The Tyrode solution was bubbled with carbogen (95% O_2/5% CO_2) and the temperature maintained at 35±0.5ºC. Force of contraction was measured isometrically with Grass FT03C force transducers

connected via amplifiers to a Macintosh computer via a MacLab system (Brown *et al*., 1991). Cumulative concentration-response curves were performed for noradrenaline and either acetylcholine or sodium nitroprusside in the presence of a submaximal (approximately 70%) contraction to noradrenaline.

Statistical Analysis

All values are presented as mean ± standard error of the mean (SEM). The -log EC_{50} was determined from the concentration giving half-maximal responses in individual concentration-response curves. Statistical comparisons of the group means were made by one-way analysis of variance (ANOVA) with a Bonferroni post-test analysis for multiple groups or by paired or unpaired Student's t-test as appropriate for two group comparison. $P<0.05$ was considered statistically significant.

Drugs

Aminoguanidine hemisulfate, deoxycorticosterone acetate, heparin, noradrenaline, acetylcholine and sodium nitroprusside were purchased from Sigma-Aldrich Chemical Company, St Louis, MO, USA. Noradrenaline, acetylcholine and sodium nitroprusside were dissolved in distilled water; deoxycorticosterone acetate was dissolved in dimethylformamide with mild heating.

RESULTS

Physiological parameters

Daily aminoguanidine doses remained relatively constant over the 4 weeks with an average daily dose for UNX+AG rats of 97±4 mg kg^{-1} day^{-1} (n=16-28), and an average daily dose for DOCA+AG rats of 182±17 mg kg^{-1} day^{-1} (n=16-28) (Figure 1). DOCA-salt rats developed hypertension; aminoguanidine treatment significantly lowered systolic blood pressures in both UNX+AG and DOCA+AG groups (Table 1).

Structural parameters

DOCA-salt rats exhibited marked cardiovascular remodelling, including left and right ventricular hypertrophy, increased left ventricular wall thickness, increased thoracic aortic wall thickness and decreased ascending aortic arch diameter but without dilatation of the left ventricular chamber (Table 1). Furthermore, DOCA-salt rats showed increased left ventricular interstitial inflammatory cell infiltration together with left ventricular interstitial fibrosis (Table 1). Treatment with aminoguanidine in the DOCA-salt rats attenuated the increase in left and right ventricular wet weights and prevented the decrease in ascending aortic arch diameter (Table 1). Aminoguanidine did not significantly alter inflammatory cell infiltration or left ventricular fibrosis (Table 1).

Functional parameters

DOCA-salt hypertensive rats exhibited significantly increased passive diastolic stiffness (Table 1), reduced maximal rates of contraction and relaxation together with prolongation of the action potential durations at 20% and 90% of repolarization (Table 1). Vascular dysfunction was also evident in the DOCA-salt rats as shown by reduced maximal contractile responses to noradrenaline and reduced maximal endothelium-dependent relaxation to acetylcholine and endothelium-independent relaxation to sodium nitroprusside (Figure 2).

Treatment with aminoguanidine in the DOCA-salt rats prevented the increased diastolic stiffness and improved rates of contraction and relaxation (Table 1). In addition, aminoguanidine increased action potential durations at 50% of repolarization but did not affect action potential durations at 20% and 90% of repolarization (Table 1). Finally, aminoguanidine treatment prevented the decreased maximal vascular contractile and relaxant responses in the DOCA-salt hypertensive rats (Figure 2).

DISCUSSION

Long-lived proteins such as collagen undergo continual non-enzymatic cross-linking with advanced glycation end-products (AGEs) during ageing (Lakatta, 1993; Corman *et al*., 1998) as well as in diabetes (Brownlee, 1995; Norton *et al*., 1996) and hypertension (Mizutani *et al*., 2002; Wu & Juurlink, 2002). Aminoguanidine inhibits the formation of collagen cross-linking by reacting with the reactive glucose-protein Amadori product and forming an unreactive substituted Amadori product that cannot form AGEs (Brownlee *et al*., 1986; Edelstein & Brownlee, 1992). Increased collagen cross-linking has been shown to correlate with increased myocardial and vascular stiffness (Corman *et al*., 1998; Badenhorst *et al*., 2003). Thus, inhibition of collagen cross-linking and AGE formation should decrease AGE-induced cross-linking and decrease stiffness. Aminoguanidine has additional actions that may be of relevance in the prevention of cardiovascular disease. It is a selective inhibitor of the endotoxin-induced or cytokine-induced inducible nitric oxide synthase (iNOS) isoform (Griffiths *et al*., 1993). By inhibiting iNOS, aminoguanidine may also decrease the formation of peroxynitrite (Hong *et al*., 2000), a pro-oxidant and an effective contributor to endothelial dysfunction (Kojda & Harrison, 1999). Aminoguanidine may also act an as antioxidant in the DOCA-salt rat, a model of increased superoxide production (Somers *et al*., 2000; Wu *et al*.,

2001), by quenching hydroxyl radicals and inhibiting free radical formation (Giardino *et al*., 1998). Further, AGEs binding to their receptor RAGE triggers a cascade of events leading to intracellular generation of free radicals, oxidative stress and activation of the transcription factor NF-κB (Yan *et al*., 1994; Lander *et al*., 1997); inhibition of AGE formation may prevent this cascade.

One possible mechanism of action of aminoguanidine in DOCA-salt rat hearts is as an iNOS inhibitor (Nilsson, 1999). Inflammatory cell infiltration has been demonstrated in the left ventricles of DOCA-salt rats (Fujisawa *et al*., 2001; Ammarguellat *et al*., 2002) and iNOS has been implicated in inflammation (Salvemini *et al*., 1995) and myocardial dysfunction (Salvemini *et al*., 1995; Oyama *et al*., 1998). However, it is important to note that as increased iNOS activity is characteristic of inflammation, a selective reduction in iNOS activity should also result in a reduction in inflammation. In this study, aminoguanidine did not reduce inflammatory cell infiltration into the myocardium, suggesting that iNOS inhibition by aminoguanidine is unlikely to be a major mechanism in the cardiac responses in the current study. The reduced response in thoracic aorta from UNX rats to acetylcholine could be due to inhibition of NO synthase in endothelial cells by aminoguanidine; however, this does not seem to apply to aorta from DOCA-salt rats where the endothelium is damaged.

The dosage of aminoguanidine used for this study was based on previous studies using 0.1% in the drinking water (Li *et al*., 1996; Reckelhoff *et al*., 1999). Since dose depends on water intake, this protocol produced different doses to DOCA-salt and control (uninephrectomized) rats. However, a wide range of aminoguanidine doses has been reported (25mg kg^{-1} (Brownlee *et al*., 1986), 100mg kg^{-1} (Kochakian *et al*., 1996), 250mg kg^{-1} (Bucala *et al*., 1991) and 7.35mmol kg^{-1} (which as a free base equates to approximately 545mg kg^{-1})

(Norton *et al*., 1996)), all shown to be effective in inhibiting the formation of AGEs and cross-linking. Thus, it is likely that the average aminoguanidine dose administered in this study would be effective in inhibiting collagen cross-linking.

Most experimental studies have investigated the role of increased collagen cross-linking in ageing and diabetes, rather than in hypertension. An increased cross-linking was shown in DOCA-salt hypertensive rats (Ooshima & Midorikawa, 1977) and in Spontaneously Hypertensive Rats (Badenhorst *et al*., 2003) but not in aortic-banded pressure overload hypertrophy (Badenhorst *et al*., 2003). Methylglyoxal, the precursor of AGEs, and AGE formation were increased in the vascular tissues of Spontaneously Hypertensive Rats (Mizutani *et al*., 2002; Wu & Juurlink, 2002); inhibition of AGE formation lowered systolic blood pressure and glycated albumin concentrations (Mizutani *et al*., 2002). As far as we are aware, this is the first study to show that a compound known to reduce collagen cross-linking can improve cardiac function in hypertensive rats.

Endothelial dysfunction is characteristic of the DOCA-salt rat (Kirchner *et al*., 1993; Somers *et al*., 2000). Numerous studies have shown favourable effects of aminoguanidine on the vasculature of ageing and diabetic rats by decreasing cross-linking, reducing reactive oxygen species and inhibiting quenching of nitric oxide (Bucala *et al*., 1991; Li *et al*., 1996; Corman *et al*., 1998). This study shows that this improvement can also be demonstrated in the DOCA-salt hypertensive rat. Changes in blood pressure as in the DOCA-salt hypertensive rat may lead to vascular remodelling to maintain the wall stress, according to the LaPlace relationship. An analysis of wall thicknesses at the operating pressure in treated and untreated rats, rather than *in vitro* in an isolated vessel, may allow estimation of changes in wall stress during treatment.

Action potential prolongation in hypertrophied DOCA-salt hearts is probably caused by a reduction in the transient outward potassium current (I_{to}) (Momtaz *et al*., 1996). However, aminoguanidine appeared to have minimal effects on regulating potassium currents in our study. We have shown prolongation of the APD_{50} in DOCA-salt rats treated with aminoguanidine. Prolongation of APD_{50} is believed to be primarily due to an increased L-type Ca^{2+} inward current (I_{Ca}) (Hart, 1994). Aminoguanidine has been shown to reverse the depression of the L-type calcium current in transplanted myocytes (Ziolo *et al*., 2001). Thus, this could be one mechanism by which aminoguanidine treatment selectively prolonged APD_{50} in these rats.

In summary, it is likely that inhibition of AGE-induced collagen cross-linking as well as its antioxidant properties contributed to the favourable effects of aminoguanidine on hypertension, hypertrophy, cardiac contractility and vascular reactivity without necessarily reducing fibrosis, inflammatory cell infiltration or iNOS activity in the DOCA-salt rats. Furthermore, this study clearly supports the concept that improvement in function is not necessarily dependent on a reduced content of collagen but rather reduced collagen cross-linking. Indeed, if collagen cross-linking plays such an important role in hypertension, the use of selective AGE cross-link breakers such as ALT-711 (Vasan *et al*., 2003; Bakris *et al*., 2004) could be of significant therapeutic importance in reversing end-organ damage in hypertension. A recent study by Little and coworkers (2005) demonstrated that treatment with ALT-711 decreased left ventricular mass and improved left ventricular diastolic filling parameters and quality of life in patients with diastolic heart failure. Although experience with ALT-711 in clinical studies is still limited, this compound is a potential treatment for the symptoms of diastolic heart failure (Little *et al*., 2005; Redfield, 2005).

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Table 1: Physiological parameters

Parameter	UNX 4wk	UNX + aminoguanidine	DOCA-salt 4wk	DOCA-salt + aminoguanidine
Systolic blood pressure (mmHg)	128 ± 5 (12)	117 ± 3* (11)	176 ± 4* (11)	144 ± 5** (12)
Initial body weight (g)	326 ± 3 (17)	338 ± 8 (21)	341 ± 8 (17)	344 ± 6 (26)
Final body weight (g)	431 ± 8 (17)	440 ± 10 (19)	354 ± 10* (16)	369 ± 5 (21)
LV+septum weight (mg g^{-1} bodywt)	1.97 ± 0.04 (15)	2.08 ± 0.04 (12)	3.17 ± 0.07* (14)	2.66 ± 0.08** (10)
RV weight (mg g^{-1} bodywt)	0.57 ± 0.02 (15)	0.53 ± 0.02 (12)	0.65 ± 0.02* (14)	0.56 ± 0.03** (10)
Thoracic aortic wall thickness (μm)	123 ± 4 (6)	121 ± 5 (8)	157 ± 6* (7)	145 ± 5* (8)
LV interstitial inflammation (grade)	0.8 ± 0.4 (5)	0.8 ± 0.4 (5)	2.8 ± 0.4* (5)	2.8 ± 0.6* (5)
LV interstitial area of collagen (%)	2.71 ± 0.21 (7)	4.06 ± 0.77 (8)	14.43 ± 1.75* (8)	10.96 ± 1.42* (8)
Diastolic stiffness (κ)	22.2 ± 1.2 (12)	19.4 ± 0.9 (12)	30.1 ± 1.2* (12)	24.3 ± 1.2** (10)
$+dP/dT_{max}$ (mmHg sec^{-1})	2080 ± 70 (8)	2340 ± 130* (12)	1610 ± 130* (13)	2370 ± 100** (10)
$-dP/dT_{max}$ (mmHg sec^{-1})	-1590 ± 50 (9)	-1550 ± 60 (12)	-1320 ± 70* (12)	-1480 ± 40** (10)
LVPWd (mm)	1.5 ± 0.1 (15)	1.7 ± 0.1* (11)	2.0 ± 0.1* (14)	2.0 ± 0.1* (14)
LVIDd (mm)	6.8 ± 0.2 (17)	7.1 ± 0.3 (13)	6.6 ± 0.2 (15)	6.6 ± 0.2 (15)
Ejection fraction (%)	91.0 ± 1.5 (16)	92.6 ± 1.4 (13)	93.8 ± 0.9 (15)	93.3 ± 1.4 (15)
Ascending aortic arch diameter (mm)	3.3 ± 0.1 (7)	3.6 ± 0.1* (12)	2.9 ± 0.1* (5)	3.3 ± 0.1** (14)
APD20 (msec)	6.8 ± 1.1 (8)	6.9 ± 1.1 (4)	10.1 ± 1.5* (7)	10.7 ± 1.0* (4)
APD50 (msec)	16.4 ± 2.0 (8)	22.8 ± 4.9 (4)	19.8 ± 2.7 (7)	42.4 ± 6.2** (4)
APD90 (msec)	34.4 ± 3.5 (8)	38.4 ± 7.7 (4)	59.1 ± 10.3* (7)	76.8 ± 11.2* (4)

All values shown represent the mean ± SEM (n value); LV=left ventricular, RV=right ventricular, LVPWd = left ventricular posterior wall thickness in diastole; LVIDd = left ventricular internal diameter in diastole; APD=action potential duration at 20, 50 or 90% of repolarization. Significance: *$p < 0.05$ vs UNX rats; **$p < 0.05$ vs DOCA-salt rats.

Legends to figures:

Figure 1. Daily aminoguanidine dose (mg kg^{-1}) of UNX rats treated with aminoguanidine and DOCA-salt rats treated with aminoguanidine. Values are mean±SEM; n=16-28 for all groups.

Figure 2. Concentration-response curves to noradrenaline (A) for UNX rats (-log EC50 7.4±0.1, n=9), UNX rats treated with aminoguanidine (-log EC50 7.7±0.1, n=7), DOCA-salt rats (-log EC50 7.8±0.1, n=9) and DOCA-salt rats treated with aminoguanidine (-log EC50 8.0±0.1 n=7). Concentration-response curves to acetylcholine (B) for UNX rats (-log EC50 7.0±0.1, n=9), UNX rats treated with aminoguanidine (-log EC50 7.1±0.2, n=7), DOCA-salt rats (-log EC50 7.0±0.1, n=9) and DOCA-salt rats treated with aminoguanidine (-log EC50 7.6±0.2 n=7). Concentration-response curves to sodium nitroprusside (C) for UNX rats (-log EC50 7.8±0.1, n=9), UNX rats treated with aminoguanidine (-log EC50 7.7±0.1, n=7), DOCA-salt rats (-log EC50 7.4±0.1, n=9) and DOCA-salt rats treated with aminoguanidine (-log EC50 8.0±0.2 n=7). Values are mean±SEM; Significance: *$p < 0.05$ vs UNX rats; **$p < 0.05$ vs DOCA-salt rats.

Figure 1

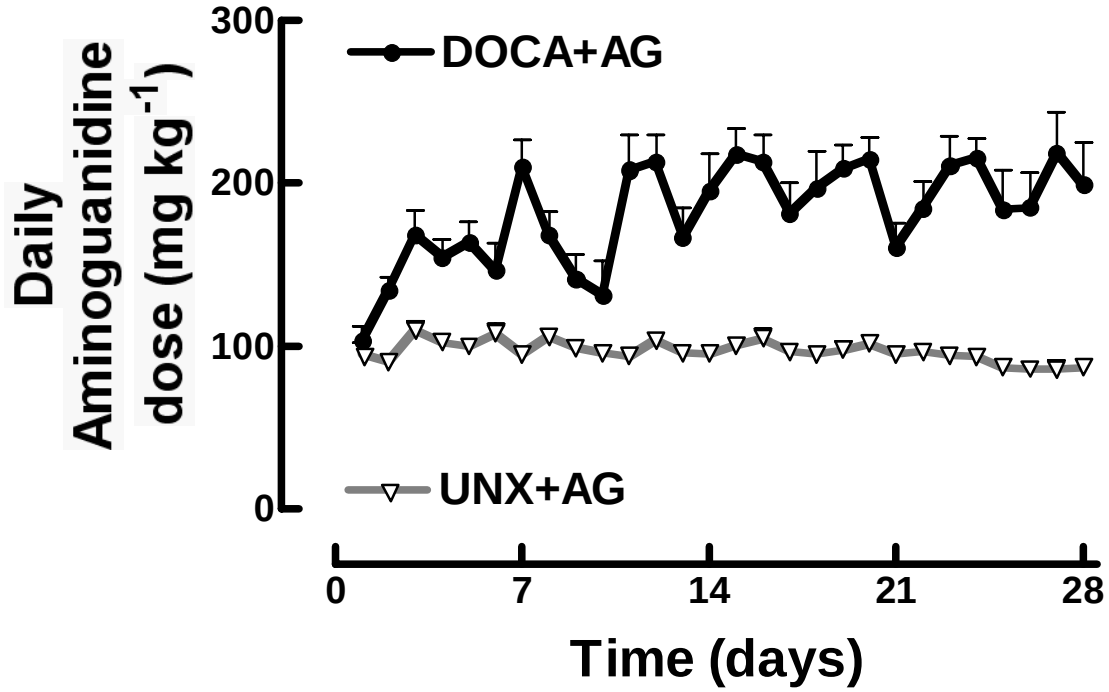

Figure 2a

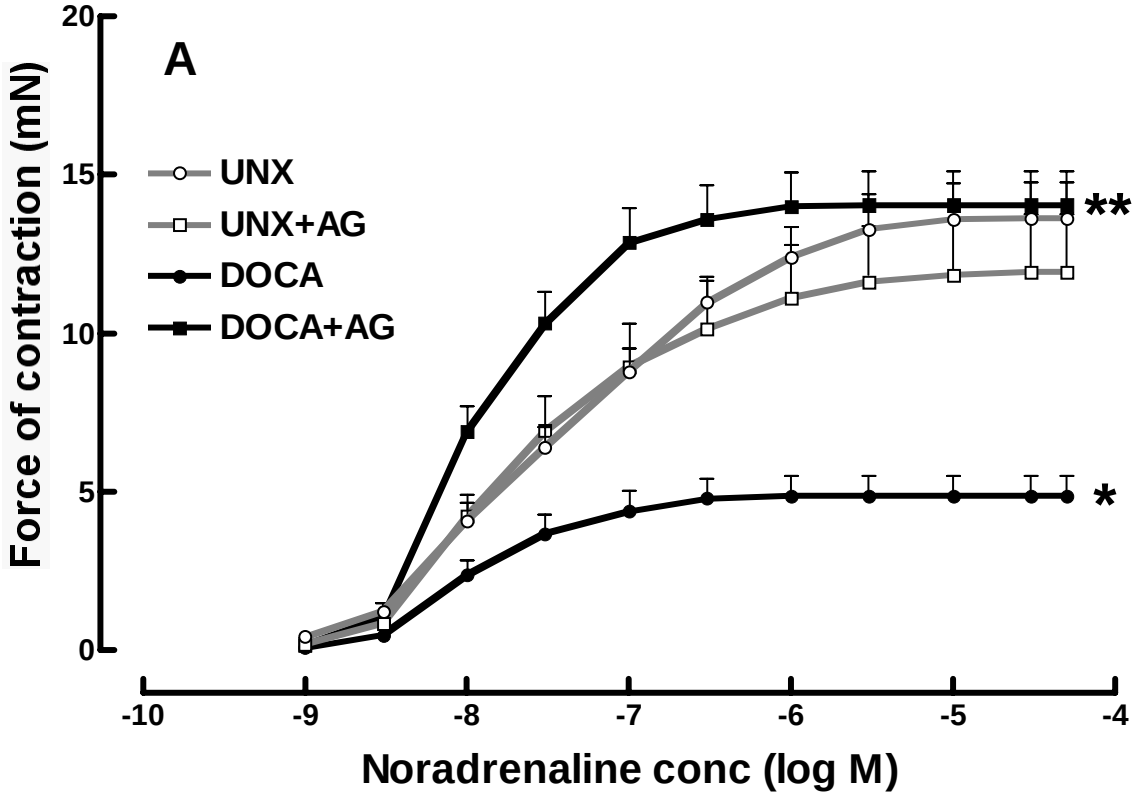

Figure 2b

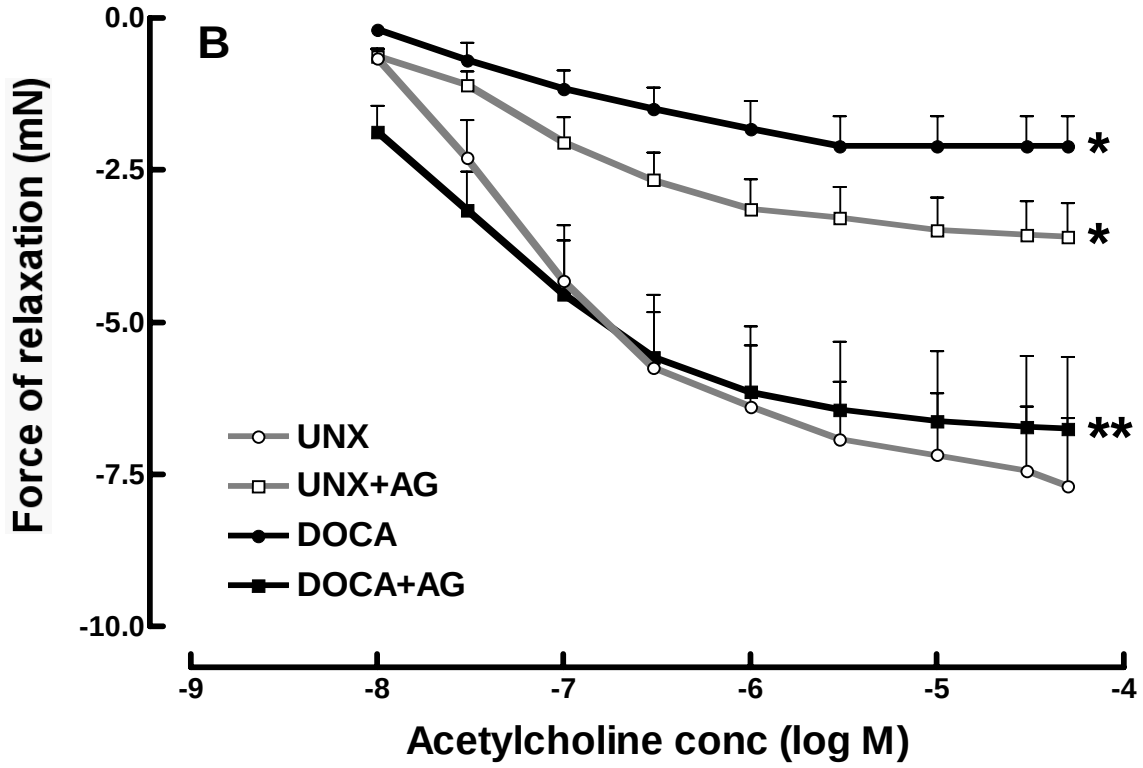

Figure 2c