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RESEARCH PAPER

Aminoguanidine prevents the impairment of cardiac pumping mechanics in rats with streptozotocin and nicotinamide-induced type 2 diabetes

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Background and purpose: Aminoguanidine (AG), an inhibitor of advanced glycation endproducts, has been shown to prevent arterial stiffening and cardiac hypertrophy in streptozotocin (STZ) and nicotinamide (NA)-induced type 2 diabetes in rats. Our aims were to examine whether AG produced benefits on cardiac pumping mechanics in the STZ and NA-treated animals in terms of maximal systolic elastance (E_{max}) and theoretical maximum flow (Q_{max}).
Experimental approach: After induction of type 2 diabetes, rats received daily injections of AG (50 mg kg^{-1}, i.p.) for 8 weeks and were compared with age-matched, untreated, diabetic controls. Left ventricular (LV) pressure and ascending aortic flow signals were recorded to calculate E_{max} and Q_{max}, using the elastance-resistance model. Physically, E_{max} reflects the contractility of the myocardium as an intact heart, whereas Q_{max} has an inverse relationship with the LV internal resistance.
Key results: Both type 2 diabetes and AG affected E_{max} and Q_{max}, and there was an interaction between diabetes and AG for these two variables. The E_{max} and Q_{max} were reduced in rats with type 2 diabetes, but showed a significant rise after administration of AG to these diabetic rats. Moreover, the increase in Q_{max} corresponded to a decrease in total peripheral resistance of the systemic circulation when the STZ and NA-induced diabetic rats were treated with AG.
Conclusions and implications: AG therapy prevented not only the contractile dysfunction of the heart, but also the augmentation in LV internal resistance in rats with STZ and NA-induced type 2 diabetes.
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Keywords: advanced glycation end products; aminoguanidine; maximal systolic elastance; theoretical maximum flow; streptozotocin–nicotinamide diabetic rats

Abbreviations: AG, aminoguanidine; AGEs, advanced glycation end products; E_{max}, maximal systolic elastance; NA, nicotinamide; P_{isomax}, peak isovolumic pressure of the left ventricle; Q_{max}, theoretical maximum flow; R, internal resistance of the left ventricle; R_p, total peripheral vascular resistance; STZ, streptozotocin; V_{eed}, effective end-diastolic volume of the left ventricle

Introduction

Numerous studies have demonstrated disturbances in cardiac calcium homoeostasis as well as an abnormal myosin isoenzyme profile in diabetes mellitus (Dillmann, 1980; Malhotra *et al*., 1981; Penpargkul *et al*., 1981). The pathogenesis and mechanisms underlying these abnormalities in the diabetic heart have not been fully elucidated. However, recent evidence has indicated that advanced glycation end products (AGEs) are pivotal mediators of cardiovascular dysfunction in diabetes. Persistent hyperglycaemia, dyslipidaemia and oxidative stress can act in concert to induce the formation of AGEs and cause cardiovascular inflammation, fibrosis and damage (Bucala, 1997; Baynes and Thrope, 1999; Brownlee, 2005; Thomas *et al*., 2005). The ability of AGEs to modify intracellular ryanodine receptors and sarcoplasmic reticulum Ca^{2+}-ATPase impairs the amplitude of Ca^{2+} transients in the diabetic myocardium (Bidasee *et al*., 2003, 2004). Moreover, activation of AGE receptors on cardiac myocytes directly influences calcium homoeostasis

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(Petrova *et al*., 2002) and contributes to interstitial fibrogenesis (Norton *et al*., 1996; Candido *et al*., 2003). Such changes in the cellular physiology of the diabetic heart may lead to contractile dysfunction and suppress the velocity of shortening.

Aminoguanidine (AG), a nucleophilic hydrazine compound, is a prototype of a scavenging agent that prevents glucose-induced formation of AGEs by reacting with electrophilic, carbonyl intermediates, thereby protecting nucleophilic residues on proteins and lipids from modification by chemicals (Brownlee *et al*., 1986; Edelstein and Brownlee, 1992; Nilsson, 1999; Thornalley, 2003). Inhibition of the accumulation of AGE by AG has been shown to retard the diabetes-associated reduction in myocardial compliance, by improving collagen digestibility (Norton *et al*., 1996). AG therapy also prevents cardiac hypertrophy by blocking protein carbonylation in the diabetic heart (Stadler *et al*., 2005). But little attention has been given to the cardiodynamic response to AG in that it might provide significant protection against the deterioration in systolic pumping mechanics induced in type 2 diabetes in rats.

Masiello *et al*. (1998) described a new rat model of type 2 diabetes that shares a number of features with human diabetes mellitus type 2. The diabetic syndrome is experimentally induced in adult rats by administration of streptozotocin (STZ) and partially protected with a suitable dose of nicotinamide (NA). This model is characterized by a 40% reduction in β-cell mass (Novelli *et al*., 2001), which results in moderate and stable hyperglycaemia, glucose intolerance, altered but significant glucose-stimulated insulin secretion, and *in vivo* and *in vitro* responsiveness to tolbutamide (Masiello *et al*., 1998). Our previous work demonstrated that treatment of the STZ and NA-induced diabetic rats with AG attenuates arterial stiffening and cardiac hypertrophy, in part through inhibition of the accumulation of AGE on collagen within the arterial wall (Chang *et al*., 2006a, b). However, the effects of type 2 diabetes and AG on cardiac pumping mechanics have never been examined.

The present study was designed to determine the effects of AG on the systolic mechanical behaviour of the ventricular pump in terms of maximal systolic elastance (E_{max}) and theoretical maximum flow (Q_{max}) in STZ and NA-induced type 2 diabetic rats. Left ventricular (LV) pressure and ascending aortic flow signals were measured to evaluate E_{max} and Q_{max}, by making use of the elastance-resistance model (Campbell *et al*., 1986; Shroff *et al*., 1992). E_{max} is an indicator of the elasticity and this reflects subtle changes in contractile status, and is independent of preload, afterload and heart rate in a given contractile state of the ventricle (Suga *et al*., 1973; Hunter *et al*., 1983). The value of E_{max}, therefore, represents the contractility of the myocardium as an intact heart. However, the LV end-systolic pressure–volume relationship may not be as independent of load as is often claimed, because van der Velde *et al*. (1991) found that (at a given end-systolic volume) the end-systolic pressure increased as total peripheral resistance increased. On the other hand, the Q_{max} is the amount of outflow generated by the ventricle if it were to eject under zero load conditions and has an inverse relationship with the LV internal resistance (Shroff *et al*., 1990, 1992). Our data suggest that AG therapy protects the myocardial contractility and internal resistance of the left ventricle (R) from deteriorating in rats administered STZ and NA.

Methods

Animals and catheterization

Male Wistar rats (2 months old) were randomly divided into four groups ($n = 15$ in each group) as follows: (i) normal controls (NC); (ii) rats of type 2 diabetes (STZ-NA); (iii) NC treated with AG (NC + AG); (iv) STZ-NA treated with AG. The diabetes mellitus type 2 was induced by i.p. administration of 180 mg kg^{-1} NA, 30 min before an i.v. injection of 50 mg kg^{-1} STZ (Masiello *et al*., 1998). STZ was dissolved in 0.1 M citrate buffer (pH 4.5). After the induction of diabetes, the STZ-NA rats were randomly allocated into a vehicle-treated diabetic group and a treated group, which received daily injections of AG 50 mg kg^{-1}, i.p. The animals were studied 8 weeks after the induction of type 2 diabetes to determine the effects of AG on the systolic mechanical behaviour of the ventricular pump. Insulin concentrations in the plasma were measured by the ELISA method. The development of hyperglycaemia was confirmed by blood glucose determination using a SURESTEP Test Strip. All rats were allowed free access to the Purina chow and water and housed two to three per cage, kept in an animal room with a 12 h light/dark cycle. The animal experiments were conducted according to the 'Guide for the Care and Use of Laboratory Animals', and were approved by the Animal Care and Use Committee of the National Taiwan University.

The general surgical procedures and method used to measure the haemodynamic variables in anaesthetized rats were as described previously (Chang *et al*., 2002). In brief, the rats were anaesthetized with sodium pentobarbital (50 mg kg^{-1}, i.p.), placed on a heating pad, intubated and ventilated with a Model 131 rodent respirator (New England Medical Instruments, Medway, MA, USA). The chest was opened through the second intercostal space of the right side. An electromagnetic flow probe, Model 100 series of internal circumference 8 mm (Carolina Medical Electronics, King, NC, USA), was positioned around the ascending aorta to record the pulsatile aortic flow. A high-fidelity pressure catheter, Model SPC 320 of size 2F (Millar Instruments, Houston, TX, USA), was inserted via the isolated right carotid artery into the left ventricle to measure the LV pressure. The ECG of lead II was recorded with an ECG/Biotach amplifier (Gould, Cleveland, OH, USA). The selective LV pressure and aortic flow signals of 5–10 beats were averaged in the time domain, using the peak R wave of ECG as a fiducial point. A single-beat estimation technique was performed to calculate the systolic elastance and resistance that characterize the pumping mechanics of the diabetic heart (Chang *et al*., 2002).

Prediction of the LV pressure using the elastance-resistance model

Model-derived pressure of the left ventricle $\hat{P}(t)$ can be predicted by using the elastance-resistance model if the

model parameters are previously identified (Campbell *et al*., 1986; Shroff *et al*., 1992). The relationship between instantaneous LV pressure, flow and isovolumic pressure can be written as follows:

$$\hat{P}(t) = P_{\mathrm{iso}}(t)\left[1 - \frac{V_{\mathrm{ej}}(t)}{V_{\mathrm{eed}}}\right]\left[1 - \frac{Q(t)}{Q_{\mathrm{max}}}\right] \quad (1)$$

where $V_{\mathrm{ej}}(t)$ is instantaneously ejected volume computed by numerically calculating the running integral of the aortic flow signal $Q(t)$. Q_{max} is the theoretical maximum flow, and V_{eed} is the effective LV end-diastolic volume that is the volume difference between LV end-diastolic volume and the zero-pressure volume axis intercept. $P_{\mathrm{iso}}(t)$ is the isovolumic pressure obtained by occluding the ascending aorta near the sinuses of Valsalva at the end of diastole. Herein, $P_{\mathrm{iso}}(t)$ was derived from the measured pressure of an ejection contraction by making use of a nonlinear least-squares approximation technique as follows (Sunagawa *et al*., 1980):

$$P_{\mathrm{iso}}(t) = \frac{1}{2}P_{\mathrm{idmax}}[1 - \cos(\omega t + c)] + P_{\mathrm{d}} \quad (2)$$

where P_{idmax} is a peak-developed isovolumic pressure, ω is an angular frequency, c is a phase-shift angle of the sinusoidal curve and P_{d} is the LV end-diastolic pressure. $P_{\mathrm{iso}}(t)$ in Figure 1b was obtained by fitting the measured LV pressure curve segments from the end-diastolic pressure point to the peak $+\mathrm{d}P/\mathrm{d}t$ and from the pressure point of the peak $-\mathrm{d}P/\mathrm{d}t$ to the same level as the end-diastolic pressure of the preceding beat (Takeuchi *et al*., 1991). The peak of the ECG R wave was used to identify the LV end-diastolic point. The estimated peak isovolumic pressure of the left ventricle, P_{isomax}, is the pressure sum of P_{idmax} and P_{d}.

Both V_{eed} and Q_{max} are the model parameters that remain to be determined by curve-fitting techniques. Campbell *et al*. (1986) found that Eq. 1 can be used to fit the measured LV pressure of an ejecting beat very well, if the fitting interval is $t_{\mathrm{ej}} < t < t_{\mathrm{pisomax}}$, where t_{ej} is the onset of ventricular ejection and t_{pisomax} is the time of peak isovolumic pressure. Initial values of V_{eed} and Q_{max} are chosen first. The Nelder–Meade simplex algorithm (Dennis and Woods, 1987) is then used to adjust V_{eed} and Q_{max} iteratively, to minimize the root mean square error (e_{p}) (Chang *et al*., 2002). The parameters coincident with the minimum objective function are taken as the model estimates of the systolic pumping mechanics of the left ventricle (Figure 1c). Thus, the LV systolic elastance can be calculated by making use of $E(t) = P_{\mathrm{iso}}(t)/V_{\mathrm{eed}}$, and its maximal value is the maximal systolic elastance ($E_{\mathrm{max}} = P_{\mathrm{isomax}}/V_{\mathrm{eed}}$). The internal resistance of the left ventricle can be expressed as $R(P_{\mathrm{iso}}) = P_{\mathrm{iso}}(t)/Q_{\mathrm{max}}$. In addition, total peripheral resistance of the systemic circulation (R_{p}) was calculated as the mean aortic pressure/mean aortic flow.

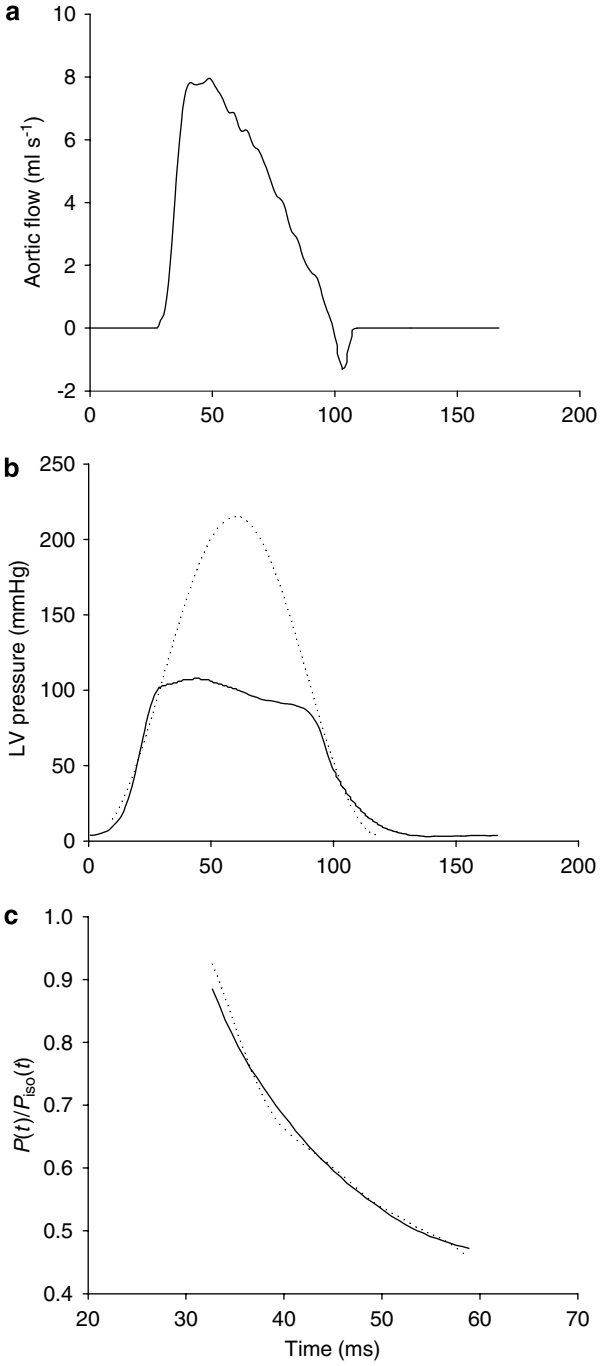

Figure 1 The solid curves show the measured ascending aortic flow signal (**a**) and left ventricular pressure waveform (**b**) in one control rat. (**b**) The dashed line represents the isovolumic pressure curve at an end-diastolic volume, which is estimated by fitting a sinusoidal function to the isovolumic portions of the measured left ventricular pressure. (**c**) Shows the measured data (solid line) and model-generated data (dashed line) when the elastance-resistance model is fitted over $t_{\mathrm{ej}} < t < t_{\mathrm{pisomax}}$, where t_{ej} is the onset of ventricular ejection and t_{pisomax} is the time of peak isovolumic pressure. $P(t)$, the measured left ventricular pressure; $P_{\mathrm{iso}}(t)$, the estimated isovolumic pressure.

Statistics

All data are expressed as means ± s.e.mean A two-way ANOVA was employed to determine the effects of type 2 diabetes and AG on the systolic mechanical behaviour of the ventricular pump in rats administered STZ and NA. Simple effects analysis was used when a significant interaction between type 2 diabetes and AG occurred. Differences between means within levels of a factor were determined

by Tukey's honestly significant difference method (Kirk, 1982). Significant differences were assumed at the level of $P<0.05$.

Drugs and materials

NA, STZ and AG were obtained from Sigma (St Louis, MO, USA). The ELISA method used was from Mercodia AB (Uppsala, Sweden) and the SURESTEP Test Strip from Lifescan Inc. (Milpitas, CA, USA).

Results

The solid curves in Figures 1a and b show the ascending aortic flow signal measured and LV pressure waveform, respectively, in one control rat. In Figure 1b, the dashed line represents the isovolumic pressure curve at the end-diastolic volume measured, which was estimated by fitting a sinusoidal function to the isovolumic portions of the LV pressure measured. Figure 1c demonstrates the similarity between the computed and measured LV pressure waveforms during the fitting interval $t_{ej}<t<t_{pisomax}$. The averaged value over all animals studied for e_p as an indication of the quality of fit was 0.0043 ± 0.0003. Goodness of fit for the model was also reflected in a high coefficient of determination (0.9899 ± 0.0019) as well as a relatively low standard error of the estimate ($2.24\pm0.13\%$). These indicate that the estimated parameters, V_{eed} and Q_{max}, were of good quality for analysing the cardiac pumping mechanics, by making use of the elastance-resistance model.

Figure 2 demonstrates the effects of type 2 diabetes and AG on the estimated P_{isomax}, effective LV end-diastolic volume V_{eed} and E_{max}. Both diabetes and AG affected E_{max} and there was an interaction between diabetes and AG for this variable. In the type 2 diabetic rats, the V_{eed} was increased 18.8% compared to control ($P<0.01$) (Figure 2b) in the absence of any significant changes in P_{isomax} (Figure 2a), leading to a significant fall of 21.9% in E_{max} ($P<0.01$) (Figure 2c). After treatment with AG, the STZ- and NA-induced diabetic rats showed a significant decline in V_{eed} without changes in P_{isomax}, and exhibited a marked rise in E_{max} of 26.8% ($P<0.01$). When normalized for LV weight, E_{maxn} (that is, E_{max}/LV weight in Figure 2d) of the diabetic heart was still

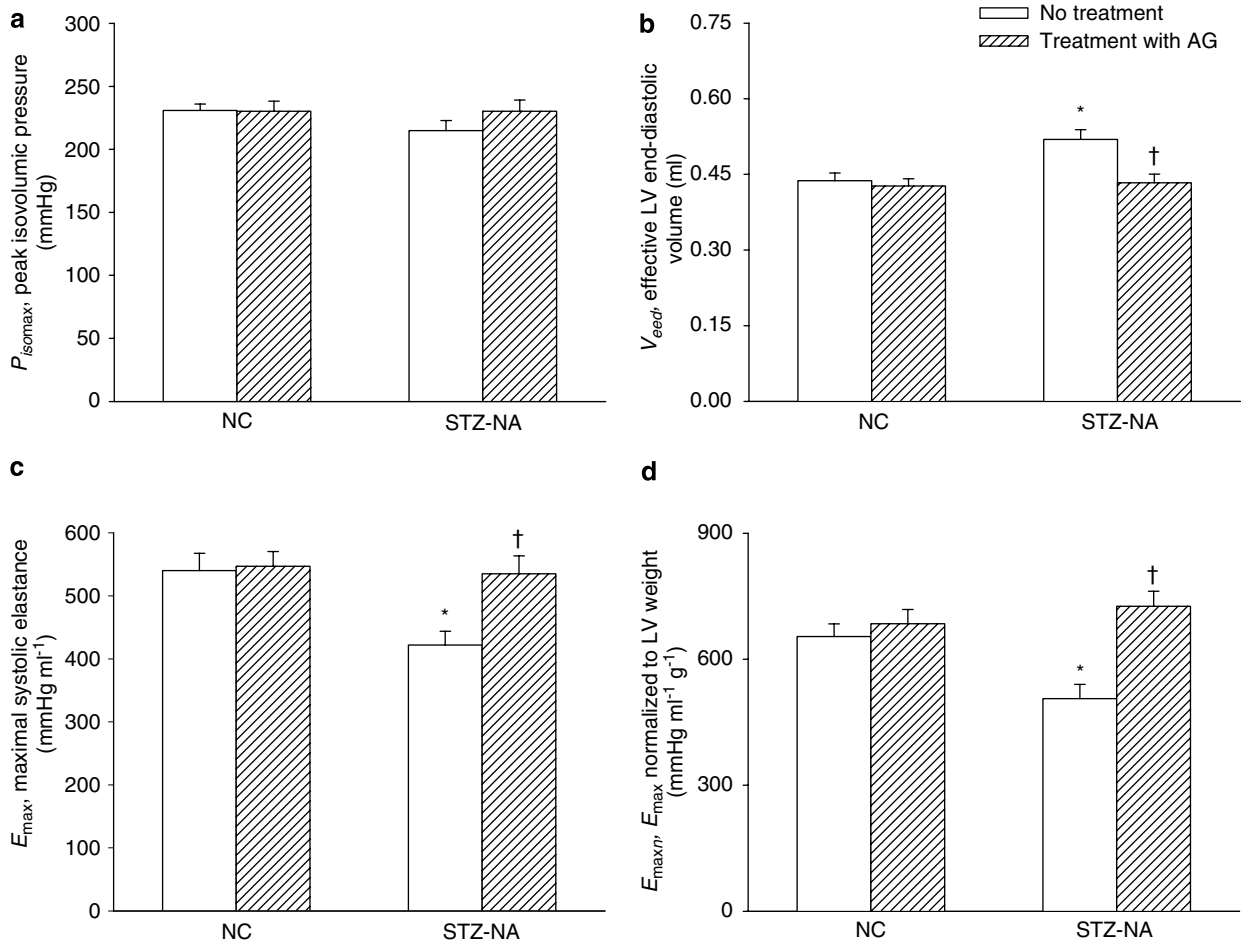

Figure 2 Effects of type 2 diabetes and AG on (**a**) estimated peak isovolumic pressure P_{isomax}, (**b**) effective LV end-diastolic volume V_{eed} and (**c**) maximal systolic elastance E_{max} ($n=15$ per group). E_{max} can be determined by the ratio of P_{isomax} to V_{eed} and (**d**) E_{maxn} is the value of E_{max} normalized for LV weight. AG, aminoguanidine; LV, left ventricular; NC, normal controls; STZ-NA, diabetic rats at 8 weeks after being administered streptozotocin and nicotinamide. *Significant difference ($P<0.05$) from the control group (NC). †Significant difference ($P<0.05$) from the STZ-NA group.

significantly lower than that of the control heart, and this diminished E_{maxn} was prevented by administration of AG to the diabetic animals.

The effects of type 2 diabetes and AG on Q_{max} are depicted in Figure 3a. Q_{max} was also plotted against total peripheral resistance of the systemic circulation R_p (Figure 3b). Both type 2 diabetes and AG affected Q_{max}, and there was an interaction between type 2 diabetes and AG for this variable. Q_{max} was lower in type 2 diabetic rats compared to controls but when AG was administered to these STZ- and NA-treated rats, this variable increased significantly by 15.2% ($P<0.05$) (Figure 3a). This increase in Q_{max} paralleled the decrease in R_p observed in diabetic animals treated with AG (Figure 3b).

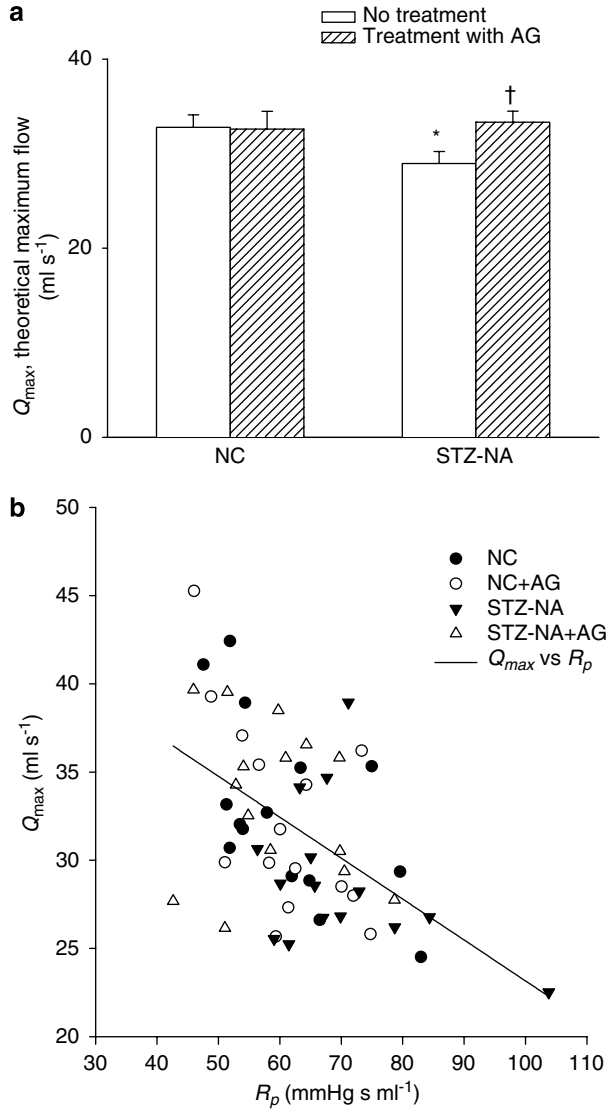

Figure 3 (**a**) The effects of type 2 diabetes and AG on theoretical maximum flow Q_{max} ($n=15$ per group). Q_{max} was also plotted against total peripheral resistance of the systemic circulation R_p (**b**). An inverse relationship between Q_{max} and R_p is evident after pooling the data from all groups. The solid line is obtained when a linear regression of Q_{max} on R_p is performed on data from all the rats studied, having the linear equation $Q_{max}=46.3601-0.2320\times R_p$ with $r=0.5098$; $P<0.0001$. AG, aminoguanidine; NC, normal controls; STZ-NA, diabetic rats at 8 weeks after being administered streptozotocin and nicotinamide. *Significant difference ($P<0.05$) from the control group (NC). †Significant difference ($P<0.05$) from the STZ-NA group.

Discussion and conclusions

Previous work from our laboratory demonstrated that the STZ- and NA-induced type 2 diabetes includes moderate and stable hyperglycaemia without changes in the plasma insulin level, which is not affected by AG treatment (Chang *et al*., 2006b). Moreover, treatment of the diabetic rats with AG attenuates arterial stiffening and cardiac hypertrophy, in part through inhibition of the accumulation of AGE on collagen within the arterial wall. The results from the present study suggest that AG therapy provides significant protection against deterioration of the contractile status and R in rats administered STZ and NA.

Effects of type 2 diabetes and AG on the contractile status of the left ventricle

As mentioned earlier, the LV E_{max} can be determined by the ratio of P_{isomax} to V_{eed}. The increased V_{eed} in the absence of any significant changes in P_{isomax} primarily acts to diminish E_{max} in STZ- and NA-induced type 2 diabetic rats. This suggests that the diabetic myocardium may be incapable of producing enough pressure force to support E_{max} along with the increased V_{eed}. Treatment with AG significantly affected the STZ- and NA-derived impairment in V_{eed}, leading to an increase in E_{max} (Figure 2c). A major role of AGEs in the pathogenesis of various cardiac disorders in diabetes has been implicated. AGEs act by modifying cardiac proteins to cause abnormalities in calcium metabolism and/or defects in matrix molecules, thereby impairing the contractile status of the left ventricle in diabetes (Norton *et al*., 1996; Petrova *et al*., 2002; Bidasee *et al*., 2003, 2004; Candido *et al*., 2003). Moreover, the worsened $P_{isomax}-V_{eed}$ relationship in diabetes suggests that the underlying cooperative mechanisms in cardiac muscle such as length sensitivity (Rice *et al*., 1999) may be impaired, leading to cardiac dysfunction. Inhibition of the AGE-induced collagen crosslinking by AG has been demonstrated to improve the contractile dysfunction of the left ventricle in rats with hypertension (Chan *et al*., 2006) as well as in animals with volume-overload hypertrophy (Herrmann *et al*., 2003). These findings are in keeping with our data showing that collagen glycated within the aortic wall was reduced by administration of AG to the STZ- and NA-treated rats (Figure 3; Chang *et al*., 2006b). We concluded that AG therapy would improve the $P_{isomax}-V_{eed}$ relationship, thereby attenuating the impairment of the contractile status of the left ventricle in the rats administered STZ and NA.

Effects of type 2 diabetes and AG on the systolic resistance of the left ventricle

Another aspect of cardiac mechanics that is altered in type 2 diabetic rats is Q_{max}, which is reduced (Figure 3a). We have demonstrated that an inverse relationship between Q_{max} and

arterial load exists in rats (Chang *et al*., 2002). Hence, the STZ- and NA-induced rise in R_p (Chang *et al*., 2006b) may be one of the major factors responsible for the decline in Q_{max} (Figure 3b). After exposure to AG, the internal resistance of the diabetic heart was attenuated in the STZ- and NA-treated animals, as reflected by a rise in Q_{max} (Figure 3a). The increase in Q_{max} corresponded to the decrease in R_p when the diabetic rats were administered AG (Figure 3b). This suggests that the beneficial effect of AG on the reduction in arterial load is due to its favourable effects on Q_{max} in diabetes. Another important factor influencing Q_{max} is the shift of the myosin isoenzyme profile from the fast V1 isoform towards the slow V3 isoform in the diabetic heart (Dillmann, 1980; Malhotra *et al*., 1981; Penpargkul *et al*., 1981), because there is an inverse relationship between Q_{max} and per cent of slow V_3 isoform (Shroff *et al*., 1990). In skeletal muscle fibres, the targeting of reducing sugars to the lysine-rich ATPase catalytic site causes a decrease in actin-activated ATPase activity (Brown and Knull, 1992; Avigad *et al*., 1996; Ramamurthy *et al*., 2001). However, the molecular basis for the inhibitory effect of AG on glycation in myosin isoenzyme molecules of the left ventricle remains to be determined.

Ventricular–arterial coupling in rats with STZ- and NA-induced type 2 diabetes

An electrical model of the left ventricle coupled to its arterial circulation can be constructed to quantify the integrative nature of overall cardiovascular performance (Shroff *et al*., 1985, 1992; Campbell *et al*., 1986). As mentioned earlier, changes that take place in the diabetic heart include a decline in myocardial contractility (that is, the reduced E_{max} in Figure 2c) and an increase in R (that is, the diminished Q_{max} in Figure 3a). In addition, the vasculature is also altered in the diabetic rats, increasing the arterial load imposed on the heart (that is, the augmented R_p in Figure 3b). Therefore, the STZ- and NA-induced impairment of the mechanical properties of both the left ventricle and the vasculature reduced the blood flow in arteries of diabetic rats when compared with the normal controls (1.51 ± 0.04 vs 1.78 ± 0.07 ml s^{-1}, respectively; $P<0.05$). Administration of AG to the STZ- and NA-treated rats not only prevented the damage to the cardiac pumping mechanics, but also retarded the augmentation in arterial load, optimizing the integrative nature of overall cardiovascular function, as evidenced by an increase of 14.6% in cardiac output ($P<0.05$). However, it should be noted that our inferred changes in internal resistance cannot be extrapolated simply to apply to how the ventricle will react to changes in the systemic vascular resistance. Moreover, even though AG inhibits protein carbonylation in the diabetic heart (Stadler *et al*., 2005), it also has a reducing effect on the afterload, which would prevent cardiac hypertrophy in rats with STZ- and NA-induced type 2 diabetes (Chang *et al*., 2006b).

Limitations

It is noteworthy that our approach is highly dependent on the elastance-resistance model, which is not a perfect model for the evaluation of the LV systolic mechanics. Hunter *et al*. (1983) demonstrated that, in addition to elastance and resistance, there are at least two or more processes involved that determine systolic mechanical behaviour of the ventricular pump. These processes include the effect of volume and the deactivation factor. However, Campbell *et al*. (1986) showed that the elastance-resistance model could be used to fit the measured LV pressure of an ejecting beat very well, if the fitting interval is $t_{ej}<t<t_{pisomax}$. Also, Shroff *et al*. (1992) believed that the elastance-resistance model is a useful model to quantify the systolic pumping mechanics of the left ventricle, provided one clearly understands its limitations.

In addition to being an AGE blocker, AG is an inhibitor of , a potent inhibitor of the inducible isoform, and a weaker inhibitor of endothelial isoform (Nilsson, 1999; Thornalley, 2003). AG, by suppressing activation of vascular NADPH oxidase by cell-surface receptors for AGEs and by inhibition of uncoupled endothelial isoform of NOS (Thornalley, 2003), may also reduce superoxide production. Thus, AG might act as a protective agent in type 2 diabetes-induced cardiovascular complications by decreasing peroxynitrite formation as a consequence of its effects mentioned above. As the ability of AG to suppress superoxide production in the STZ- and NA-induced diabetic rats was not investigated in the present study, we cannot conclude that the effects of AG observed are only due to inhibition of the formation of AGE.

Taken together, the alterations that take place in the left ventricle include a decline in E_{max} as well as Q_{max} in the rats with type 2 diabetes induced by STZ and NA. An increase in V_{eed} in the absence of any significant changes in P_{isomax} primarily acts to reduce E_{max} so that the contractility of the diabetic heart is impaired. Treatment with AG for 8 weeks ameliorated the contractile dysfunction of the left ventricle in this new rat model of type 2 diabetes, as evidenced by the increase of E_{max}. Moreover, administration of AG to the diabetic animals attenuated the augmented internal resistance of the diabetic heart, as reflected by the rise of Q_{max}. AG induced an increase in Q_{max} in the rats with STZ- and NA-induced diabetes and this corresponded with a decrease in R_p in the diabetic rats. From these results, we suggest that AG therapy may impart significant protection against the worsened contractile status and R in STZ- and NA-induced type 2 diabetes in rats.

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Conflict of interest

The authors state no conflict of interest.

References

Avigad G, Kniep A, Bailin G (1996). Reaction of rabbit skeletal myosin with D-glucose 6-phosphate. *Biochem Mol Biol Int* **40**: 273–284.

Baynes JW, Thrope SR (1999). Role of oxidative stress in diabetic complications. A new perspective on an old paradigm. *Diabetes* **48**: 1–9.

Bidasee KR, Nallani K, Yu Y, Cocklin RR, Zhang Y, Wang M *et al*. (2003). Chronic diabetes increases advanced glycation end products on cardiac ryanodine receptors/calcium-release channels. *Diabetes* **52**: 1825–1836.

Bidasee KR, Zhang Y, Shao CH, Wang M, Patel kp, Dincer ÜD *et al*. (2004). Diabetes increases formation of advanced glycation end products on sarco(endo)plasmic reticulum Ca^{2+}-ATPase. *Diabetes* **53**: 463–473.

Brown MR, Knull HR (1992). Effects of nonenzymatic glycation of subfragment-1 of myosin on interaction with actin. Biochem. *Cell Biol* **70**: 617–622.

Brownlee M (2005). The pathobiology of diabetic complications. *Diabetes* **54**: 1615–1625.

Brownlee M, Vlassara H, Kooney A, Ulrich P, Cerami A (1986). Aminoguanidine prevents diabetes-induced arterial wall protein cross-linking. *Science* **232**: 1629–1632.

Bucala R (1997). Lipid and lipoprotein modification by advanced glycosylation end-products: role in atherosclerosis. *Exp Physiol* **82**: 327–337.

Campbell KB, Ringo JA, Knowlen GG, Kirkpatrick RD, Schmidt SL (1986). Validation of optimal elastance-resistance left ventricle pump models. *Am J Physiol* **251**: H382–H397.

Candido R, Forbes JM, Thomas MC, Dean RG, Burns WC, Tikellis C *et al*. (2003). A breaker of advanced glycation end products attenuates diabetes-induced myocardial structure changes. *Circ Res* **92**: 785–792.

Chan V, Hoey A, Brown L (2006). Improved cardiovascular function with aminoguanidine in DOCA-salt hypertensive rats. *Br J Pharmacol* **148**: 902–908.

Chang KC, Lo HM, Tseng YZ (2002). Systolic elastance and resistance in the regulation of cardiac pumping function in early streptozotocin-diabetic rats. *Exp Biol Med* **227**: 251–259.

Chang KC, Tseng CD, Chou TF, Cho YL, Chi TC, Su MJ *et al*. (2006a). Arterial stiffening and cardiac hypertrophy in e new rat model of type 2 diabetes. *Eur J Clin Invest* **36**: 1–7.

Chang KC, Tseng CD, Wu MS, Liang JT, Tsai MS, Cho YL *et al*. (2006b). Aminoguanidine prevents arterial stiffening in a new rat model of type 2 diabetes. *Eur J Clin Invest* **36**: 528–535.

Dennis JE, Woods DJ (1987). New computing environments. In: Wouk A (ed). *Microcomputers in Large-Scale Computing*. SIAM: Philadelphia. pp 116–122.

Dillmann WH (1980). Diabetes mellitus induces changes in cardiac myosin in the rat. *Diabetes* **29**: 579–582.

Edelstein D, Brownlee M (1992). Mechanistic studies of advanced glycosylation end product inhibition by aminoguanidine. *Diabetes* **41**: 26–29.

Herrmann KL, McCulloch AD, Omens JH (2003). Glycated collagen cross-linking alters cardiac mechanics in volume-overload hypertrophy. *Am J Physiol (Heart Circ Physiol)* **284**: H1277–H1284.

Hunter WC, Janicki JS, Weber KT, Noordergraaf A (1983). Systolic mechanical properties of the left ventricle: effects of volume and contractile state. *Circ Res* **52**: 319–327.

Kirk RE (1982). *Experimental Design: Procedures for the Behavioral Sciences*, 2nd edn. Brooks/Cole: Belmont, California. pp 121.

Malhotra A, Penpargkul S, Fein FS, Sonnenblick EH, Scheuer J (1981). The effects of streptozotocin-induced diabetes in rats on cardiac proteins. *Circ Res* **49**: 1243–1250.

Masiello P, Broca C, Gross R, Roye M, Manteghetti M, Hillaire-Buys D *et al*. (1998). Experimental NIDDM. Development of a new model in adult rats administered streptozotocin and nicotinamide. *Diabetes* **47**: 224–229.

Nilsson BO (1999). Biological effects of aminoguanidine: an update. *Inflamm Res* **48**: 509–515.

Norton GR, Candy G, Woodiwiss AJ (1996). Aminoguanidine prevents the decreased myocardial compliance produced by streptozotocin-induced diabetes mellitus in rats. *Circulation* **93**: 1905–1912.

Novelli M, Fabregat ME, Fernandez-Alvarez J, Gomis R, Masiello P (2001). Metabolic and functional studies on isolated islets in a new rat model of type 2 diabetes. *Mol Cell Endocrinol* **175**: 57–66.

Penpargkul S, Fein FS, Sonnenblick EH, Scheuer J (1981). Depressed cardiac sarcoplasmic reticular function from diabetic rats. *J Mol Cell Cardiol* **13**: 303–309.

Petrova R, Yamamoto Y, Muraki K, Yonekura H, Sakurai S, Watanabe T *et al*. (2002). Advanced glycation end-product calcium handling impairment in mouse cardiac myocyte. *J Mol Cell Cardiol* **34**: 1425–1431.

Ramamurthy B, Höök P, Jones AD, Larsson L (2001). Changes in myosin structure and function in response to glycation. *FASEB J* **15**: 2415–2422.

Rice JJ, Winslow RL, Hunter WC (1999). Comparison of putative cooperative mechanisms in cardiac muscle: length dependence and dynamic responses. *Am J Physiol* **276** (Heart Circ Physiol 45): H1734–H1754.

Shroff SG, Janicki JS, Weber KT (1985). Evidence and quantitation of left ventricular systolic resistance. *Am J Physiol* **249**: H358–H370.

Shroff SG, Janicki JS, Weber KT (1992). Mechanical and energetic behavior of the intact left ventricle. In: Fozzard HA (ed). *The Heart and Cardiovascular System*, 2nd edn. Raven: NY. pp 129–150.

Shroff SG, Naegelen D, Clark W (1990). Relation between left ventricular systolic resistance and contractile rate processes. *Am J Physiol* **258**: H381–H394.

Stadler K, Jenei V, Somogyi A, Jakus J (2005). Beneficial effects of aminoguanidine on the cardiovascular system of diabetic rats. *Diabetes Metab Res Rev* **21**: 189–196.

Suga H, Sagawa K, Shoukas AA (1973). Load independence of the instantaneous pressure–volume ratio of the canine left ventricle and effects of epinephrine and heart rate on the ratio. *Circ Res* **32**: 314–322.

Sunagawa K, Yamada A, Senda Y, Kikuchi Y, Nakamura M, Shibahara T *et al*. (1980). Estimation of the hydromotive source pressure from ejection beats of the left ventricle. *IEEE Trans Biomed Eng* **27**: 299–305.

Takeuchi M, Igarashi Y, Tomimoto S, Odake M, Hayashi T, Tsukamoto T *et al*. (1991). Single-beat estimation of the slope of the end-systolic pressure-volume relation in the human left ventricle. *Circulation* **83**: 202–212.

Thomas MC, Baynes JW, Thorpe SR, Cooper ME (2005). The role of AGEs and AGE inhibitors in diabetic cardiovascular disease. *Curr Drug Targets* **6**: 453–474.

Thornalley PJ (2003). Use of aminoguanidine (Pimagedine) to prevent the formation of advanced glycation endproducts. *Arch Biochem Biophys* **419**: 31–40.

van der Velde ET, Burkhoff D, Steendijk P, Karsdon J, Sagawa K, Baan J (1991). Nonlinearity and load sensitivity of end-systolic pressure–volume relation of canine left ventricle *in vivo*. *Circulation* **83**: 315–327.