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# Angiotensin II acutely decreases myocardial stiffness: a novel AT1, PKC and $Na^+/H^+$ exchanger-mediated effect

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**1** Acute effects of angiotensin II (AngII) on diastolic properties of the myocardium were investigated.

**2** Increasing concentrations of AngII ($10^{-9}$ to $10^{-5}$ M) were added to rabbit papillary muscles in the absence ($n=11$) or presence of: (i) AT1 receptor antagonists, losartan ($10^{-6}$ M; $n=7$) or ZD-7155 ($10^{-7}$ M; $n=8$); (ii) ZD-7155 ($10^{-7}$ M) plus AT2 receptor antagonist PD-123,319 ($2\times10^{-6}$ M; $n=6$); (iii) PKC inhibitor, chelerythrine ($10^{-5}$ M; $n=8$); or (iv) $Na^+/H^+$ exchanger (NHE) inhibitor, 5-(*N*-methyl-*N*-isobutyl)-amiloride ($10^{-6}$ M; $n=10$). Passive length–tension relations were constructed before and after a single concentration of AngII ($10^{-5}$ M, $n=6$). Effects of AngII infusion ($10\,\mu g\,kg^{-1}\,min^{-1}$) were evaluated in *in situ* rabbit hearts.

**3** AngII concentration dependently increased inotropy and resting muscle length (RL). At $10^{-5}$ M, active tension increased $43.3\pm6.25\%$ and RL $1.96\pm0.4\%$. Correcting RL to its initial value resulted in a $46\pm4\%$ decrease of resting tension, indicating decreased muscle stiffness, as confirmed by the right and downward shift of the passive length–tension relation promoted by AngII. In the intact heart, at matched systolic pressures of 112 mmHg, AngII decreased end-diastolic pressures from $10.3\pm0.3$ to $5.9\pm0.5$ mmHg, and minimal diastolic pressures from $8.4\pm0.5$ to $4.6\pm0.6$ mmHg.

**4** AT1 blockade inhibited AngII effects on myocardial inotropy and stiffness, while PKC or NHE inhibition only significantly attenuated its effects on resting length and tension.

**5** In conclusion, AngII decreases myocardial stiffness, an effect that requires AT1 receptor activation and is mediated by PKC and NHE. This represents a novel mechanism of acute neurohumoral modulation of diastolic function, suggesting that AngII is a powerful regulator of cardiac filling.
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**Keywords:** Angiotensin II; diastolic function; distensibility; myocardial stiffness; AT1 receptor

**Abbreviations:** AngII, angiotensin II; BDM, 2,3-butanedione monoxime; CHE, chelerythrine; ET-1, endothelin-1; LV, left ventricular; LVP, left ventricular pressure; MIA, 5-(*N*-methyl-*N*-isobutyl)-amiloride; NHE, $Na^+/H^+$ exchanger; NO, nitric oxide; PKC, protein kinase C; PV, pressure–volume

## Introduction

The octapeptide angiotensin II (AngII) has a central role in cardiovascular homeostasis exerting its effects by binding to two different subtypes of G protein-coupled receptors: AT1 and AT2 (Allen *et al*., 1999; De Gasparo *et al*., 2000). Both subtypes are expressed in the heart of several animal species. The AT1 receptor is generally considered to account for most of the classical effects of the renin–angiotensin system, such as vasoconstriction and positive inotropy (Ahmed *et al*., 1975; Moravec *et al*., 1990; Ishihata & Endoh, 1995; De Gasparo *et al*., 2000; Modesti *et al*., 2000; Basso & Terragno, 2001). These acute actions of AT1 stimulation are mediated by phosphoinositide hydrolysis and activation of protein kinase C (PKC)-dependent pathways (Ishihata & Endoh, 1993; Petroff *et al*., 2000; Kim & Iwao, 2001). Also an increase of calcium transients, through the L-type $Ca^{2+}$ current (Skolnick *et al*., 1998; Petroff *et al*., 2000; Aiello & Cingolani, 2001) and myofilament sensitization (Watanabe & Endoh, 1998), are apparently involved, although the precise mechanisms are still controversial, in part because of species differences.

Chronically, AT1 stimulation by locally or systemically produced AngII (Modesti *et al*., 2000) promotes mitogenesis (Sudgen, 2002), growth and extracellular matrix synthesis (Brooks *et al*., 1997), thereby contributing to ventricular remodelling (Weber *et al*., 1995), cardiac hypertrophy (Batenburg *et al*., 2004), and, ultimately, to the deterioration of systolic and diastolic functions (McElmurray *et al*., 1999).

These chronic effects have, so far, been considered the main mechanisms through which AngII and other neurohumoral agents may influence the diastolic properties of the myocardium. Some studies, however, suggest that diastolic stiffness may be actively modulated by NO (Shah *et al*., 1994; Heymes *et al*., 1999) and endotelin-1 (ET-1) (Leite-Moreira *et al*., 2003). In isolated myocytes, NO increases resting diastolic cell length, and in intact hearts, shifts downward the diastolic PV loop during filling, both indicating decreased myocardial stiffness. In isolated cardiac muscle, we showed that ET-1 increases distensibility of acutely loaded myocardium through

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ET-A receptors and $Na^+/H^+$ exchanger (NJE) activation. As AngII shares some of the subcellular pathways and widely interacts with ET-1 and NO (Berthold *et al*., 1999; Rossi *et al*., 1999), we hypothesized that it might as well acutely modulate the diastolic properties of the myocardium. In this paper, we tested this hypothesis and investigated possible underlying mechanisms of the observed effects.

## Methods

The investigation conforms to the *Guide for the Care and Use of Laboratory Animals* published by the National Institutes of Health (NIH Publication No. 85-23, revised 1996).

The study was carried out in two different experimental models: isolated papillary muscles and *in situ* intact heart.

### *Isolated papillary muscles*

*Experimental preparation* Male New Zealand White rabbits (*Oryctolagus cuniculus*; 2.0–3.0 kg; $n=32$) were anesthetized with intravenous pentobarbital sodium (25 mg kg$^{-1}$). A left thoracotomy was performed, and beating hearts were quickly excised and immersed in modified Krebs–Ringer solution (composition in mM: 98 NaCl, 4.7 KCl, 2.4 $MgSO_4 \cdot 7H_2O$, 1.2 $KH_2PO_4$, 4.5 glucose, 1.8 $CaCl_2 \cdot 2H_2O$, 17 $NaHCO_3$, 15 $C_3H_3NaO_3$, 5 $CH_3COONa$, 0.02 atenolol) at 35°C with cardioplegic 2,3-butanedione monoxime (BDM; 3%) and 5% newborn calf serum. Atenolol was used to prevent $\beta$-mediated effects. Solutions were in equilibrium with 95% $O_2$ and 5% $CO_2$, maintaining the pH between 7.38 and 7.42. After dissection, papillary muscles ($n=56$; length: $3.2\pm2$ mm; weight: $2.8\pm0.3$ mg; preload: $4.6\pm0.1$ mN) were vertically mounted in a 10 ml plexiglas organ bath containing the aforementioned solutions. The lower muscular end was fixed in a phosphorbronze clip, and the upper tendinous end was attached to an electromagnetic length–tension transducer (University of Antwerp, Belgium). Preload was initially set between 3 and 4 mN according to muscle dimensions. The preparations were stimulated at 0.6 Hz with a voltage of 10% above threshold (typically 3–6 mV) by rectangular pulses of 5 ms duration through two platinum electrodes arranged longitudinally alongside the entire muscle. After 20 min later, bathing solutions were replaced by corresponding ones without BDM, and 1 h later, replaced by corresponding ones without calf serum. During the next 2 h, the muscles were stabilized. Finally, the muscles were stretched to a muscle length at which active force development was maximal. This length (mm) is known as maximum physiological length ($L_{max}$). Protocols were initiated after two superimposable isotonic and isometric control twitches separated by a 10 min interval were obtained. At the end of the experiment, the muscles were lightly blotted and then weighed. Muscle cross-sectional area was calculated by dividing the weight of the muscle by its length at $L_{max}$. A cylindrical shape and a specific gravity of 1.0 were assumed (Gillebert & Raes, 1994; Brutsaert *et al*., 1988). Muscle tension was then expressed as force normalized per cross-sectional area (mN/mm$^2$).

*Experimental protocol* Effects of increasing concentrations of AngII ($10^{-7}$, $10^{-6}$ and $10^{-5}$ M) on contraction, relaxation and diastolic properties of the cardiac muscle were studied in rabbit papillary muscles in the absence ($n=11$) or presence of (i) losartan ($10^{-6}$ M; $n=7$), a selective AT1 receptor competitive antagonist; (ii) ZD-7155 ($10^{-7}$ M; $n=8$), another AT1 receptor competitive antagonist that is approximately 10 times more potent than losartan in suppressing the AngII-induced pressor response (Junggren *et al*., 1996); (iii) ZD-7155 ($10^{-7}$ M) plus the AT2 receptor antagonist PD-123,319 ($2\times10^{-6}$ M; $n=6$); (iv) an inhibitor of PKC, chelerythrine (CHE, $10^{-5}$ M; $n=8$); and (v) an inhibitor of the NHE, 5-(*N*-methyl-*N*-isobutyl)-amiloride (MIA; $10^{-6}$ M; $n=10$). These substances were dissolved in the Krebs–Ringer solution and muscle twitches were recorded after a stable response was obtained, typically 15–20 min later. Finally, passive length–tension relations were constructed before and after a single concentration of AngII ($10^{-5}$ M, $n=6$). Notably in each experimental protocol, all papillary muscles were obtained from different animals.

All chemicals were obtained from Sigma-Aldrich, except ZD-7155 and losartan that were obtained from Tocris Bioscience and Cayman Chemical Company Europe, respectively.

*Data acquisition and analysis* Isotonic, afterloaded, and isometric twitches were recorded and analyzed. Selected parameters include the following: resting tension (RT; mN mm$^{-2}$), active tension (AT; mN mm$^{-2}$); maximum velocity of tension rise ($dT/dt_{max}$; mN mm$^{-2}$ s$^{-1}$); maximum velocity of tension decline ($dT/dt_{min}$; mN mm$^{-2}$ s$^{-1}$); peak isotonic shortening (PS; $\%L_{max}$); maximum velocity of shortening ($dL/dt_{max}$; $L_{max}$ s$^{-1}$); maximum velocity of lengthening ($dL/dt_{min}$; $L_{max}$ s$^{-1}$); time to half-relaxation (tHR, ms).

### In situ *heart model*

*Experimental preparation* Male New Zealand White rabbits (*O. cuniculus*, $2.0\pm0.2$ kg, $n=6$) were premedicated with ketamine hydrochloride (50 mg kg$^{-1}$ i.m.) and xylazine hydrochloride (5 mg kg$^{-1}$ i.m.). An auricular vein was cannulated, and a prewarmed solution containing 20 meq KCl and 40 meq $NaHCO_3$ in 500 ml of 0.9% NaCl was administrated to compensate for perioperative fluid losses. A tracheostomy was performed, and mechanical ventilation was initiated (Harvard Small Animal Ventilator, model 683), delivering oxygen-enriched air. Respiratory rate and tidal volume were adjusted to keep arterial blood gases and pH within physiological limits. Anesthesia was maintained with ketamine hydrochloride (33 ml kg h$^{-1}$ i.m.) and pentobarbital sodium (12.5 mg kg$^{-1}$ i.v. before opening the chest, and then 2.5 mg kg$^{-1}$ i.v. as needed). A 20-gauge catheter was inserted in the right femoral artery and connected to a pressure transducer to monitor heart rate and arterial pressure, and to obtain samples for blood gas analysis. The heart was exposed by a median sternotomy, and the pericardium was widely opened. A silk suture was placed around the ascending aorta to perform an aortic banding during the experimental protocol. A 3-F high-fidelity micromanometer (SPR-524, Millar Instruments, Houston, TX, U.S.A.) was inserted through an apical puncture wound into the left ventricular (LV) cavity, positioned at the midventricular level, and secured in place with a purse-string suture to measure LVP. The manometer was calibrated against a mercury column and zeroed after stabilization for 30 min in a water bath at body temperature. A limb electrocardiogram (DII) was recorded throughout.

*Experimental protocol* After complete instrumentation, we allowed the animal preparation to stabilize for 30 min before the beginning of the experimental protocol. Recordings were made with respiration suspended at end expiration. After obtaining the baseline recordings a banding was applied to constrict the ascending aorta in order to elevate peak systolic LV pressure by ~50% of its baseline value. After a stabilization period of 15 min new recordings were obtained. The aortic banding was then released and the hemodynamic parameters allowed returning to baseline values. After a new period of 15 min, AngII infusion was started at a rate of $10\,\mu g\,kg^{-1}\,min^{-1}$, which we found in preliminary experiments to elevate, after stabilization, peak systolic LV pressure by ~50% of its baseline value as well. Stabilization of systolic pressures during AngII infusion was achieved in less than 5 min. Recordings were then made 15 min after starting AngII infusion.

*Data acquisition and analysis* Parameters were converted on-line to digital data with a sampling frequency of 500 Hz. LVP was measured at end-diastole ($LVP_{ED}$), at peak systole ($LVP_{max}$) and at its minimum diastolic value ($LVP_{min}$).

*Statistical methods*

Values are means±s.e. Effects of increasing concentrations of AngII alone on the various experimental parameters were analyzed by one-way repeated-measures ANOVA. Effects of increasing concentrations of AngII under various experimental conditions were analyzed with a repeated-measures two-way ANOVA. Effects on the various parameters of a single concentration of the antagonists, as well as the effects of AngII on muscle length at each RT were analyzed with a paired *t*-test. Hemodynamic measurements at baseline, during aortic banding and during AngII infusion were analyzed with a repeated-measures two-way ANOVA. When significant differences were detected with any of the ANOVA tests, the Student–Newman–Keuls test was selected to perform pairwise multiple comparisons. $P<0.05$ was accepted as significant.

## Results

Baseline performance of rabbit papillary muscles was similar in all experimental protocols. Mean values of the contractile parameters from the 56 papillary muscles were as follows: AT $27.3\pm3.1\,mN\,mm^{-2}$; $dT/dt_{max}$ $169\pm18\,mN\,mm^{-2}\,s^{-1}$; $dT/dt_{min}$ $-131\pm13\,mN\,mm^{-2}\,s^{-1}$; PS 13.0±1.4% of $L_{max}$; $dL/dt_{max}$, $0.9\pm0.1\,L_{max}\,s^{-1}$; $dL/dt_{min}$, $-3.1\pm0.4\,L_{max}\,s^{-1}$; tHR, 428±14 ms.

Effects of increasing concentrations of AngII ($10^{-7}$, $10^{-6}$ and $10^{-5}$ M) on papillary muscles function are summarized and illustrated in Figure 1, where it can be seen that these concentrations increased myocardial contractility, lusitropy and distensibility. The highest dose of AngII ($10^{-5}$ M) increased 43.3±6.25% AT, 58.6±9.6% $dT/dt_{max}$ and 49.2±9.8% $dT/dt_{min}$ ($P<0.05$). Effects on tHR were not statistically significant. With regard to the diastolic properties of the myocardium, in addition to increasing relaxation rate ($dT/dt_{min}$), we observed that AngII progressively increased resting muscle length (RL) (Figure 1, bottom). Correcting muscle length, at the end of the experiment, to its initial value resulted in a 46±4% decrease of RT, without altering the other contractile parameters. These results indicate an increase in muscle distensibility, or conversely, a decrease in muscle stiffness.

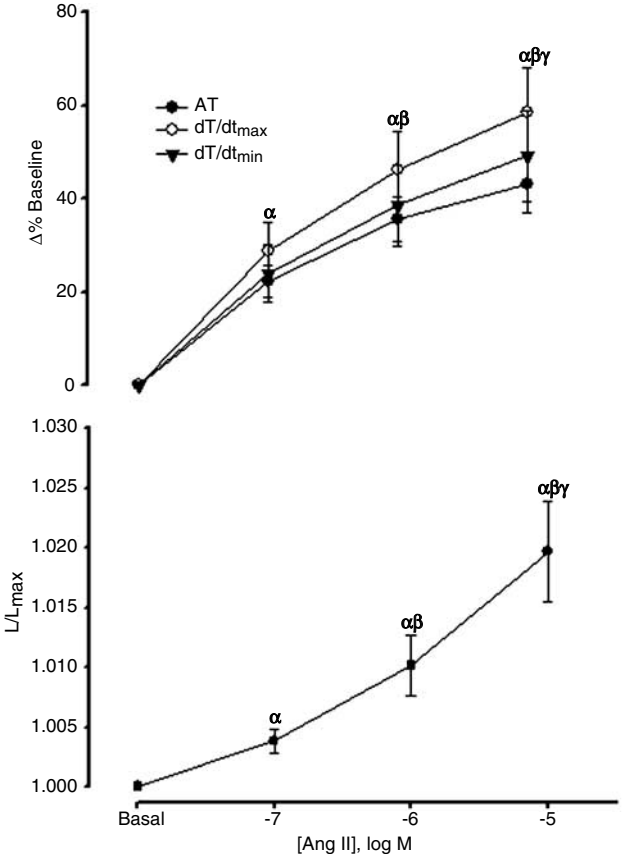


**Figure 1** Effect of increasing concentrations of angiotensin II (AngII) on active tension (AT), $dT/dt_{max}$ and $dT/dt_{min}$ (top) and muscle length ($L/L_{max}$, bottom). Data are means±s.e.; $P<0.05$: $\alpha$ vs baseline, $\beta$ vs $10^{-7}$ M AngII, $\gamma$ vs $10^{-6}$ M AngII. In the upper panel the statistical significance symbols apply to the three displayed curves.

This aspect is further explored in Figure 2 where passive length–tension relations at baseline and in the presence of AngII ($10^{-5}$ M) are depicted. In this figure, it can be seen that this relation is right and downward shifted by AngII. In other words, at each RT, muscle length was always significantly greater in the presence of AngII, indicating that this peptide acutely increases distensibility and lowers stiffness of the myocardium.

Effects of AngII in the absence or presence of selective AT1 receptor antagonists (losartan or ZD-7155 alone or with the selective AT2 receptor antagonist, PD-213,319), PKC inhibition (CHE) or NHE inhibition (5-*N*-methyl-*N*-isobutyl-amiloride (MIA)) are illustrated in Figures 3 and 4. None of these agents significantly modified *per se* any of the analyzed contractile parameters. For instance, for AT the results were as follows: losartan −1.3±3.2% ($P=0.9$); ZD-7155 −1.9±1.2% ($P=0.1$); ZD-7155 plus PD-213,319 −5.8±3.4% ($P=0.9$); CHE −1.3±3.2% ($P=0.3$); MIA +9.1±4.6% ($P=0.6$).

The myocardial effects of AngII were, however, significantly altered by these agents. Losartan and ZD-7155 blunted both

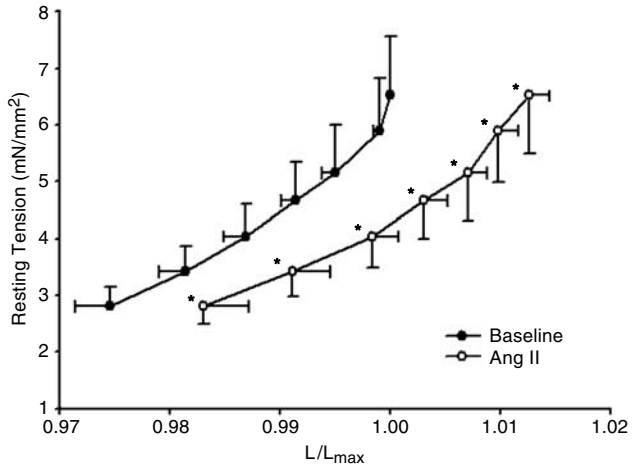


**Figure 2** Passive length–tension relations at baseline and in the presence of angiotensin II (AngII, $10^{-5}$ M). Data are means±s.e.; $P<0.05$: * AngII vs baseline.

the positive inotropic effects of AngII and its effects on resting length and tension. However, as can be seen in Figures 3 and 4, even if both blockers completely abolished the effects of AngII on muscle length and RT (myocardial distensibility), their interference with the positive inotropic effect of AngII was not entirely superposable. Whereas losartan completely abolished this effect, ZD-7155 reversed it at the highest concentration of AngII ($10^{-5}$ M), decreasing 29.9±8.6% AT, 25.4±7.6% $dT/dt_{max}$, 26.9±8.3% $dT/dt_{min}$ and 9.6±2.8 tHR% ($P<0.05$). This negative inotropic effect of the highest concentration of AngII in the presence of ZD-7155 was attenuated by the concomitant inhibition of AT2 receptors with PD-123,319. In these circumstances, AngII ($10^{-5}$ M) only decreased 14.6±3.9% AT, 11.5±3.1% $dT/dt_{max}$, 13.3±3.0% $dT/dt_{min}$ and 3.1±1.4 tHR% ($P<0.05$), without affecting myocardial stiffness.

On the other hand, CHE and MIA did not change significantly the effects of AngII on AT, $dT/dt_{max}$ or $dT/dt_{min}$, but significantly attenuated the effects of AngII on RL and tension. In the presence of CHE, AngII ($10^{-5}$ M) increased 35.5±8.5% AT, 48.3±10.9% $dT/dt_{max}$ and 50.0±10% $dT/dt_{min}$ ($P<0.05$). The effect of AngII on muscle length was markedly reduced, leading to a decrease in passive tension of only 10.9±4.5%. The same concentration of AngII, in the presence of MIA, increased 31.6±9.2% AT, 37.1±6.8% $dT/dt_{max}$ and 20.5±5.7% $dT/dt_{min}$. This blocker attenuated the effects of AngII on myocardial stiffness, although to a smaller extent than CHE. In these circumstances passive tension decreased by 23.2±5.0% ($P<0.05$).

The effects of AngII infusion on LV systolic and diastolic pressures of the *in situ* intact heart are illustrated in Figure 5. The dose used in this study increased systolic LV pressures from 74±2 to 111±4 mmHg, while decreasing end-diastolic pressures from 7.1±0.3 to 5.9±0.5 mmHg ($P<0.05$) and minimal pressures from 6.0±0.3 to 4.6±0.6 mmHg ($P<0.05$). On the contrary, a similar increase in systolic LV pressure from 74±2 to 112±4 mmHg, promoted by an aortic banding, increased end-diastolic pressures from 7.1±0.3 to 10.0±0.3 mmHg ($P<0.05$) and minimal pressures from 6.0±0.3 to 8.4±0.5 mmHg ($P<0.05$).

## Discussion and conclusions

This study provides strong evidence that AngII induces a significant concentration dependent decrease of myocardial stiffness in a very short time frame. Our data suggest that such an effect is mediated by AT1 receptors and is dependent on the activation of PKC and NHE (NHE).

AT1, $\alpha_1$-adrenoceptors and $ET_A$ receptors belong to a family of G protein (Gq)-coupled receptors, which are linked to phospholipase C activation and the consequent production of inositol triphosphate and diacylglicerol. In the rabbit heart, the positive inotropic effect of AngII is associated with an increase in phosphoinositide turnover (Ishihata & Endoh, 1993; Kim & Iwao, 2001). Recently, PKC-$\alpha$ has been identified as a fundamental regulator of cardiac contractility and calcium handling in cardiac myocytes (Wehrens & Marks, 2004). It can directly phosphorylate protein phosphatase inhibitor-1, augmenting the activity of protein phosphatase-1 and causing hypophosphorylation of phospholamban, resulting in inhibition of SERCA2a and impaired calcium reuptake into the sarcoplasmic reticulum (Braz *et al*., 2004). PKC also increases intracellular pH through the activation of the NHE (Takabashi *et al*., 1997; Wakabayashi *et al*., 1997; Snabaitis *et al*., 2000; Salas *et al*., 2001). Intracellular alkalinization is associated with increased calcium sensitivity of the contractile proteins (Mayoux *et al*., 1994), therefore resulting in a positive inotropic effect (Salas *et al*., 2001), although this might vary according to the animal species considered (Kim & Iwao, 2001).

In the present study, the positive inotropic effect of AngII was abolished by AT1 blockade using both losartan or ZD-7155, but not significantly influenced by PKC and NHE inhibition. In the presence of ZD-7155, the highest concentration of AngII used in the present study even caused a significant negative inotropic effect. Given that ZD-7155 is a more potent AT1 receptor antagonist than losartan it may have uncovered a negative inotropic effect because of AT2 receptor stimulation, because this effect was attenuated by the concomitant presence of the AT2 receptor antagonist PD-123,319.

On the other hand, AngII-induced decrease of myocardial stiffness was significantly blunted by inhibition of AT1 receptor, PKC and NHE, although to a lesser extent with the latter. This indicates that AngII increases inotropy and decreases myocardial stiffness by distinct subcellular mechanisms.

The effects of AngII on myocardial stiffness were shown both in the isolated papillary muscle and in the *in situ* intact heart. In the latter, AngII infusion induced a significant increase of LV systolic pressures, while decreasing LV diastolic filling pressures. We have previously shown, in the same animal species, that an elevation of systolic LV pressures of such magnitude significantly increases LV diastolic pressures (Leite-Moreira *et al*., 1999; Leite-Moreira & Correia-Pinto, 2001). In the present study, systolic LV pressure elevation, induced by an aortic banding, also significantly increased LV diastolic pressures. Therefore, it is not surprising that when the effects of AngII on diastolic LV pressures were evaluated at matched systolic LV pressures a bigger effect could be detected. In fact, in these circumstances, LV end-diastolic pressures decreased by 40.4±5.0% and minimal pressures by 43.0±9.7%.

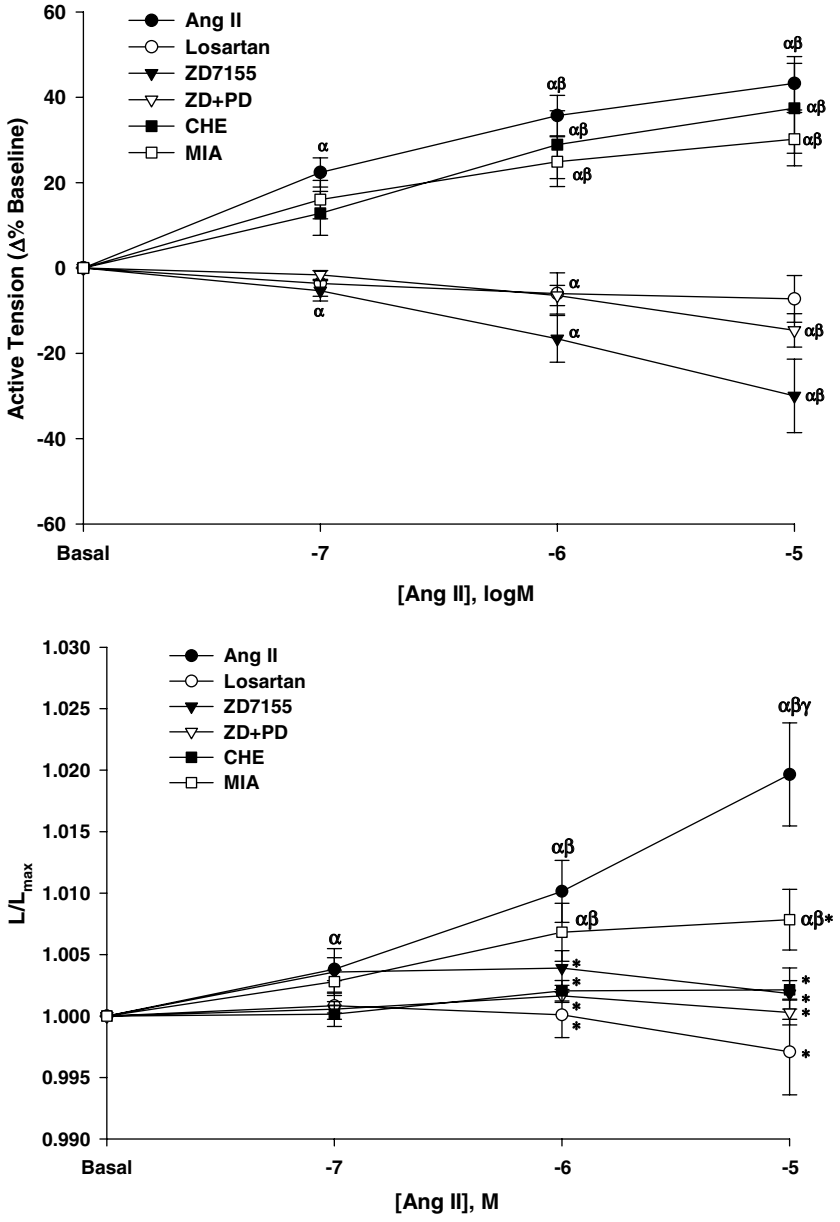


**Figure 3** Effect of increasing concentrations of angiotensin II (AngII) on active tension (top) and muscle length (bottom) in the absence or presence of losartan ($10^{-6}$ M), ZD-7155 ($10^{-7}$ M), ZD-7155 plus PD-123,319 ($10^{-7}$ and $2 \times 10^{-6}$ M, respectively), the PKC inhibitor chelerythrine (CHE, $10^{-5}$ M) or the $Na^+/H^+$ exchanger inhibitor 5-(*N*-methyl-*N*-isobutyl)-amiloride (MIA, $10^{-6}$ M). Data are means ± s.e. $P<0.05$: $\alpha$ vs baseline, $\beta$ vs $10^{-7}$ M AngII, $\gamma$ vs $10^{-6}$ M AngII, * vs AngII alone.

Myocardial stiffness is an important determinant of ventricular filling, and, therefore, of diastolic function. As outlined in the introduction, until recently, it was considered that neurohumoral agents only could influence the diastolic properties of the myocardium through chronic changes, as those induced by fibrosis and hypertrophy (Hood *et al*., 1970; Leite-Moreira & Correia-Pinto, 2001). More recent studies, however, have shown that diastolic stiffness may be acutely modulated by NO (Shah *et al*., 1994; Heymes *et al*., 1999), endothelin-1 (ET-1) (Leite-Moreira, *et al*., 2003) and $\beta$-adrenoceptors stimulation (Yamamoto *et al*., 2002; Borbely *et al*., 2005), while the present study demonstrates that the same is true for AngII.

AngII can increase NO production and release through AT2 receptor stimulation (Zhang *et al*., 2003; Batenburg *et al*., 2004). Although NO has been previously shown to decrease myocardial stiffness, presumably as a result of PKG-mediated phosphorylation of myofilaments, this mechanism should not have accounted for our findings, because the effects of AngII on myocardial stiffness were abolished by selective AT1 inhibition.

ET-1 also interacts with AngII (Herizi *et al*., 1998; Rossi *et al*., 1999). With regard to diastolic function, we have recently shown, in the same animal species, that ET-1 increases myocardial distensibility (Leite-Moreira *et al*., 2003). There are, however, important differences between the effects of

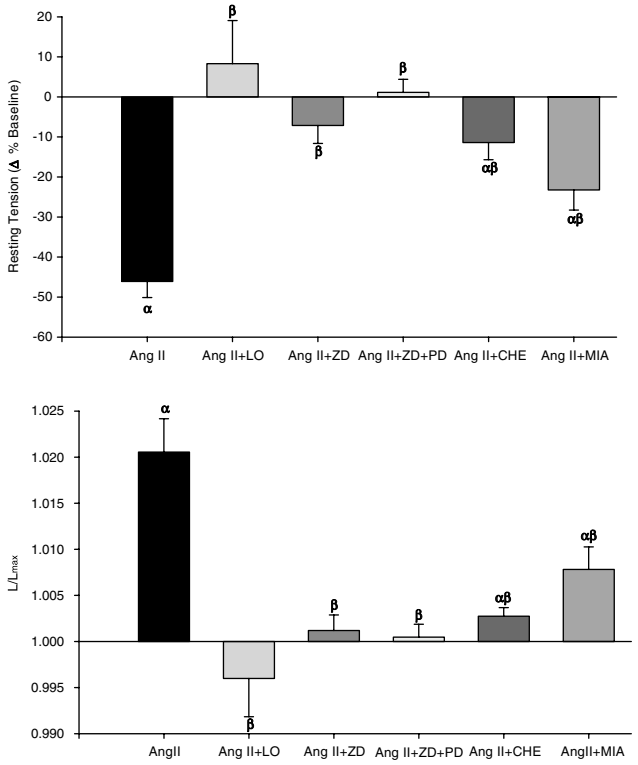


**Figure 4** Effects of angiotensin II (AngII, $10^{-5}$ M) on resting tension (top) and resting muscle length (bottom, $L/L_{max}$) in the absence or presence of losartan (LO; $10^{-6}$ M), ZD-7155 (ZD; $10^{-7}$ M), ZD-7155 $10^{-7}$ M plus PD-123,319 $2 \times 10^{-6}$ M (ZD + PD), the PKC inhibitor chelerythrine (CHE, $10^{-5}$ M) or the $Na^+/H^+$ exchanger inhibitor 5-(*N*-methyl-*N*-isobutyl)-amiloride (MIA, $10^{-6}$ M). Data are means ± s.e.; $P<0.05$: $\alpha$ vs baseline $\beta$ vs AngII alone.

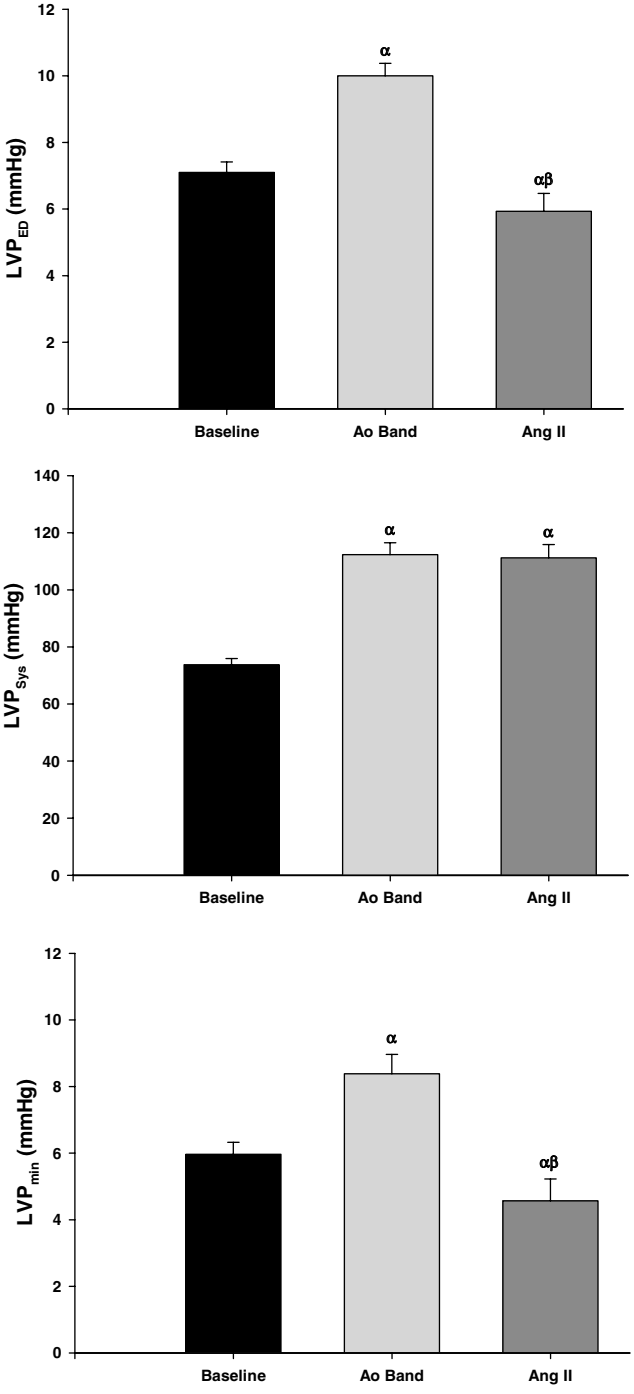


**Figure 5** Left ventricular end-diastolic ($LVP_{ED}$), peak systolic ($LVP_{sys}$) and minimal ($LVP_{min}$) pressures in the *in situ* intact heart at baseline, during aortic banding (AoBand) and during the infusion of angiotensin II (AngII, $10\,\mu g\,kg^{-1}\,min^{-1}$). Data are means ± s.e.; $P<0.05$: $\alpha$ vs baseline $\beta$ vs aortic banding.

AngII and ET-1. In fact, increased myocardial distensibility in response to ET-1 was observed only in acutely loaded cardiac muscles and was completely abolished by NHE inhibition, indicating that the two effects are not identical.

With regard to $\beta$-adrenoceptors stimulation it decreases myocardial stiffness through protein kinase A-induced phosphorylation of titin (Yamasaki *et al*., 2002). This post-translational modification of titin was shown to acutely shift the diastolic length tension relation downward (i.e., decrease stiffness) both in animal models (Yamasaki *et al*., 2002) and in human myocardium (Borbely *et al*., 2005).

It should be emphasized that the observed effects of AngII on myocardial stiffness cannot be ascribed to creep or stress relaxation, as AngII increased muscle length at constant RT even in isotonic twitches. Interestingly, even if muscle length increased beyond $L_{max}$, following AngII administration, no impairment in contractile performance was observed.

Finally, concerning the pathophysiologic relevance of our findings, we must point out that decreases of 46% in passive tension of the isolated muscle or of 40–44% in LV diastolic pressures of the intact heart indicate that AngII might allow the ventricle to reach high filling volumes at almost half filling pressures, which is undoubtedly a quite powerful adaptation mechanism. These acute beneficial effects of AngII on diastolic function are overcome by its role in progression to cardiac fibrosis and ventricular remodelling when its levels remain chronically elevated (Weber *et al*., 1995; McElmurray *et al*., 1999; Berry *et al*., 2001; Yamamoto *et al*., 2002).

In conclusion, besides its well-known effects in myocardial contractility, AngII decreases myocardial stiffness, an effect that requires the activation of AT1 receptors and is mediated by PKC and NHE. This novel effect of AngII broadens our concepts with regard to the acute neurohumoral modulation of diastolic function and represents a potentially powerful regulator of cardiac filling.

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