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Modulação aguda da função cardíaca diastólica e suas implicações fisiopatológicas

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Acute modulation of diastolic cardiac function and its pathophysiological implications

Running title: Acute modulation of diastolic function

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ABSTRACT

Myocardial function is regulated by several acute and chronic neurohumoral mediators. Furthermore, mechanical stretch is also an important determinant of cardiac performance. This work assessed the myocardial response to an acute stretch, with special emphasis in its diastolic arm. Rabbit papillary muscles were stretched from 92% to 100% of L_{max} under basal conditions and in the presence of: i) an inhibitor of the Na^+/H^+ exchanger (NHE) (amiloride); ii) an inhibitor of the reverse mode of the Na^+/Ca^{2+} exchanger (rNCX) (KB-R7943); iii) an inhibitor of PKC (chelerythrine); iv) an inhibitor of PKA (KT-5720); v) angiotensin-II (AngII); vi) an AT1 receptor antagonist (ZD-7155); vii) ZD-7155 plus an AT2 receptor antagonist (PD123,319); and viii) after two cycles of ischemic preconditioning (IP). The myocardial response to stretch was also studied under ischemic conditions in the absence or presence of: i) ZD-7155; ii) ZD-7155 plus PD123,319; and iii) after ischemic preconditioning. Acute stretch elicited immediate and delayed increases in active tension (AT). During ischemia AT decreased instead of increasing during the delayed response, but this deterioration was blunted by ZD-7155 and IP. Regarding diastolic function, after an immediate increase upon stretch, there was a significant and time-dependent decrease in passive tension. This decrease was attenuated with PKC inhibition and blunted during ischemia. The latter effect was prevented with AT1 antagonism. These results suggest that mechanical stretch elicits not only a systolic but also a diastolic adaptive response. Both responses are impaired during ischemia, but ischemic preconditioning and AT1 antagonism seem to partially prevent this deterioration.

Keywords: myocardial stretch; Frank-Starling mechanism; Anrep effect; diastole.

INTRODUCTION

The heart has a pivotal role in cardiovascular homeostasis and represents a paradigmatic organ involved in continuous mechanical adaptation. Its remarkable biomechanical sensing apparatus allows not only chronic but also acute modulation of cardiac function(21). However, even though the diastolic effects of this modulation were overlooked during a long time, there is a growing body of evidence showing that diastolic function is acutely influenced by different loads(20) and neurohumoral conditions(18).

Myocardial stretch is one important determinant of cardiac performance. Indeed, acute myocardial stretch elicits a biphasic positive inotropic response: first, an immediate increase in contractility, also known as the Frank-Starling mechanism (described about a century ago by Otto Frank and Ernest Starling), and later, a slow time-dependent increase in developed force over the value obtained immediately after stretch, known as the Slow Force Response (SFR). The latter was first described by von Anrep in 1912 but was better characterized by the experimental work of Parmley and Chuck(29). In the last 10 years, several research groups unraveled some of the mechanisms underlying the SFR.(5, 16) Briefly, even though suggesting that species-dependent differences may exist(3-4, 23, 35), activation of the Na^+/H^+ exchanger (NHE) and the reverse mode of the Na^+/Ca^{2+} exchanger (rNCX) seems to be crucial to the systolic response to stretch.

However, all previous studies have systematically dismissed the diastolic counterpart of myocardial response to stretch. In addition, the evidence that cardiac diastolic properties could be influenced by protein kinase A (PKA) and protein kinase C (PKC)-dependent phosphorylation of myofilament proteins, especially troponins(12, 27) and titin(17, 34), suggests that these protein kinases may also modulate the diastolic response after acute myocardial stretch.

The existence of the SFR in failing human myocardium(35) pointed out that cardiac response to acute stretch may represent an important adaptive mechanism even under pathological conditions. For example, myocardial ischemia underlies several cardiac diseases (such as angina pectoris, acute myocardial infarction and heart failure), with a prominent role in the deterioration of cardiac function which is commonly associated with a state of hemodynamic overload(28). Moreover, the degree of diastolic dysfunction upon ischemia seems to be correlated with future morbidity and mortality(24). To overcome the significant health impact of ischemic heart disease, several important strategies were described. First, the local production of angiotensin-II (AngII) during cardiac ischemia(26) and the limitation of infarct size in the presence of an AT1 receptor antagonist(6) brought forward the opportunity to modulate the renin-angiotensin system with angiotensin converting enzyme (ACE) inhibitors and angiotensin receptor (AT1) blockers. Indeed, these drugs play a cardioprotective role over systolic and diastolic functions during and after ischemia(25) and are of uppermost importance to improve prognosis and decrease mortality following an acute myocardial infarction(30). On the other hand, ischemic preconditioning (IP) is also an important mechanism of cardioprotection, where short periods of ischemia “prepare” the heart to a subsequent longer ischemic episode, reducing infarct size and risk of arrhythmias(14-15). Taking this together, a better understanding of the systolic and diastolic responses to acute stretch in the ischemic setting and its modulation by ischemic preconditioning, AT1 and AT2 receptors blockers seems to be important to provide new pharmacological tools to face the major burden of ischemic heart disease in world health.

In this way, this study aims to establish whether a simultaneous and adaptive response of myocardial diastolic function may play a role in the overall cardiac response to stretch in healthy myocardium, as well as during ischemia and after IP. The importance of AngII, AT1 and AT2 receptors in its modulation will deserve a

special emphasis. Moreover, the role of several intracellular mediators in myocardial response to acute stretch will also be assessed.

MATERIALS AND 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).

Experimental preparation. Male New Zealand white rabbits (*Oryctolagus cuniculus*; 2.0-3.0 kg; n=40) were anesthetized with intravenous pentobarbital sodium (25 mg/kg). A left thoracotomy was performed and beating hearts were quickly excised and immersed in a 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 30 °C with cardioplegic 2,3-butanedione monoxime (BDM; 3%) and 5% newborn calf serum. Atenolol was used to prevent β adrenoceptor-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 = 101; length at 92% of L_{max}: 3.6±0.1 mm; length at L_{max}: 3.9±0.1 mm; weight: 2.6 ± 0.2 mg) 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 2 and 3 mN according to muscle dimensions. The preparations were stimulated at 0.2 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. Twenty minutes later, bathing solutions were replaced by corresponding ones without BDM and 30 minutes later replaced by corresponding ones without calf serum.

After stabilization and at constant length, a higher load was imposed so that the muscles could now start to contract isometrically. Then, the muscles were slowly

stretched to the length at which active force development is maximal (L_{max}). The length corresponding to 92% of L_{max} was calculated and the muscles were unstretched and led to stabilize at this new length. Protocols were started after obtaining two superimposable isometric control twitches separated by a 10 minutes interval. At the end of the experiment, the muscles were lightly blotted and then weighted. Muscle cross-sectional area was calculated by dividing the weight of the muscle by its length at either 92% or 100% of L_{max}, according to the phase of the protocol. A cylindrical shape and a specific gravity of 1.0 were assumed (7). Muscle tension was then expressed as force normalized per cross-sectional area (mN/mm^2).

Experimental protocol. The effects of acute muscle stretch from 92 to 100% of L_{max} on systolic and diastolic properties of rabbit papillary muscles were first assessed under basal conditions (control group, protocol A, n=9) and then in the presence of an inhibitor of NHE, 5-(*N*-methyl-*N*-isobutyl)-amiloride (10^{-6} M, protocol B; n=8); an inhibitor of rNCX, KB-R7943 ($5x10^{-6}$ M, protocol C; n=9); an inhibitor of PKC, chelerythrine (10^{-5} M, protocol D; n=7); an inhibitor of PKA, KT-5720 (10^{-5} M, protocol E; n=7); AngII (10^{-5} M, protocol F; n=8); an AT1 receptor antagonist, ZD-7155 (10^{-6} M, protocol G; n=8); an AT1 receptor antagonist, ZD-7155 (10^{-6} M) plus an AT2 receptor antagonist, PD123,319 (10^{-6} M, protocol H; n=7). These substances were dissolved in Krebs-Ringer solution and the papillary muscles were stretched after mechanical stabilization at least 20 min later.

In a second set of protocols, the systolic and diastolic responses to stretch were studied under ischemic conditions. After stabilization, the bathing solution was changed by another one without glucose and the O_2 source was stopped. After 5 minutes of ischemia, muscles were stretched from 92 to 100% of L_{max} in the absence (n=7, protocol I) or presence of: 1) ZD-7155 (10^{-6} M, protocol J; n=7) and 2) ZD-7155 (10^{-6} M)

plus PD123,319 (10^{-6} M, protocol K; n=7). Fifteen minutes after muscle stretch, the muscles were reperfused with a glucose containing Krebs-Ringer solution and O_2.

To assess the effect of IP on the myocardial response to stretch two more protocols were performed. After stabilization, muscles underwent two cycles of 5 minutes of ischemia and 10 minutes of reperfusion. Thereafter, they were stretched from 92 to 100% of L_{max} with glucose and O_2 (protocol L, n=9) or after 5 minutes of ischemia (protocol M, n=8). In the latter, the muscles were reperfused fifteen minutes after muscle stretch. A schematic representation of all protocols performed is showed in Figure 1.

Data acquisition and analysis. Muscles were stretched to 100% of L_{max} and maintained at this length for 60 min. Isometric twitches were recorded and analyzed with dedicated software (University of Antwerp, Belgium). Selected parameters include: active tension (AT; mN/mm^2), passive tension (PT; mN/mm^2), maximum velocity of tension rise (dT/dt_{max}; $mN/mm^2/s$), and maximum velocity of tension decline (dT/dt_{min}; $mN/mm^2/s$).

Statistical methods. Values are presented as means ± standard error (SEM). Within the same group, the effects of acute muscle stretch on the various experimental parameters at specific time points were analyzed with a paired Student's t-test. The time-dependent effects of muscle stretch within the same group were analyzed by a repeated measures one-way ANOVA, while a repeated measures two-way ANOVA was used when comparing the time-dependent effects of muscle stretch among several groups. When significant differences were detected with any of the ANOVA tests, the Holm-Sidak's method was selected to perform multiple comparisons. $P<0.05$ was accepted as significant.

RESULTS

Systolic and diastolic functions upon myocardial stretch. A representative band of a contracting muscle that was acutely stretched from 92 to 100% of L_{max} is shown in Figure 2. Acute stretch of papillary muscles elicited an immediate increase in AT from 22.1±5.3 to 28.5±6.1 mN/mm^2 (P<0.001), followed by a time-dependent increase in contractility over the initial response up to 34.4±6.2 mN/mm^2 (P=0.008), 60 minutes after stretch (Figure 3-A), representing an immediate increase in AT of 35.6±5.3% and a delayed increase over the initial response of 40.7±13.8%. Moreover, there was also a significant and simultaneous increase in contraction velocity (dT/dt_{max}; immediate increase from 107.4±36.6 to 139.9±43.3 mN/mm^2/s, P=0.003). Considering diastolic function, upon stretch there was an immediate increase in PT from 1.7±0.4 to 18.2±2.2 mN/mm^2 (P<0.001), followed by a significant and time-dependent decrease down to 8.2±1.1 mN/mm^2 (P<0.001) at the end of the protocol (Figure 3-B), representing an overall decrease of PT of 55.1±2.6%. Furthermore, there was an immediate increase in relaxation velocity (dT/dt_{min}) from 79.8±27.3 to 95.1±27.7 mN/mm^2/s (P=0.004), and a progressive improvement up to 112.7±28.4 mN/mm^2/s at the end of the protocol.

On balance, these results show that besides the stretch-induced adaptation of systolic function, there is also a prominent and synchronous adaptation of myocardial diastolic function.

NHE and rNCX-mediated effects. The immediate increase in AT upon stretch was not different between these groups and the control group (inhibition of NHE: 31.2±4.4%; inhibition of rNCX: 26.1±3.6%; control group: 35.6±5.3%; P=0.329). Nevertheless, when compared with the control group, the SFR was significantly blunted with inhibition of either NHE (SFR at 60 min: 10.3±6.1%, P=0.012) and rNCX (SFR at 60 min: 12.6±5.2%, P=0.011) (Figure 4-A). The immediate and time-dependent

increases in contraction velocity were not significantly different between these groups and the control. Regarding diastolic function, NHE inhibition did not affect the immediate increase in myocardial PT (up to 19.9±3.6 versus 18.2±2.2 mN/mm^2 in the control group, *P*=0.697) or its subsequent decrease (down to 8.8±1.2 versus 8.2±1.1 mN/mm^2 in the control group, *P*=0.724). However, the inhibition of rNCX significantly attenuated the immediate stretch-induced increase in PT (up to 11.5±2.2 versus 18.2±2.2 mN/mm^2 in the control group, *P*<0.05), as depicted in Figure 4-B, although the subsequent decrease during the 60 minutes after stretch was not significantly different from the control group (down to 5.7±1.0 versus 8.2±1.1 mN/mm^2 in control, *P*=0.113). In addition, the percentage of PT decrease at 60 min was not significantly different between these two groups and the control (inhibition of NHE: 52.8±3.7%; inhibition of rNCX: 49.0±2.5%; control group: 55.1±2.6%; *P*=0.339), nor were the immediate and delayed increases in relaxation velocity.

PKA and PKC signalling. With PKA inhibition (PKA_i) the immediate and delayed increases in AT were similar to the systolic response to stretch in the control group (immediate increase of 34.2±7.5%, *P*=0.879, and delayed increase of 36.8±7.7%, *P*=0.821). Furthermore, the immediate increase (up to 16.2±1.8 versus 18.2±2.2 mN/mm^2 in the control group, *P*=0.503) and subsequent fall (down to 6.6±0.7 versus 8.2±1.1 mN/mm^2 in the control group, *P*=0.275) in myocardial PT displayed the similar variation pattern of the control group. Likewise, no differences were observed in contraction and relaxation velocities.

With regard to systolic function, PKC inhibition (PKC_i) did not significantly influence the immediate response to stretch. However, PKC_i significantly blunted the SFR (Figure 5-A), an effect observed 40 min after stretch (9.5±7.5 versus 40.3±12.0% in the control group, *P*=0.026) and further accentuated at 60 minutes (7.4±7.5% with

PKCi versus 40.7±13.8% in the control group, *P*=0.017). Contraction velocity was not significantly affected with PKC_i. Considering the modulation of diastolic function, even at 92% of L_{max}, PKC_i was responsible for an increase in PT, from 1.83±0.3 to 3.4±0.7 mN/mm^2 (*P*=0.022). Upon stretch, the immediate increase in PT was not significantly different from the control group (up to 15.4±3.3 mN/mm^2 with PKC_i versus 18.2±2.2 mN/mm^2 in the control group, *P*=0.473). The subsequent time-dependent decrease in PT in absolute values was also not different when compared to the control group (down to 9.2±1.8 mN/mm^2 with PKC_i versus 8.2±1.1 mN/mm^2 in the control group, *P*=0.761). However, when analyzed as a percentage of PT decrease relative to the value obtained immediately upon stretch, there was a significant attenuation of myocardial PT decrease with PKCi (Figure 5-B), already significant 10 minutes after stretch (PT decrease of 31.7±2.8% with PKC_i versus 41.0±2.4% in the control group, *P*=0.026) and further accentuated until 60 minutes (39.5±2.1 % with PKCi versus 55.1±2.6 % in the control group, *P*<0.001). Relaxation velocity was not significantly affected by PKC_i. In this way, the time-dependent adaptive diastolic response to stretch may be partially dependent on PKC.

AngII, AT1 receptor and AT2 receptor-mediated effects. At 92% of L_{max}, the addition of AngII elicited a significant and positive inotropic response (67.6±12.9% increase in AT, *P*<0.001), which nearly exhausted the inotropic reserve of the muscles. Indeed, after the immediate increase in myocardial AT upon stretch (31.1±7.9%), there was no increase in AT and contraction velocity. Considering diastolic function, the addition of AngII at 92% of L_{max} induced a significant decrease in PT (from 1.63±0.8 to 1.38±1.7 mN/mm^2, *P*=0.008). Upon stretch, there was a significant attenuation of the immediate increase in PT (up to 8.1±1.3 with AngII versus 18.2±2.2 mN/mm^2 in the control group, *P*<0.001), as illustrated in Figure 6. Afterwards, there was a significant decrease in PT over time with values systematically lower than the control group (down

to 3.3±0.6 with AngII versus 8.2±1.1 mN/mm^2 in the control group, 60 minutes after stretch, *P*=0.002), although it represents a PT fall of the same magnitude as in the control group (58.3±2.7%, *P*=0.405).

Acute myocardial stretch in the presence of an AT1 receptor antagonist elicited an immediate increase in AT (from 8.9±1.5 to 11.4±1.7 mN/mm^2, *P*= 0.012) followed by a SFR (up to 14.0±2.1 mN/mm^2, *P*=0.004, 60 minutes after stretch) that was not significantly different from the control group. In addition, the immediate and delayed increases in contraction velocity were also similar to the control group. The blockade of AT1 receptors did not significantly change the diastolic response to acute stretch.

When both AT1 and AT2 receptors were blocked, the immediate and time-dependent increases in AT, contraction velocity and relaxation velocity, as well as the progressive decrease in myocardial stiffness following stretch, evidenced no significant differences from the control group.

Myocardial stretch and ischemia. At 92% of L_{max}, 5 minutes of ischemia elicited a significant decrease in myocardial contractility (decrease in AT from 22.0±2.5 to 10.9±1.5 mN/mm^2, *P*<0.001) without a concomitant significant change in PT. Upon stretch, there was an immediate increase in AT (from 10.9±1.5 to 13.3±2.1 mN/mm^2, *P*=0.020) but the slow force response was absent (Figure 7-A). In fact, there was a significant decrease in AT during the 15 minutes of ischemia after stretch (from 13.3±2.1 to 5.8±1.3 mN/mm^2, *P*<0.001), with a simultaneously decrease in contraction velocity (from 98.3±10.7 to 52.9±8.4 mN/mm^2/s, *P*<0.001). With regard to diastolic function, after the immediate increase in PT upon stretch (up to 16.3±2.4 mN/mm^2), this parameter did not change during the following 15 minutes (Figure 7-B), contrasting with the significant fall in PT when the muscles were stretched with glucose and O_2 (variation in PT of +13.4±16.7% in the ischemic muscles versus -46.2±1.8% with

glucose and O_2, *P*=0.001). Furthermore, relaxation velocity significantly decreased during this period (from 47.7±4.3 to 22.1±4.3 mN/mm^2/s, *P*<0.001). Upon reperfusion, during the remaining 45 minutes of protocol, there was a significant improvement of all parameters, both systolic (increase in AT up to 26.3±4.8 mN/mm^2 and in dT/dt_{max} up to 162.5 mN/mm^2/s, values not significantly different from the control group) and diastolic (decrease in PT down to 7.8±0.9 mN/mm^2 and increase in dT/dt_{min} up to 99.4±10.3 mN/mm^2/s, values not significantly different from the control group).

Role of AT1 and AT2 receptors in myocardial stretch during ischemia. The presence of an AT1 receptor antagonist during the first 5 minutes of ischemia at 92% of L_{max} did not modify the deterioration of both systolic and diastolic parameters. Upon stretch, there was an increase in AT (23.2±7.8%) and contraction velocity (17.9±4.8%) that was not significantly different from Protocol I. However, blocking AT1 receptors blunted the progressive decrease in AT during the 15 minutes of ischemia after stretch that was observed in Protocol I, as depicted in Figure 8-A (AT decrease of 7.9±11.3% in this group versus 70.4±5.2% in Protocol I, *P*=0.005). Furthermore, contraction velocity was also preserved during this period (variation in dT/dt_{max} of 2.2±12.9% versus –60.4±10.0% in Protocol I, *P*=0.002). Considering diastolic function, after the immediate increase in PT and relaxation velocity upon stretch, the presence of an AT1 receptor antagonist was associated with a significant decrease in PT during the following 15 minutes of ischemia (from 15.8±2.8 to 11.0±1.8 mN/mm^2, *P*=0.004). Indeed, AT1 receptor antagonism prevented the increase in PT (variation in PT 15 minutes after stretch: -28.7±3.7% in this group versus 13.5±16.7% in the absence of the AT1 receptor antagonist, *P*=0.030; Figure 8-B) and blunted the deterioration in relaxation velocity (decrease in dT/dt_{min} of 16.9±7.6% versus 75.0±11.4% in Protocol I, *P*=0.001). After reperfusion, there was a significant improvement in all parameters analyzed.

In Protocol K, the presence of both AT1 and AT2 receptors antagonists partially blunted the decrease in AT (25.6±4.7% versus 51.0±4.0% in Protocol I, *P*=0.001) observed in the first 5 minutes of ischemia at 92% of L_{max}. Subsequently, the antagonism of AT1 and AT2 receptors was associated with an AT decrease in the 15 minutes of ischemia following stretch of 32.9±12.3% (Figure 8-A). In this way, blocking AT1 and AT2 receptors significantly blunted the pronounced deterioration of AT observed in this period after stretch in Protocol I (70.4±5.2%, *P*=0.001) although not so efficaciously as when only AT1 receptors were blocked (7.9±11.3%, *P*=0.001). Moreover, the decrease in contraction velocity was also attenuated (11.2±14.1 versus 60.4±10.0%, *P*=0.015). At 15 minutes following stretch, the presence of AT1 and AT2 receptors antagonists was associated with an improvement in the diastolic parameters of the same magnitude as in Protocol J, as illustrated in Figure 8-B. Reperfusion yielded an improvement in systolic and diastolic functions, showing no differences from Protocol J.

These results evidence that the deterioration of systolic and diastolic functions upon stretch during ischemia may be prevented with AT1 receptor antagonism. This cardioprotective mechanism seems to be partially dependent on the activation of AT2 receptors, at least in its systolic arm.

Ischemic preconditioning (IP). During the 5 minutes of ischemia of each cycle of IP there was a significant decrease in AT, contraction velocity and relaxation velocity, although there was no significant variation in PT. The subsequent periods of reperfusion (10 minutes) were associated with a recovery of systolic and diastolic parameters. Upon stretch, both AT and contraction velocity showed immediate (18.4±7.4 and 21.7±6.0%, respectively) and delayed (39.4±4.9 and 37.2±10.2% over the initial response to stretch, respectively) increases, with no significant differences

from the control group (Figure 9-A). Analyzing diastolic parameters (Figure 9-B), IP before stretch significantly attenuated the immediate increase in PT (up to 13.1±2.6 versus 18.2±2.2 mN/mm^2 in the control group, *P*=0.026). This was followed by a time-dependent decrease in PT of 59.0±1.1% at the end of the protocol (versus 55.1±2.6 % in the control group, *P*=0.190). The magnitude of the improvement in relaxation velocity upon stretch and during the rest of the protocol evidenced no differences from the control group.

Stretching the muscles in the absence of glucose and O_2, after two cycles of IP, demonstrated no significant increase in AT (from 5.2±2.9 to 5.6±3.5 mN/mm^2, *P*=0.571) and in contraction velocity. However, IP attenuated the deterioration of contractility in the 15 minutes of ischemia following stretch, with a decrease in AT of 52.6±4.6% versus 70.4±5.2% in protocol I (*P*=0.023), as depicted in Figure 10-A. Considering diastolic function, although the absolute increase in PT upon stretch was not significantly different between protocols I and M (14.0 versus 12.5 mN/mm^2, respectively, *P*=0.583), IP blunted the deterioration of relaxation velocity (Figure 10-B) during the 15 minutes of ischemia after stretch, with a decrease in dT/dt_{min} just before reperfusion of 40.3±4.7 % (versus 75.0±11.4 % in protocol I, *P*=0.014). These results demonstrate that IP may improve systolic and diastolic responses to acute stretch during ischemia. With reperfusion there was a significant improvement in all parameters.

DISCUSSION

In the present study it was shown that acute stretch of rabbit papillary muscles elicited not only the already described adaptive systolic response, with immediate (FSM) and time-dependent (SFR) increases in contractility, but also a significant and still unrecognized adaptive response of diastolic function, illustrated by a time-dependent decrease in passive tension down to approximately 45% of the value obtained immediately after stretch. The experimental protocols performed demonstrated that the time-dependent decrease in myocardial stiffness is not a merely passive phenomenon but instead susceptible of active modulation. Importantly, this adaptive response is impaired by ischemia. However, IP and the modulation of the renin-angiotensin system may provide an opportunity to attenuate and even reverse the deleterious effects of ischemia upon the diastolic counterpart of the SFR.

Contribution of NHE and rNCX to stretch-induced adaptation of cardiac function. Several studies published during the last decade provided a better understanding of the SFR (5, 16). Briefly, in rabbit and human myocardium, the stretch-mediated activation of the NHE and the rNCX has been described as the most important mechanism underlying the SFR (23, 35), although the role of these exchangers on the diastolic side of myocardial response to stretch has never been addressed. In this study, the inhibition of NHE and rNCX significantly attenuated the SFR, supporting their predominant role on the adaptive systolic response to stretch. However, only the inhibition of rNCX significantly attenuated the immediate increase in diastolic stress levels, possibly due to decreased diastolic Ca^{2+} levels that result from blocking Ca^{2+} entry into cardiomyocytes during the action potential. Indeed, Ca^{2+} influx via the reverse operating mode of NCX may be sufficient to induce Ca^{2+} release from the sarcoplasmic reticulum, even though it is small under normal conditions (22). The time-

dependent decrease in PT after stretch was not significantly changed with inhibition of NHE or rNCX. In fact, a previous study demonstrated that stretch induces a slow increase in the Ca^{2+} transient that underlies the SFR but no changes in diastolic [Ca^{2+}] (13), thus suggesting that stretch-activated signaling pathways may underlie the time-dependent decrease in myocardial stiffness independently of diastolic Ca^{2+} levels.

Role of PKC on the myocardial response to acute stretch. PKC is activated by myocardial stretch (2) and is able to modulate the activity of the NHE and NCX (10, 33). Furthermore, PKC phosphorylates many other intracellular proteins that may interfere with myocardial diastolic properties (34). The results of this study show that PKC inhibition significantly attenuated the SFR only 40 min after stretch, underscoring its relevance for the maintenance but not for the initial phase of the SFR. In this way, other mechanisms initially triggered by myocardial stretch, such as NHE and rNCX, may overcome the absence of PKC activity and induce a positive inotropic response upon stretch. Possibly, the maintenance of the SFR may rely, at least in part, on PKC-mediated phosphorylation of NHE and NCX and hence higher activity levels of these exchangers.

Interestingly, inhibition of PKC attenuated the time-dependent decrease in myocardial stiffness. In this way, these results highlight that PKC plays a role on the systolic and diastolic arms of the adaptive myocardial response to acute stretch. PKC phosphorylates myofilament proteins, eliciting a decrease in their Ca2+ sensitivity (34) or a change in their tridimensional structure (9). These mechanisms may account for the contribution of PKC to the diastolic adaptive response to stretch. Considering that PKC isoforms respond to stretch in different ways (2), future studies should clarify not only the role of different PKC isoforms in the myocardial response to stretch but also the phosphorylation targets that account for its effects.

AngII enhances the adaptive diastolic response to stretch. Considering that AngII is capable of acutely decrease myocardial stiffness in an AT1 receptor-dependent manner (19), it seems reasonable to hypothesize that AngII may as well play a role in the myocardial response to acute stretch. Although it was described in rabbit and human myocardium that the SFR is independent from the local release of AngII (23, 35), the results of this study demonstrate that exogenous AngII administration significantly decreased myocardial stiffness, attenuating the immediate increase in PT upon stretch. Furthermore, diastolic stress levels were systematically lower during the remaining protocol. The activation of intracellular signaling pathways that result in PKC activation may underlie this effect. Future studies should be performed to allow the clarification of which receptor mediates the improvement of the diastolic response to acute stretch of rabbit myocardium.

Even though the SFR in rabbit and human myocardium is regarded as AngII-independent, mechanical stress may activate AT1-receptors without the involvement of AngII (36). Nevertheless, these results demonstrate that inhibition of AT1 and simultaneous blockade of AT1 and AT2 receptors did not influence systolic and diastolic responses to acute stretch, suggesting that rabbit myocardial response to acute stretch is independent of stress-induced AT1 and AT2 activation.

Deterioration of myocardial response to acute stretch during ischemia: beneficial effects of blocking AT1-receptor. The results of this study demonstrate for the first time that the SFR is abolished when rabbit myocardium is stretched during ischemia, although the immediate increase in contractility is preserved. The systolic response to acute stretch during ischemia was actually characterized by a progressive decrease in contractility. Furthermore, the novel adaptive diastolic response of rabbit papillary muscles to acute stretch described in this study was also absent when stretch

was performed during ischemia, showing no time-dependent decrease in myocardial stiffness. Not surprisingly, reperfusion evoked a marked improvement in all parameters, both systolic and diastolic.

Interestingly, the deterioration of rabbit myocardial response to stretch during ischemia was prevented in the presence of an AT1 receptor antagonist, ZD-7155. AngII is locally produced in the heart during ischemia (26) and the blockade of AT1 receptors in the context of ischemia-reperfusion exerts a cardioprotective action (32), improving both systolic and diastolic parameters (6, 11). This effect seems to be mediated by AT2 receptor activation and involves signaling via bradykinin, PKC and cGMP (11). In this study, AT1 antagonism blunted the 70% decrease in contractility observed 15 minutes after stretch during ischemia, evoking a decrease of only 8%. In addition, this effect seemed to be partially dependent upon AT2 receptor activation, because when both AT1 and AT2 receptors were blocked there was a 33% decrease in contractility. Furthermore, the presence of an AT1 receptor antagonist was associated with a decrease in myocardial stiffness after stretch, even in ischemic conditions, thus improving diastolic response to acute overload. The concomitant blockade of AT1 and AT2 receptors did not influence this response.

Overall, it is tempting to hypothesize that AngII displays a dual role, acutely modulating systolic and diastolic functions. Under basal conditions, AngII acutely decreases myocardial stiffness (19) and, as described in this study, enhances the time-dependent decrease in myocardial stiffness upon stretch. On the other hand, when AT1 receptors are blocked during ischemia, there is an improvement in systolic and diastolic responses to myocardial stretch, an effect that partially depends on the activation of AT2 receptors.

Ischemic preconditioning. IP is one important cardioprotective mechanism in which brief ischemic periods protect the heart against later and prolonged ischemic insults. Intense experimental research on this topic unraveled several intracellular signaling mechanisms that probably underlie IP (1). It is relevant to emphasize that PKC activation is regarded as one of the major mediators involved in the IP-induced cardioprotection, considering that PKC inhibitors such as chelerythrine blocks the beneficial effects of IP (8).

In this study, stretching ischemic preconditioned rabbit papillary muscles in the presence of O_2 and glucose elicited a systolic adaptive response not significantly different from the control group. However, IP improved the diastolic response to stretch, attenuating the immediate increase in stress levels. As noted above, the results of this study support that PKC seems to be important to the time-dependent decrease in myocardial stiffness after acute stretch. In this way, IP may have enhanced PKC signaling pathway, eliciting an improved diastolic adaptation to acute myocardial stretch. Indeed, IP induces a selective translocation of PKC isoforms epsilon and eta, the first step in PKC's activation (31). On the other hand, the beneficial effect of IP was present not only when the muscles were acutely stretched under basal conditions but also in the ischemic setting, blunting the progressive deterioration in contractility and relaxation velocity.

Therefore, IP not only reduces infarct size and the risk of arrhythmias during subsequent prolonged ischemic periods but may also improve myocardial response to acute stretch, attenuating the time-dependent decrease in contractility that replaces the SFR in the ischemic setting and optimizing diastolic response in either basal or ischemic settings.

CONCLUSION

Acute mechanical stretch elicits not only a systolic but also a diastolic adaptive response, with a time-dependent decrease in myocardial stiffness of about 55% of the value obtained immediately after stretch. Therefore, upon acute hemodynamic overload, the ventricle may be capable of increasing its contractility at significantly lower filling pressures, representing a favorable hemodynamic profile and decreasing the odds of congestion. This adaptive diastolic response is not a merely passive phenomenon but instead susceptible of active modulation. Indeed, it seems to partially depend upon PKC activity.

Ischemia impaired both systolic and diastolic adaptive responses to an acute mechanical load. However, the presence of an AT1 receptor antagonist partially prevented this deterioration of both systolic and diastolic functions. Furthermore, two cycles of IP evoked a cardioprotective functional effect upon mechanical stretch under ischemic conditions. These results emphasize that the blockade of the renin-angiotensin system and IP may exert a favorable effect on the myocardial response to an acute hemodynamic overload.

In conclusion, besides the well known systolic response to acute myocardial stretch, there is also a diastolic arm of this adaptive response that is actively modulated and displays important pathophysiological implications during ischemia.

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FIGURES

Figure 1. Schematic illustration of experimental protocols. White bar – presence of O_2 and glucose; gray bar: stabilization period; dark bar – absence of O_2 and glucose (ischemia)

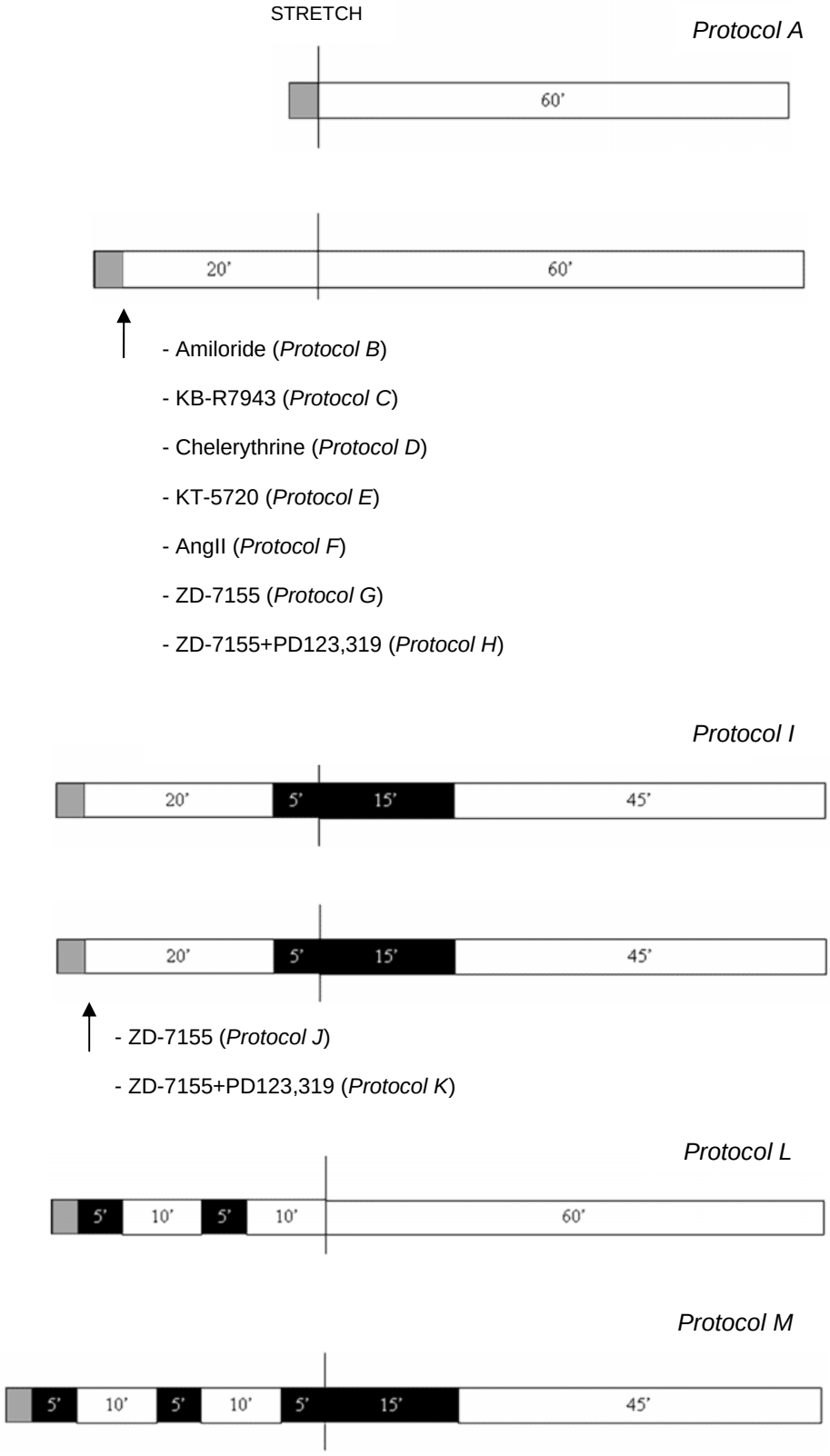

Figure 2. Representative band illustrating the acute and time-dependent effects of rabbit papillary muscle stretch on systolic and diastolic functions. AT: active tension; PT: passive tension.

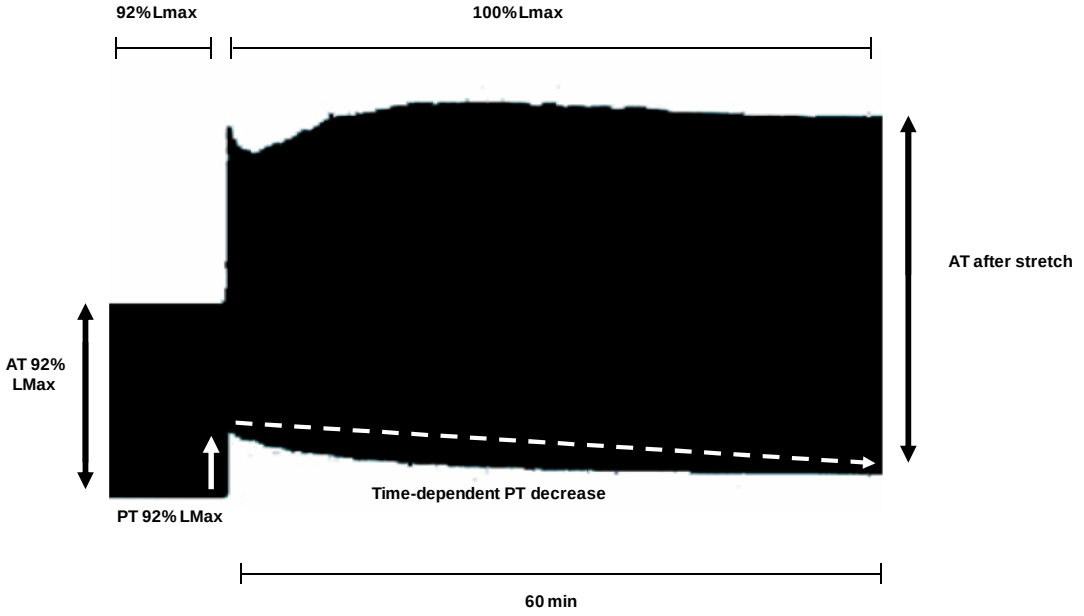

Figure 3. Systolic (**A**) and diastolic (**B**) responses of rabbit isolated papillary muscles to acute stretch (Protocol A). **P*<0.05 vs. 92% L_{max}; †*P*<0.05 vs. upon stretch.

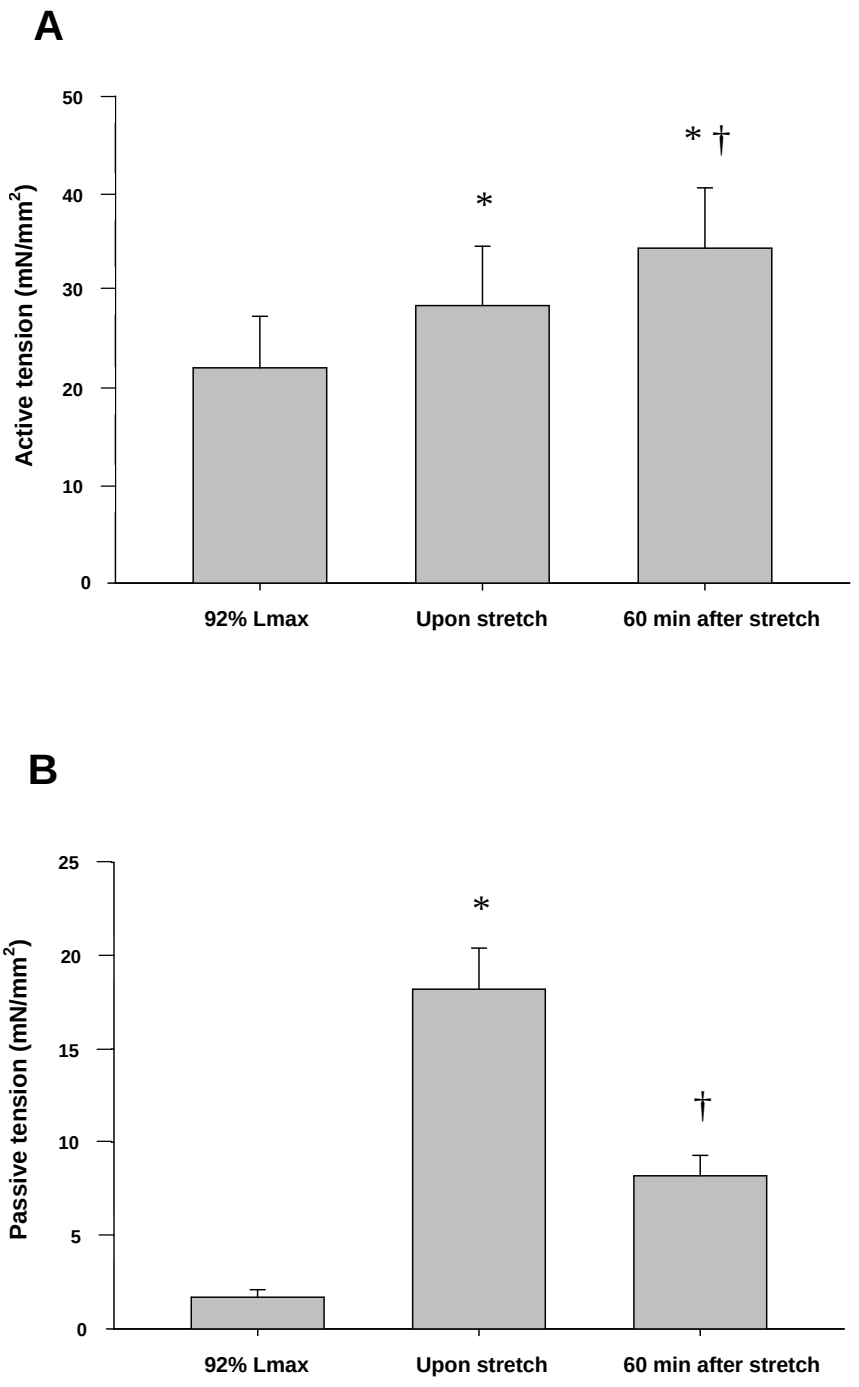

Figure 4. Functional effects of blocking NHE and rNCX upon myocardial response to stretch. **A**: Inhibition of NHE or rNCX blunts the SFR after acute myocardial stretch. FSM: Frank-Starling mechanism; SFR: Slow force response. *P<0.05 vs. control group. **B**: rNCX inhibition attenuated the immediate increase in PT upon stretch. †P<0.05 vs. Control group.

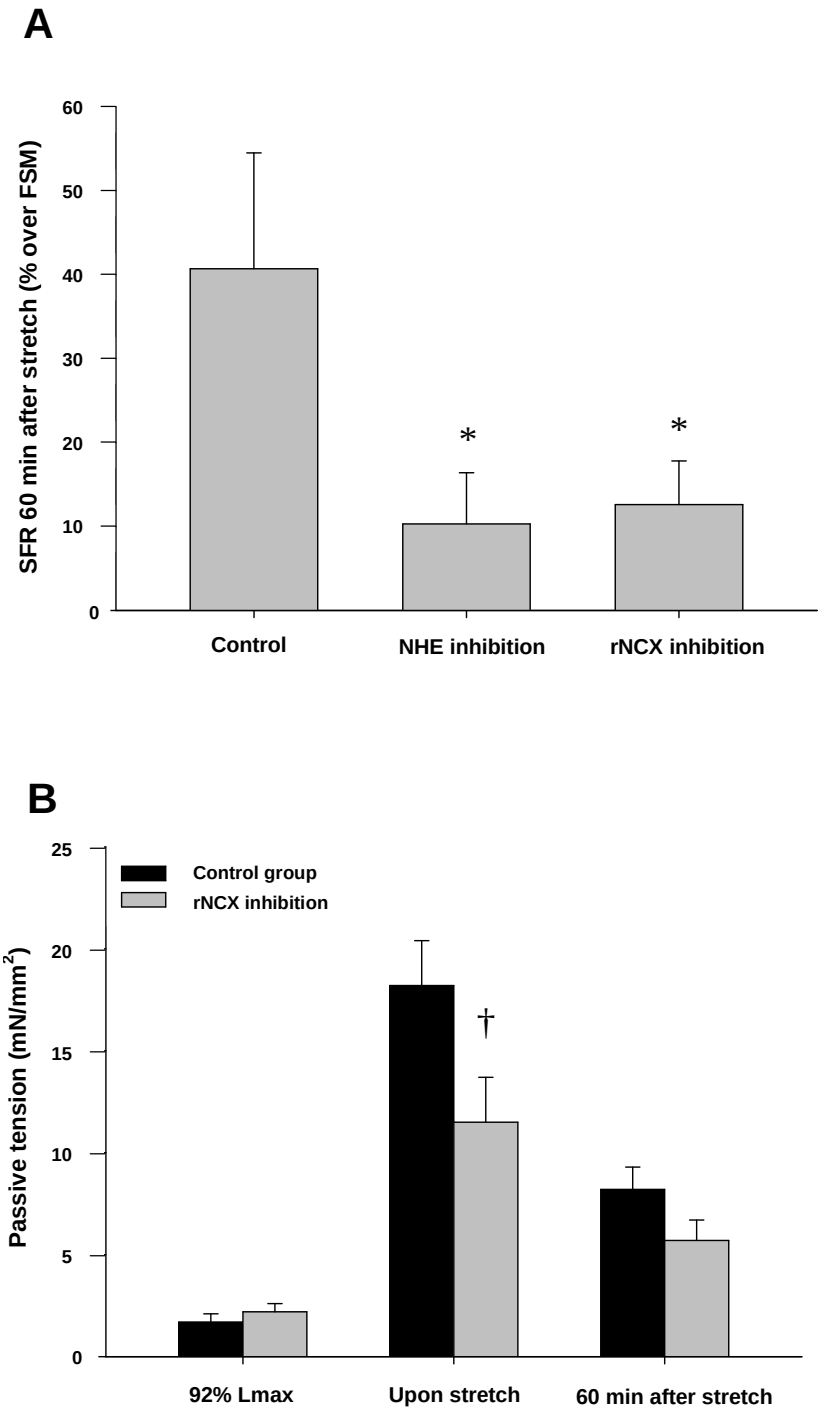

Figure 5. Functional effects of PKC inhibition upon myocardial response to stretch of isolated papillary muscles. **A**: PKC inhibition blunted the SFR. AT: active tension; FSM: Frank-Starling mechanism. **B**: The time-dependent decrease in myocardial PT after stretch was attenuated with PKC inhibition, being already significant 10 minutes after stretch to L_{max}. PT: passive tension. *P<0.05 vs. Control group.

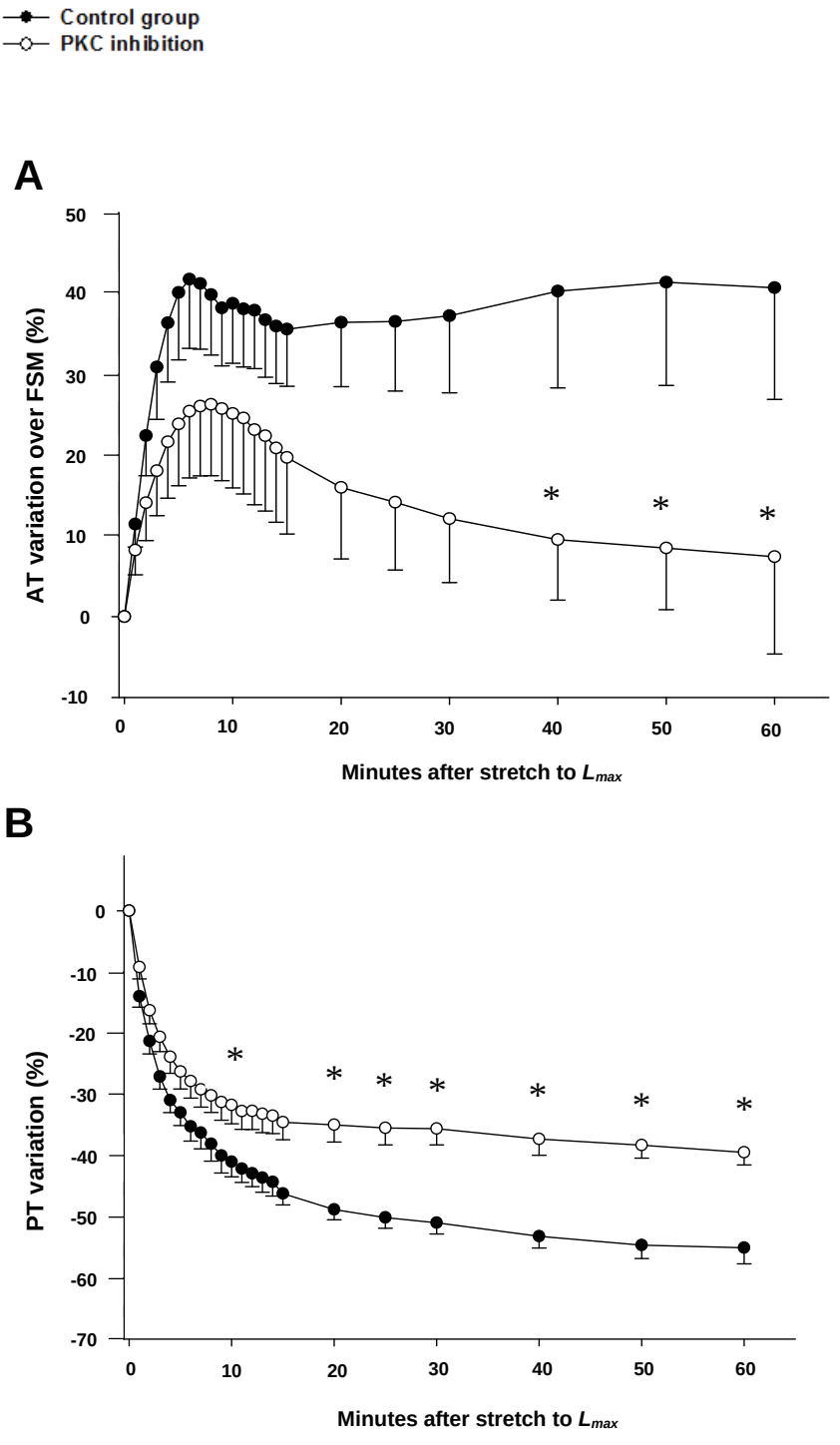

Figure 6. AngII attenuated the immediate increase in PT upon stretch and enhanced the time-dependent decrease down to a value significantly different from the control group. *P<0.05 vs. Control group.

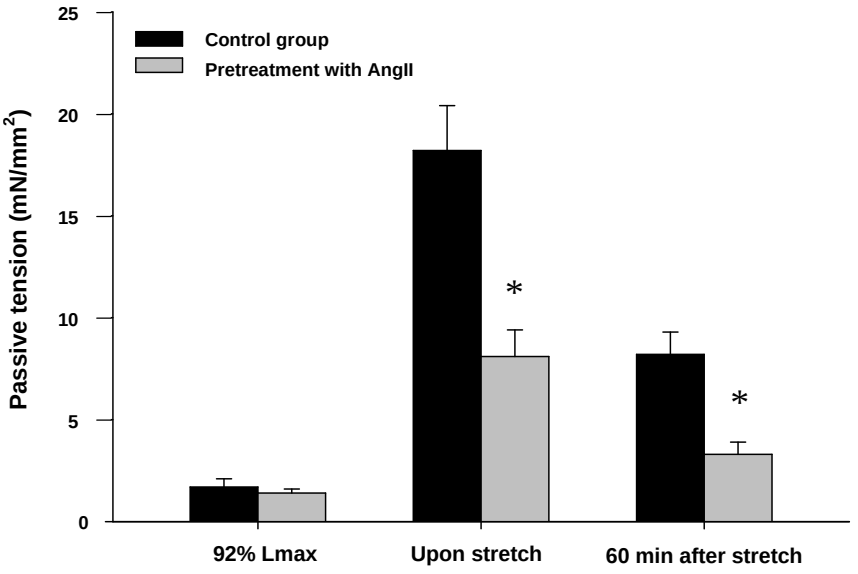

Figure 7. Ischemia impaired the adaptive systolic and diastolic responses to acute stretch. **A**: Acute stretch from 92 to 100% of L_{max} during ischemia elicited an immediate increase in active tension (AT) (Frank-Starling mechanism) but abolished the slow force response. After reperfusion, AT increased to control levels. **P*<0.05 vs. 92% L_{max} (Protocol I). †*P*<0.05 vs. upon stretch (Protocol I). ‡*P*<0.05 vs. 15 min (Protocol I). **B**: The passive tension decrease after stretch was absent during ischemia. §*P*<0.05 vs. control group.

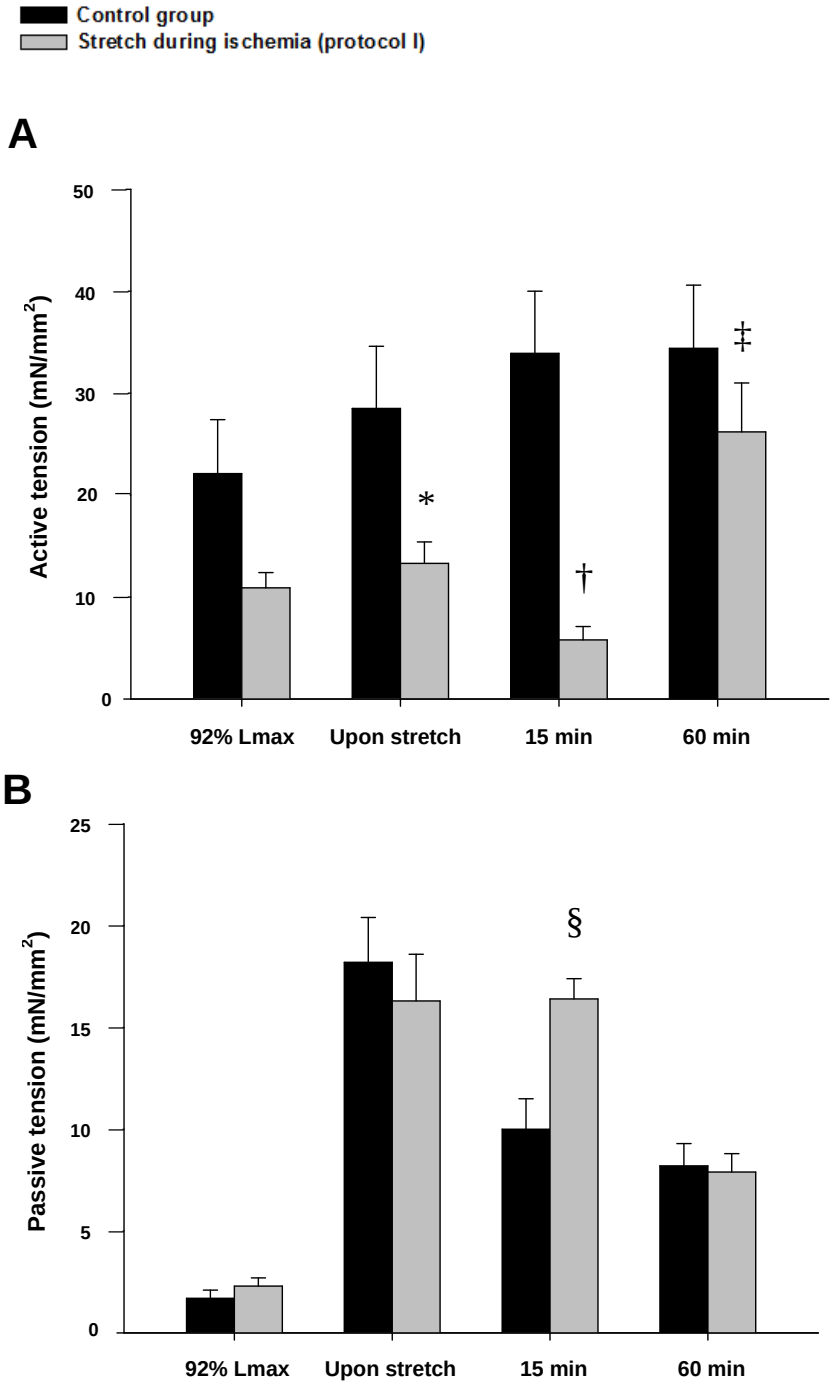

Figure 8. Impact of blocking AT1 and AT2 receptors on the systolic and diastolic responses to stretch during ischemia. **A**: Blocking AT1 receptors prevented the decrease in active tension (AT) after stretch under ischemic conditions. This effect was partially dependent on AT2 receptor activation. **B**: The blockade of AT1 receptors or concomitant blockade of AT1 and AT2 receptors improved diastolic response to stretch, eliciting a progressive decrease in passive tension (PT). **P*<0.05 vs. Protocol I. †*P*<0.05 vs. Protocol J.

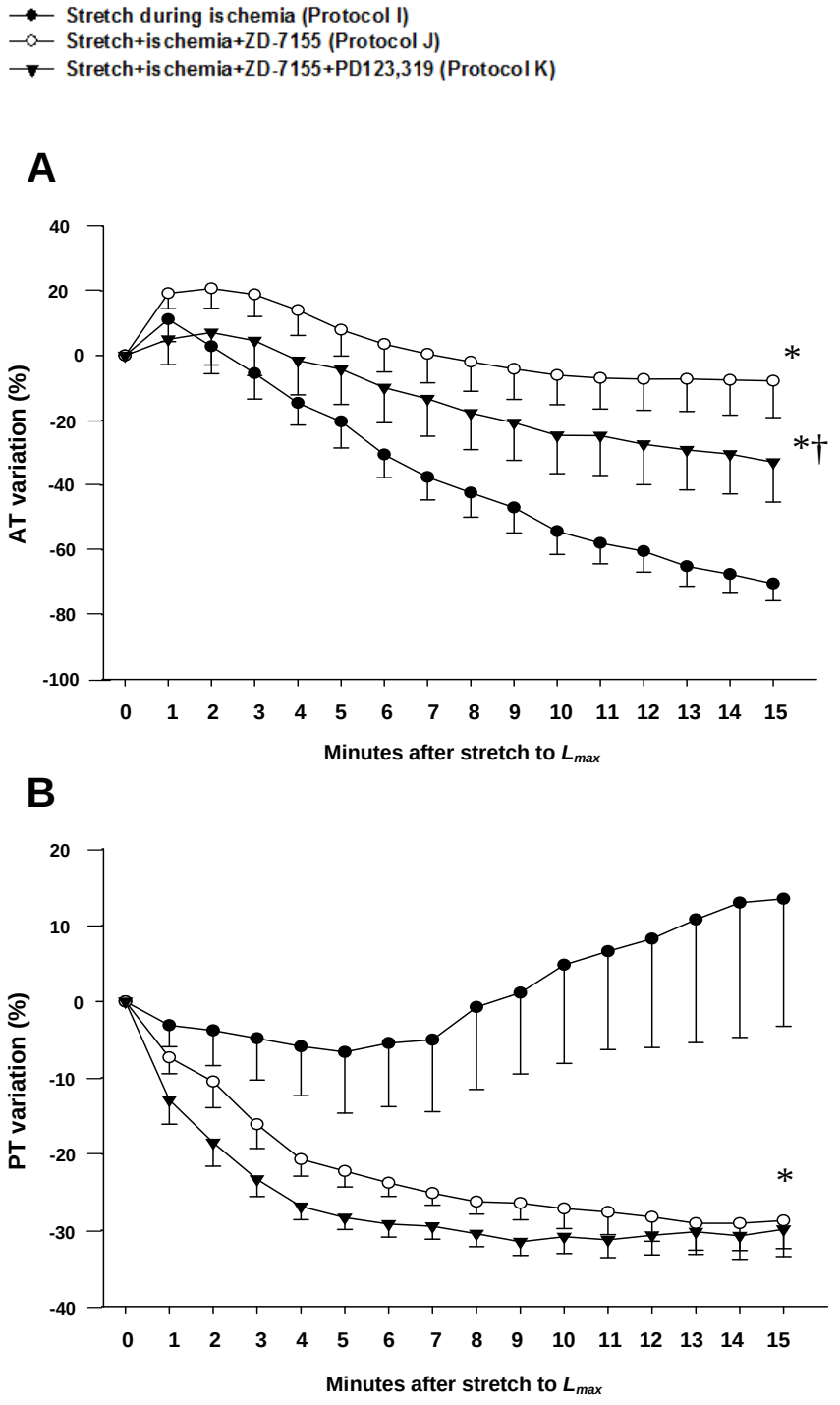

Figure 9. Myocardial response to stretch after ischemic preconditioning (Protocol L). **A**: The immediate and delayed increases in active tension were not significantly different from the control group. **B**: Ischemic preconditioning attenuated the immediate increase in passive tension. *P<0.05 vs. control group.

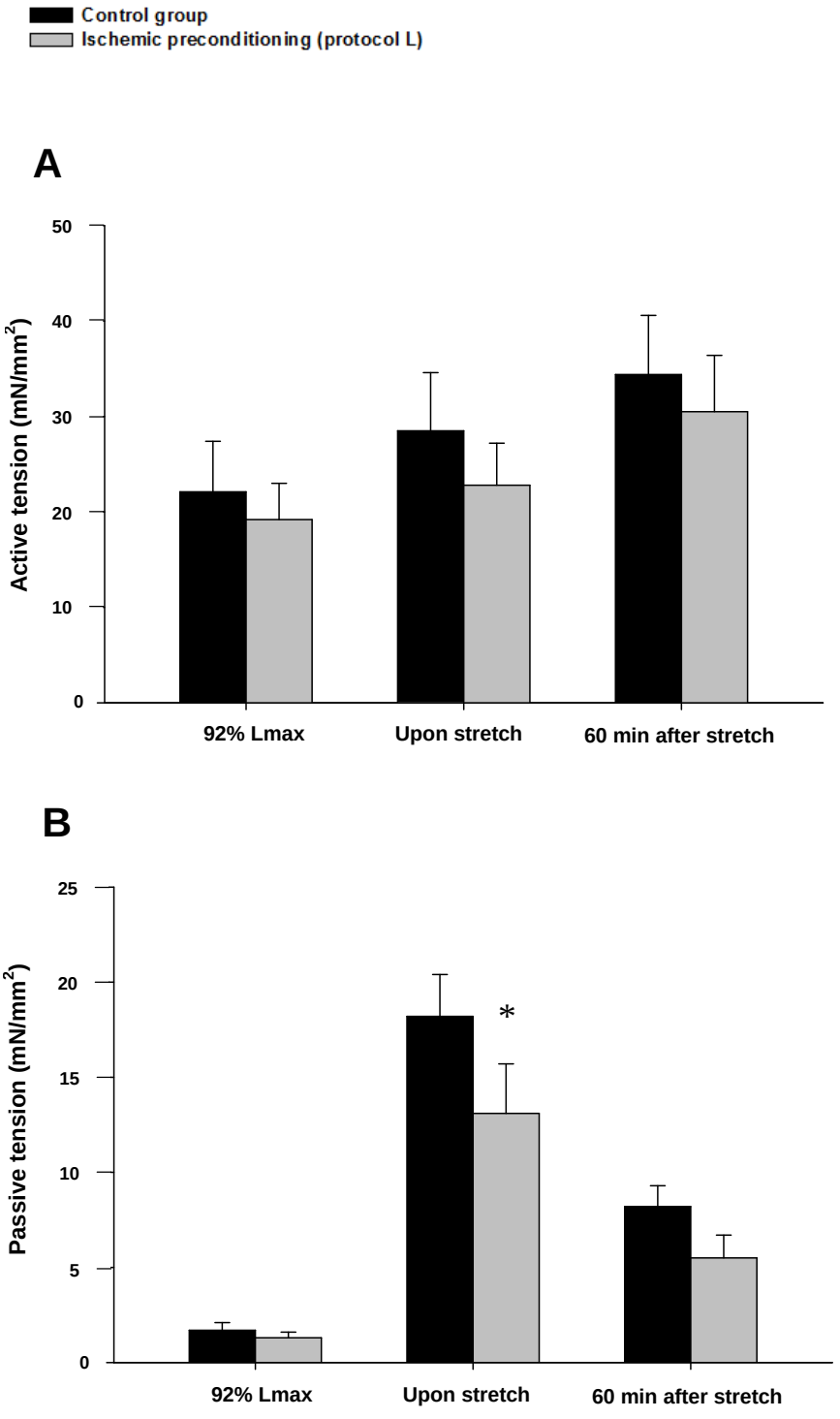

Figure 10. Ischemic preconditioning improves systolic and diastolic responses to acute stretch during ischemia. **A**: Ischemic preconditioned papillary muscles demonstrated an attenuated decrease in active tension (AT) after stretch during ischemia. **B**: Ischemic preconditioning blunted the decrease in relaxation velocity that characterized cardiac response to stretch during ischemia. *P<0.05 vs. Protocol I.