Signal Transduction and Cardiac Hypertrophy,
edited by N.S. Dhalla, L.V. Hryshko,
E. Kardami & P.K. Singal
Kluwer Academic Publishers, Boston, 2003

Stretch-Elicited Autocrine/Paracrine Mechanism in the Heart

Horacio E. Cingolani,* Néstor G. Perez,*
and María C. Camilión de Hurtado*

Centro de Investigaciones Cardiovasculares
Facultad de Ciencias Médicas
Universidad Nacional de La Plata
Calle 60 y 120, 1900 La Plata
Argentina

Summary. An autocrine/paracrine mechanism is triggered by stretching the myocardium. This mechanism involves the release of angiotensin II (Ang II), the release/increased formation of endothelin (ET), the activation of the Na^+/H^+ exchanger (NHE), the increase in intracellular Na^+ concentration ($[Na^+]_i$), and the increase in the Ca^{2+} transient that underlies the so called slow force response (SFR) to stretch. This autocrine/paracrine mechanism could explain how a change in afterload alters cardiac contractility as was reported by Anrep in 1912.

Key words: myocardial stretch, Na^+/Ca^{2+} exchange, angiotensin II, endothelin hypertrophy.

INTRODUCTION

It is known that when the length of the cardiac muscle is increased, there is first a rapid and then a slow increase in twitch force. The first phase is thought to be due to an increase in myofilament responsiveness and is considered to be the basis of the Frank-Starling mechanism (1). The second phase that develops during the next 10 minutes or so is believed to be due to an increase in the Ca^{2+} transient (2–5) and its link with autocrine/paracrine mechanisms involving the tissue renin-angiotensin system has been recently suggested (5). We will discuss here the intra-

Address for Correspondence: Dr. Horacio E. Cingolani, Centro de Investigaciones Cardiovasculares, Facultad de Ciencias Médicas, UNLP, 60 y 120 (1900) La Plata, Argentina. Fax#: (54-221) 425-5861, Phone: (54-221) 483-4833, E-mail: cicme@atlas.med.unlp.edu.ar
* Established Investigators of Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Argentina.

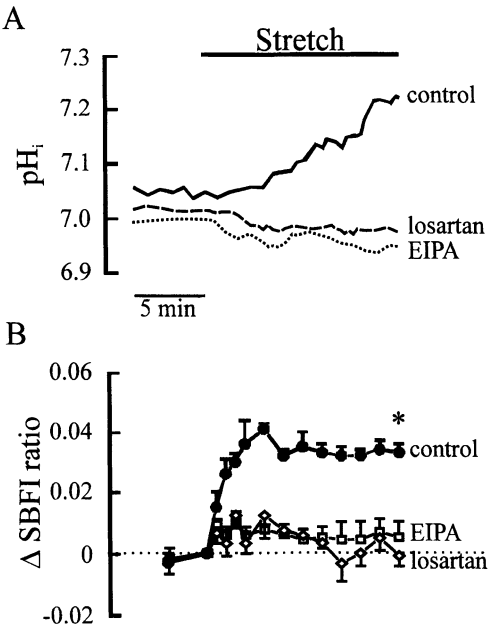

Figure 1. Stretch-induced NHE activation. Effects of EIPA and losartan. Activation of the NHE after stretching papillary muscles can be detected by both the increase in pH_i (typical experiment in panel A, "control") or in $[Na^+]_i$ (averaged experiments of panel B, "control", expressed as changes in the SBFI fluorescence ratio) in the absence of bicarbonate. When bicarbonate is present in the medium, the increase in $[Na^+]_i$ still occurs but pH_i changes are minimized. NHE blockade by EIPA as well as blockade of the Ang II AT_1 receptors by losartan abolished the changes promoted by the stretch (typical experiments in panel A and averaged data of panel B, "EIPA" and "losartan"). *indicates $p < 0.05$ vs EIPA and losartan. (Adapted from Cingolani et al. 1998 (8) and from Alvarez et al. 1999 (5) with permission).

cellular changes that follow the stretch of feline papillary muscles and the mechanical counterpart that results from the autocrine/paracrine mechanism triggered by myocardial stretch.

ACTIVATION OF THE NHE BY MYOCARDIAL STRETCH

Figure 1 shows the activation of the NHE that occurs after stretching papillary muscles from 92 to 98% of the length at which they developed the maximal twitch force (L_{max}). The activation of the NHE can be detected either by the increase in $[Na^+]_i$ (panel B) or by the increase in intracellular pH (pH_i) (panel A). When bicarbonate is present in the medium however, the changes in pH_i are minimized due to the activation of a bicarbonate acidifying mechanism (6,7) while the changes in $[Na^+]_i$ are still of similar magnitude. These changes can be abolished by NHE blockade with 5-(N-ethyl-N-isopropyl)-amiloride (EIPA) or by blockade of the Ang II AT_1 receptors with losartan (panels A and B) (8).

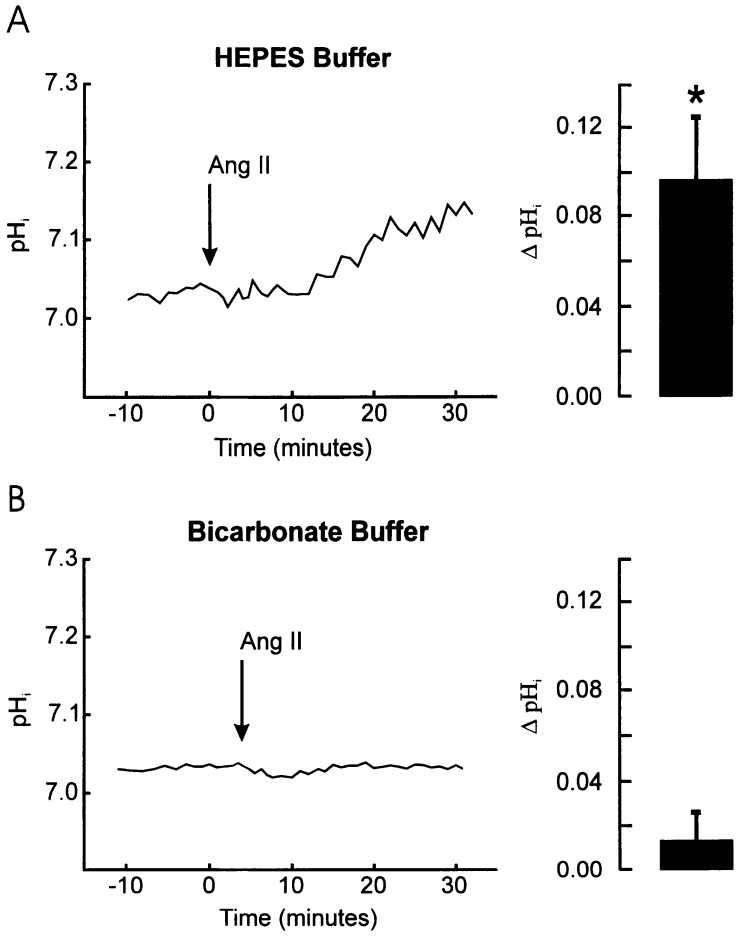

Figure 2. Exogenous Ang II can mimic the stretching effects. Ang II activates the NHE promoting an increase in pH_i that is prominent in the absence of bicarbonate (HEPES buffer) as shown in panel A (a typical experiment at left and the averaged pH_i value after 30 minutes in the column graph). In the presence of the physiological buffer (bicarbonate buffer) the changes in pH_i are minimized as shown in panel B (as in panel A, a typical experiment at left and the averaged pH_i value after 30 minutes in the column graph). *indicates $P < 0.05$ vs pre-Ang II control. (Adapted from Camilión de Hurtado et al. 1998 (6)).

ROLE OF ANG II IN THE ACTIVATION OF THE NHE

Since previous studies have shown that the stretch of isolated neonatal cardiomyocytes promotes the release of preformed Ang II (9), we were tempted to conclude that in our multicellular adult preparation the myocardial stretch releases preformed Ang II that, through the AT_1 receptors, stimulates the NHE in an autocrine or paracrine fashion (8–9). Figure 2 shows that in agreement with this, exogenous Ang II can mimic the effects of stretch. Ang II activates the NHE being reflected by an

increase in pH_i (panel A). Note that the change in pH_i is minimized in the presence of bicarbonate (panel B), because of the already mentioned simultaneous activation of a bicarbonate acidifying mechanism by Ang II (6,7).

ROLE OF ET IN THE ANG II-INDUCED ACTIVATION OF THE NHE

Based in the experiments by Ito et al. (10) and considering that several effects previously attributed to Ang II are actually the result of the formation/release of ET (7,11,12), the possibility that Ang II-induced release/formation of ET mediates the effects of stretch was explored. Figure 3 shows that it was indeed the case, since the activation of the NHE by stretching papillary muscles was abolished by both nonselective and selective ET_A receptor blockade, and by inhibition of the endothelin-converting enzyme (ECE) activity with phosphoramidon. Similarly the activation of the NHE by exogenously applied Ang II was abolished by ET receptors blockade (8), as shown in Figure 4.

CROSS TALK BETWEEN ANG II AND ET

The fact that ET receptors blockade inhibits the activation of the NHE by Ang II, together with the notion that the NHE activation induced by ET is not abolished by AT_1 receptor blockade as previously reported by us (8), indicate that Ang II-induced release/formation of ET is the valid way of the cross talk between both peptides and not the opposite. These results suggest that it should be also the pathway for stretch, ruling out the possibility that myocardial stretch releases ET which in turn would release Ang II and cause the activation of the NHE. Figure 5 schematizes the complete sequence of events that are triggered by the stretch.

THE MECHANICAL COUNTERPART OF THE AUTOCRINE/PARACRINE EFFECTS

Figure 6 shows the typical changes in developed force that follows myocardial stretch (panel A) and the suppression of the second phase (the SFR) that causes the interference of the chain of the autocrine/paracrine events that follows myocardial stretch by NHE inhibition or the blockade of either Ang II AT_1 or ET receptors (panels B–D).

THE Na^+/Ca^{2+} EXCHANGER (NCX) AS THE CULPRIT OF THE INCREASE IN THE Ca^{2+} TRANSIENT

Since it was previously reported that the progressive increase in force during the SFR is due to an increase in the Ca^{2+} transient (2–5), we tested the hypothesis that the increase in the Ca^{2+} transient could be secondary to the increase in $[Na^+]_i$ that follows the NHE activation. It can be argued that the increase in pH_i after the NHE activation could mediate or contribute to the increase in contractility through an increase in the myofilament responsiveness to Ca^{2+}. However we should keep in mind that the changes in pH_i are minimized when bicarbonate is present in the medium and that previous studies have shown the absence of changes in myofilament Ca^{2+} responsiveness during the development of the SFR (4).

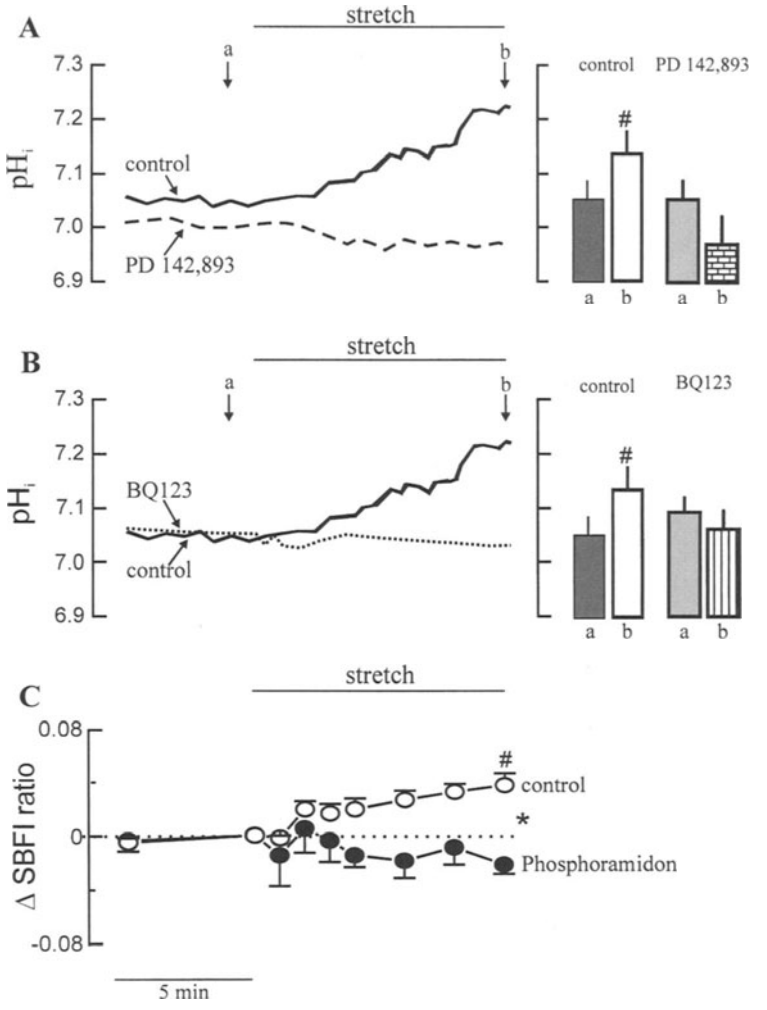

Figure 3. Formation/release of ET mediates the effects of Ang II after stretch. The stretch-induced NHE activation in isolated papillary muscles was abolished after non-selective ET receptor blockade (PD 142,893) and selective ET_A receptor blockade (BQ123), as detected by the lack of changes in pH_i after the stretch in both conditions (panels A and B, typical experiments and averaged data in the column graphs). Inhibition of the endothelin-converting enzyme activity by phosphoramidon gives further support to the hypothesis, as shown by the lack of changes in $[Na^+]_i$ (expressed as changes in the SBFI fluorescence ratio) after stretch under this condition (averaged data of panel C). "a" and "b" indicate the time at which the average for the column graphs were made. #indicates $p < 0.05$ vs pre-stretched control, *indicates $P < 0.05$ between curves. (Adapted from Cingolani et al. 1998 (8) and from Pérez et al. 2001 (15) with permission).

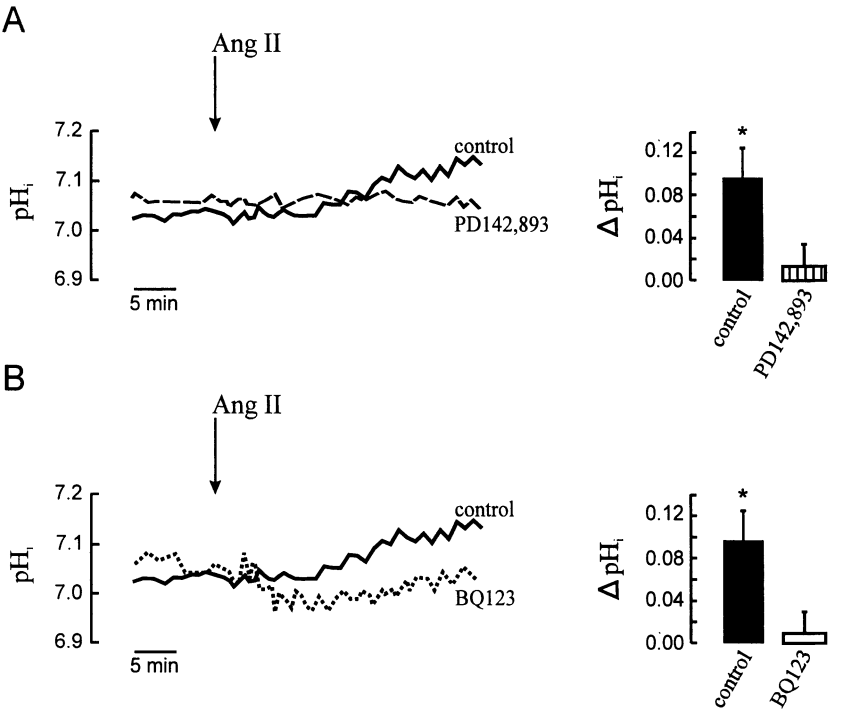

Figure 4. ET receptors blockade suppresses the activation of the NHE by exogenous Ang II. Similarly to the results for the stretch presented in Figure 3, pretreatment of the papillary muscles with a non-selective ET receptor blocker (PD 142,893, panel A) or with a selective ET_A receptor blocker (BQ123, panel B) prevented the expected increase in pH_i due to addition of Ang II. *indicates $p < 0.05$ vs pre-Ang II control. (Adapted from Cingolani et al. 1998 (8) with permission).

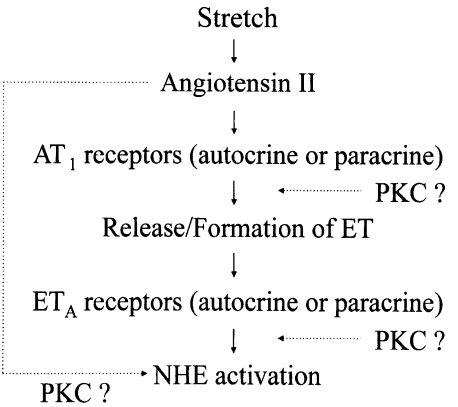

Figure 5. Chain of events triggered by the stretch. Myocardial stretch promotes the release of Ang II that will bind to the AT_1 receptors by an autocrine or paracrine mode and will stimulate the release/formation of ET probably by a protein kinase C (PKC)-mediated pathway. ET will bind to ET_A receptors (autocrine or paracrine) that will promote the activation of the NHE probably through another PKC dependent pathway. It is not possible to rule out a direct activation of the NHE by Ang II.

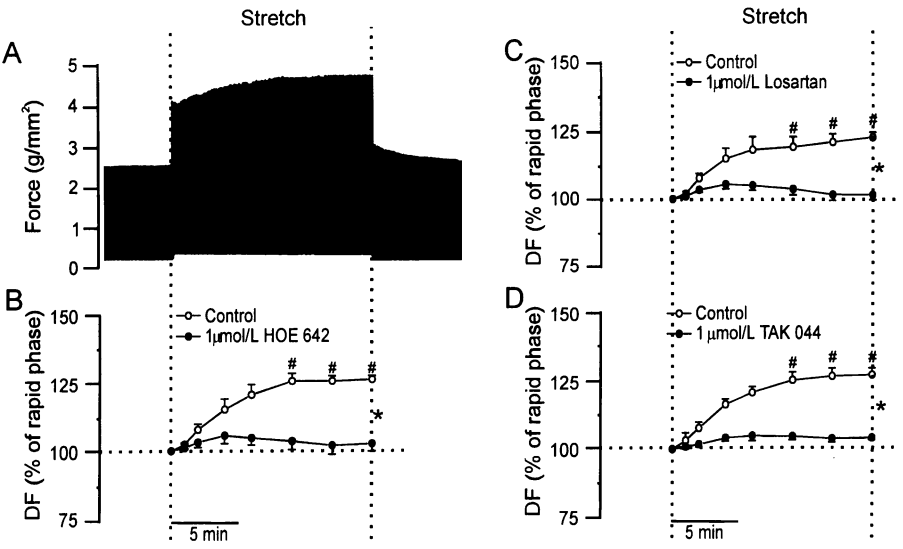

Figure 6. Typical force response to the stretch in isolated cat papillary muscle and effects of different interventions on the SFR. An original record showing the characteristic two-phase increase in force after the stretch is shown in panel A. The initial rapid phase is attributed to an increase in myofilament Ca^{2+} responsiveness, and the second one slowly developing (known as SFR) to an increase in Ca^{2+} transient. Panels B-D show the effect of three different interventions on the SFR, expressed as percent of the initial rapid phase. The SFR is abolished by NHE blockade (panel B), by Ang II AT_1 receptor blockade (panel C) and by non-selective ET receptors blockade (panel D). Taken together these results indicate that NHE activation is a key step for the development of the SFR, through a mechanism that needs the participation of Ang II and ET. #indicates $P < 0.05$ vs rapid phase, *indicates $P < 0.05$ between curves. (Adapted from Pérez et al. 2001 (15) with permission).

It is interesting that in an ionic model of cardiac myocytes where the potential contribution of sarcolemmal ion fluxes to the SFR development was analyzed, Bluhm et al. (13) found that the SFR could be mimicked by an increase in $[Na^+]_i$ that concurred with an increase in Ca^{2+} entry through the NCX.

Figure 7 shows that when extracellular Na^+ was replaced either by equimolar amounts of choline chloride and N-methyl-D-glucamine (NMG) or by equimolar amounts of lithium chloride, the SFR was abolished. These experiments suggested that the NCX either by decreasing Ca^{2+} efflux (forward mode) or by increasing Ca^{2+} influx (reverse mode) could be responsible for the increase in the Ca^{2+} transient during the SFR. Experiments using KB-R7943, a blocker of the NCX preferentially in its reverse mode showed complete suppression of the SFR, as it can be appreciated in Figure 8A, indicating that Ca^{2+} increases by this way during the SFR. These results are in agreement with those predicted by the computerized model mentioned above. Interestingly, the compound also abolished the SFR either applied immediately or 15 minutes after the stretch (Figure 8B). However, since it has been reported a direct action of Ang II and ET on the NCX (14) we can not rule out the possibility that in addition to the increase in $[Na^+]_i$, a direct effect on the NCX could be necessary to drive the exchanger in reverse.

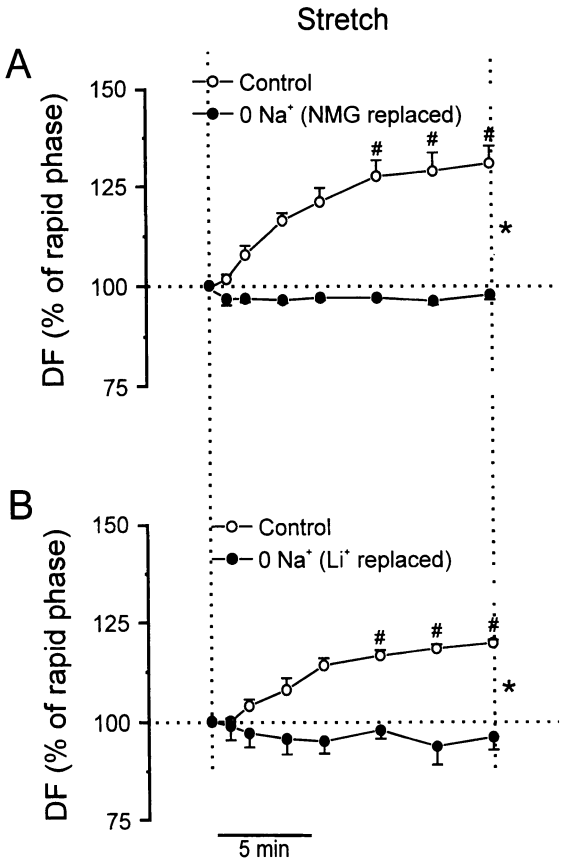

Figure 7. NCX in the generation of the SFR. Extracellular Na^+ replacement by N-methyl-D-glucamine (NMG, panel A) or by lithium (Li^+, panel B) abolished the SFR. These results provide conclusive evidence that the NCX is involved in the development of the SFR, however they do not allow ascertaining whether the forward or the reverse mode of operation mediates the effect. #indicates $P < 0.05$ vs rapid phase, *indicates $P < 0.05$ between curves. (Adapted from Pérez et al. 2001 (15) with permission).

AUTOCRINE OR PARACRINE MECHANISM?

Since our experiments were performed in a multicellular preparation in which coexist myocytes, fibroblasts and endothelial cells, it is difficult to ascertain at present whether the elicited mechanism is autocrine or paracrine. To address whether endothelial cells were the source of ET, we performed experiments in which endocardial and vascular endothelial cells were functionally inactivated with triton X100 (15). After endothelial inactivation, the SFR and the increase in $[Na^+]_i$ after the stretch both persisted and both were blocked by phosphoramidon. These data suggest that endothelial cells are not the source for ET. In the light of these results, Figure 9 summarizes two possible alternatives for the chain of events detected in a multicellular adult feline

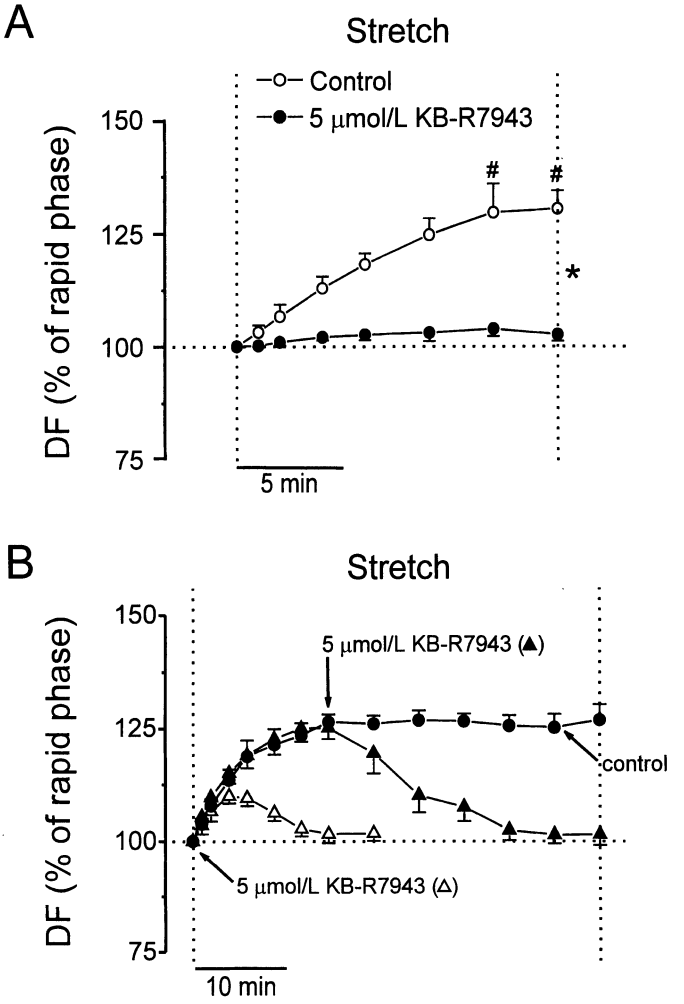

Figure 8. Effect of reverse mode NCX blockade by KB-R7943 on the development of the SFR. Panel A shows that pretreatment with KB-R7943 prevented the development of the SFR, giving strong support to the idea that Ca^{2+} influx through the NCX is the responsible for the increase in the Ca^{2+} transient that underlies the development of the SFR. Panel B shows that acute addition of the compound either at the beginning of the stretch or after 15 minutes also cancelled the SFR. #indicates $P < 0.05$ vs rapid phase, *indicates $P < 0.05$ between curves. (Adapted from Pérez et al. 2001 (15) with permission).

preparation that determines the development of the SFR. The upper panel schematizes the autocrine-signaling pathway to explain the SFR. In this alternative, stored Ang II is released from the myocyte and binds to its AT_1 receptors. The myocyte will form/release ET that, through the ET_A receptors, will stimulate the NHE activity by a PKC-dependent pathway. In the lower panel (paracrine) the mechanism is similar, but the source of ET would be the fibroblast instead of the myocyte. Ang II released

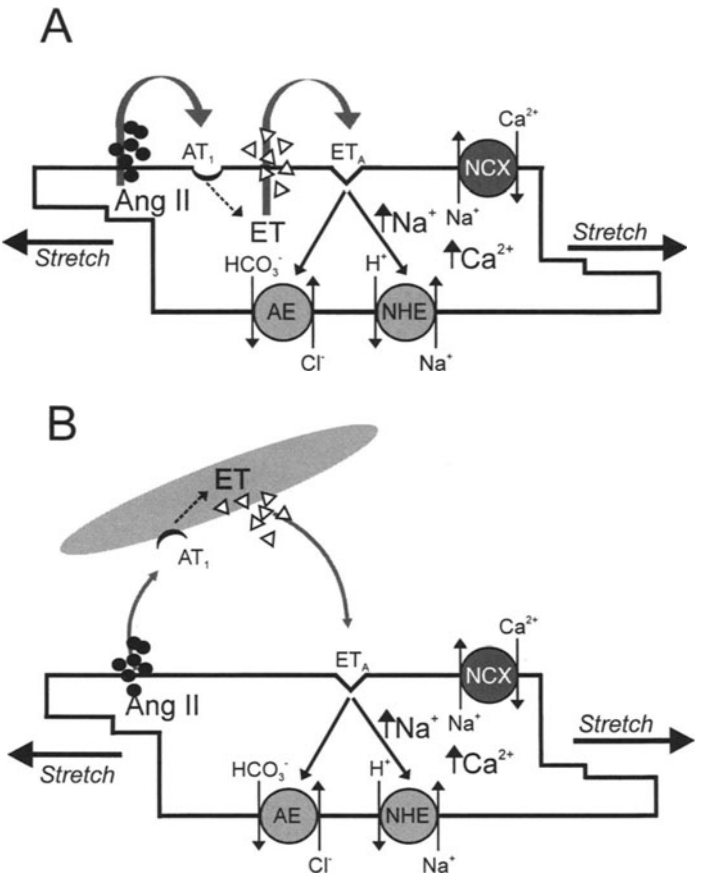

Figure 9. Hypothetical signaling pathways triggered by the stretch. Panel A schematizes the autocrine alternative in which the myocyte is the source and the target of Ang II. Ang II binds to AT_1 receptors and promotes the formation/release of ET, which, through the ET_A receptors, stimulates NHE activity by a PKC-dependent pathway. Simultaneous activation of the Na^+ independent Cl^-/HCO_3^- exchanger (AE) minimizes the increase in pH_i, but not in $[Na^+]_i$, which will activate the NCX in its reverse mode promoting the increase in the Ca^{2+} transient. Panel B schematizes the paracrine alternative in which the mechanism is essentially the same, but the source of ET is the fibroblast instead of the myocyte. Ang II released by the myocyte stimulates AT_1 receptors on fibroblasts, this promotes the formation/release of ET that will bind on the myocytes's ET_A receptors in a cross-talked paracrine loop. (Adapted from Pérez et al. 2001 (15) with permission).

by the myocyte will stimulate AT_1 receptors on fibroblasts in a paracrine fashion inducing the formation/release of ET and the ET released by fibroblasts will act on the myocytes's ET_A receptors in a cross-talked paracrine loop. Whatever the case, simultaneous activation of the acidifying mechanism Na^+ independent Cl^-/HCO_3^- exchanger (6,7) will preclude changes in pH_i, but not in $[Na^+]_i$ that will rise. The increase in $[Na^+]_i$ will favor the NCX in its reverse mode (Ca^{2+}_{in}-Na^+_{out}) and this will increase the $[Ca^{2+}]_i$ transient determining the development of the SFR.

CONCLUSIONS

The results presented herein are providing evidence of the autocrine/paracrine role played by the local myocardial renin-angiotensin system in the adult multicellular preparation.

The mechanical counterpart of this chain of events is an increase in contractility mediated by an increase in the Ca^{2+} transient through the NCX operating in reverse mode and can be the explanation for the Anrep effect (16) and the SFR.

The heart makes use of a dual mechanism for adapting its working output to changing hemodynamic conditions. First, there is a rapid increase in myofilament sensitivity, which depends on the stretch. This is followed by a slow increase in the Ca^{2+} transient, a response that results from a stretch-induced autocrine/paracrine loop involving the tissue renin-angiotensin system.

Although it is well known that myocardial stretch elicits an increase in protein synthesis leading to hypertrophy (9) and that the hypertrophic response is induced at least partly through an increase in secretion/synthesis of Ang II and ET (9,17), it still remains to be determined how the mechanical stretch is converted into biochemical signals that activate protein synthesis. Protein kinase cascades (18,19), calcium handling (20), calcineurin (21) and other mechanisms (22) have been extensively analyzed as possible mediators of the hypertrophic response. Whether or not the autocrine/paracrine system described here is linked to the development of myocardial hypertrophy is examined in another chapter of this book.

REFERENCES

1. Lakatta EG. 1992. Length modulation of muscle performance. Frank-Starling law of the heart. In: Fozzard HA, ed. The Heart and Cardiovascular System, 2nd ed. New York, NY: Raven;1325–1351.
2. Allen DG, Nichols CG, Smith GL. 1988. The effects of changes in muscle length during diastole on the calcium transient in ferret ventricular muscle. J Physiol (Lond) 406:359–370.
3. Parmley WW, Chuck L. 1973. Length-dependent changes in myocardial contractile store. Am J Physiol 224:1195–1199.
4. Kentish JC, Wrzosek A. 1998. Changes in force and cytosolic Ca^{2+} concentration after length changes in isolated rat ventricular trabeculae. J Physiol (Lond) 506:431–444.
5. Alvarez BV, Pérez NG, Ennis IL, Camilión de Hurtado MC, Cingolani HE. 1999. Mechanisms underlying the increase in force and Ca^{2+} transient that follow stretch of cardiac muscle. A possible explanation of the Anrep Effect. Circ Res 85:716–722.
6. Camilión de Hurtado MC, Alvarez BV, Pérez NG, Ennis IL, Cingolani HE. 1998. Angiotensin II Activates Na^+-independent Cl^--HCO_3^- Exchange in Ventricular Myocardium. Circ Res 82:473–481.
7. Camilión de Hurtado MC, Alvarez BV, Ennis IL, Cingolani HE. 2000. Stimulation of Myocardial Na^+-independent Cl^--HCO_3^- Exchanger by Angiotensin II is Mediated by Endogenous Endothelin. Circ Res 86:622–627.
8. Cingolani HE, Alvarez BV, Ennis IL, Camilión de Hurtado MC. 1998. Stretch-Induced Alkalinization of Feline Papillary Muscle. An autocrine-Paracrine System. Circ Res 83:775–780.
9. Sadoshima J, Xu Y, Sleiter HS, Izumo S. 1993. Autocrine release of angiotensin II mediates stretch-induced hypertrophy of cardiac myocytes in vitro. Cell 75:977–984.
10. Ito H, Hirata Y, Adachi S, Tanaka M, Tsujino M, Kioke A, Nogami A, Marumo F, Hiroe M. 1993. Endothelin-1 is an autocrine-paracrine factor in the mechanism of angiotensin II-induced hypertrophy in cultured rat cardiomyocytes. J Clin Invest 92:398–403.
11. Rajagopalan S, Laursen JB, Borthayre A, Kurz S, Keiser J, Haleen S, Glaid A, Harrison DG. 1997. Role for endothelin-1 in Angiotensin II-mediated hypertension. Hypertension 30:29–34.
12. Ortiz MC, Sanabria E, Manriquez MC, Romero JC, Juncos LA. 2001. Role of endothelin and isoprostanes in slow pressor responses to angiotensin II. Hypertension 37:505–510.

13. Bluhm WF, Lew WY, Garfinkel A, McCulloch AD. 1998. Mechanism of length history-dependent tension in an ionic model of the cardiac myocyte. Am J Physiol (Heart Circ Physiol) 274:H1032–H1040.
14. Ballard C, Schaffer S. 1996. Stimulation of the Na^+/Ca^{2+} exchanger by phenylephrine, angiotensin II and endothelin 1. J Mol Cell Cardiol 28:11–17.
15. Pérez NG, Camilión de Hurtado MC, Cingolani HE. 2001. Reverse mode of the Na^+/Ca^{2+} exchange following myocardial stretch. Underlying mechanism of the slow force response. Circ Res 88: 376–382.
16. von Anrep G. 1912. On the part played by the suprarenals in the normal vascular reactions on the body. J Physiol (Lond) 45:307–317.
17. Yamazaki T, Komuro I, Kudoh S, Zou Y, Shiojima I, Hiroi Y, Mizuno T, Maemura K, Kurihara M, Aikawa R, Takano H, Yazaki Y. 1996. Endothelin-1 is involved in mechanical stress-induced cardiomyocyte hypertrophy. J Biol Chem 271:3221–3228.
18. Komuro I, Yazaki Y. 1993. Control of cardiac gene expression by mechanical stress. Annu Rev Physiol 55:55–75.
19. Chien KR, Grace AA, Hunter JJ. 1998. Molecular biology of cardiac hypertrophy and heart failure. In: Molecular Basis of Cardiovascular Disease. Ed, KR Chien. Philadelphia, Pa: WB Saunders Co.
20. Marbán E, Koretsune Y. 1990. Cell calcium, oncogenes, and hypertrophy. Hypertension 15:652–658.
21. Zou Y, Hiroi Y, Uozumi H, Takimoto E, Toko H, Zhu W, Kudoh S, Mizukami M, Shimoyama M, Shibasaki F, Nagai R, Yazaki Y, Komuro I. 2001. Calcineurin plays a critical role in the development of pressure overload-induced cardiac hypertrophy. Circulation 104:97–101.
22. Molkentin JD. 2000. Calcineurin and beyond. Cardiac hypertrophic signaling. Circ Res 87:731–738.