Potentiation of stretch-induced atrial natriuretic peptide secretion by intracellular acidosis

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Tavi, Pasi, Mika Laine, Sari Voutilainen, Petri Lehenkari, Olli Vuolteenaho, Heikki Ruskoaho, and Matti Weckstrom. Potentiation of stretch-induced atrial natriuretic peptide secretion by intracellular acidosis. Am. J. Physiol. 277 (Heart Circ. Physiol. 46): H405–H412, 1999.—We sought to investigate whether atrial myocyte contraction and secretion of the atrial natriuretic peptide (ANP) are affected in the same manner by intervention in intracellular Ca2+ handling by acidosis. The effects of propionate (20 mM)-induced intracellular acidosis on the stretch-induced changes in ANP secretion, contraction force, and intracellular Ca2+ concentration ([Ca2+]i) were studied in the isolated rat atrium. The stretch of the atrium was produced by increasing the intra-atrial pressure of the paced and superfused preparation. Contraction force was estimated from pressure pulses generated by the contraction of the atrium. Intracellular Ca2+ was measured from indo 1-AM-loaded atria, and ANP was measured by radioimmunoassay from the perfusate samples collected during interventions. Intracellular pH of the atrial myocytes was measured by a fluorescent indicator (BCECF)-based imaging system. Intracellular acidification caused by 20 mM propionic acid (0.18 pH units) potentiated the stretch-induced (intra-atrial pressure from 1 to 4 mmHg) ANP secretion, causing a twofold secretion compared with nonacidotic controls. Simultaneously, the responsiveness of the atrial contraction to stretch was reduced (P < 0.05, n = 7). Stretch augmented the systolic indo 1-AM transients in acidic (P < 0.05, n = 6) and nonacidic atria (P < 0.05, n = 6). However, during acidosis this was accompanied by an increase of the diastolic indo 1-AM ratio (P < 0.05, n = 6). Cooccurrence of stretch and acidosis caused an increase in systolic and diastolic [Ca2+]i and potentiated the stretch-induced ANP secretion, whereas the contraction force and its stretch sensitivity were decreased. This mechanism may be involved in ischemia-induced ANP secretion, suggesting a role for ANP secretion as an indicator of contractile dysfunction.

atrial function; calcium; contractile function; hormones

ATRIAL NATRIURETIC PEPTIDE (ANP) is a natriuretic, diuretic, and vasorelaxant cardiac hormone secreted in response to atrial stretch (37). Myocardial stretch rapidly triggers the exocytosis of ANP from the heart atria and ventricles both in vivo (11, 26) and in vitro (9, 28, 38, 43). In addition to stretch, myocardial ischemia is also a potent stimulus for ANP release in vivo. Increased plasma ANP levels have been found in patients with ischemic heart disease and myocardial infarction (25) and found in response to transient left ventricular ischemia induced by percutaneous transluminal coronary angioplasty (12, 18). Elevated levels of ANP in ischemia have been shown to correlate positively with ventricular dysfunction and elevated atrial pressure (25). However, in vitro studies suggest that myocardial ischemia may also directly cause ANP release (48). The cellular mechanisms of this are not known, but they may be related to changes in several intracellular signaling pathways activated in ischemia (37). One of these could be acidosis, because acidification of intracellular pH (pHi) is one of the consequences of ischemia (29). Acidosis alone decreases the contraction force of the cardiac muscle (33). It may also predispose the cardiac muscle to arrhythmias and change the Ca2+ balance of the cardiac myocytes (3). On the premise that stretch is the main stimulus of ANP secretion, we hypothesized that modulation of the stretch-stimulated secretion by induced acidosis could provide a mechanism contributing to the ischemia-induced ANP secretion.

Therefore, we studied what kind of mechanisms are involved when intracellular acidosis changes the atrial responses to stretch. Using an isolated rat left atrial model in which stretch of the tissue was produced by changing the intra-atrial pressure, we studied and characterized the combined effects of stretch and propionate-induced intracellular acidosis on ANP secretion. Acidosis might mediate its effects via changes in the intracellular diastolic and systolic Ca2+ concentration ([Ca2+]i), which, in addition to the regulation of contraction force, also seems to be involved in ANP secretion (23). Thus we also measured indo 1-AM fluorescence and contraction-induced pressure pulses together with sampling the secreted ANP from the atria.

METHODS

Drugs and chemicals. HEPES and propionate (propionic acid) were obtained from Sigma (St. Louis, MO); KCl, glucose, CaCl2, and MgCl2 were from E. Merck (Darmstadt, Germany); NaCl was from FF-Chemicals (Sweden); and 2',7'-bis(2-carboxyethyl)-5(6)-carboxyfluorescein (BCECF), nigericin, indo 1-AM, Pluronic F-127, probenecid [p-(dipropylsulfonyl)-benzoic acid] were from Molecular Probes Europe (Leiden, The Netherlands).

Animals, in vitro atrial preparation, and measurement of contraction force. Male Sprague-Dawley rats weighing 290–400 g were used. The rats were decapitated, and the heart from each rat was rapidly removed and placed in oxygenated (−10°C) buffer solution (in mM: 137 NaCl, 5.6 KCl, 2.2 CaCl2, 5.0 HEPES, 1.2 MgCl2, and 2.5 glucose; pH 7.4), which was also used at 37°C for superfusion of the atrium. In the buffer
solution containing sodium salt of the propionic acid (20 mM), the amount of NaCl was reduced by 20 mM, but otherwise the solutions were identical. The experimental model used in this study was the isolated rat atrial appendix, prepared as described previously (45). Briefly, a cross-branch polyethylene adapter was inserted into the lumen of the left appendix, and the tissue was placed in a constant temperature (37°C) organ bath. Another tube with a smaller diameter was inserted inside the adapter to carry the perfusate inflow into the lumen of the atrium. The outflow from the lumen came from one cross branch of the cross cannula. The stretch of the tissue was controlled by the intra-atrial pressure simply by adjusting the height of the tip of the outflow tube. One cross branch of the cross cannula was connected to a pressure transducer (TCB 100, Millar Instruments) so that the pressure in the lumen of the atrium could be recorded. Inflow and outflow (3 ml/min) to both the atrial lumen and the organ bath at a constant temperature were controlled by a peristaltic pump (7553-85, Cole-Parmer Instrument). The contraction force (as pressure generated by the contraction) was recorded concomitantly with the sampling of the perfusate.

Measurement of ANP secretion. The rat left atria were preincubated in normal HEPES buffer for 30 min before we recorded concomitantly with the sampling of the perfusate. When propionate was used in ANP preincubated in normal HEPES buffer for 30 min before we recorded concomitantly with the sampling of the perfusate. Tissue force (as pressure generated by the contraction) was recorded concomitantly with the sampling of the perfusate.

Calcium measurements. Intracellular Ca$^{2+}$ was determined by using indo 1-AM fluorescence. For indo 1-AM loading, Langendorff-perfused hearts were superfused for 25–40 min (flow 7 ml/min) with HEPES buffer (30 ml) containing 10 μmol of indo 1-AM dissolved in 200 μl of DMSO with 20% Pluronic, 0.5 mmol/l probenecid, and 1.5% BSA. After a 20-min washout period, the left atrial appendix was cut off and attached to the perfusion system. The atrium was paced with two platinum electrodes at 1 Hz. The excitation and emission of indo 1-AM was performed with a xenon light source and a suitably branched fiber optic silica cable (excitation at 355 nm and the emission monitored at 405 and 495 nm) and was detected with photomultiplier tubes. The emission signal was amplified (10×) and low-pass filtered at 50 Hz with an adjustable filter (Kemo). The indo 1-AM emission ratio (405/480 nm) was calculated on-line from a digitized signal by custom-created software (with Testpoint, Capital Equipment). When the indo 1-AM ratio is used to estimate the free [Ca$^{2+}$], as in this study, possible sources of errors have to be specified. The relationship between the indo 1-AM ratio and [Ca$^{2+}$] is not linear (13). This nonlinearity causes an underestimation of the true [Ca$^{2+}$] at high Ca$^{2+}$ levels. The difference between the indo 1-AM ratio and true [Ca$^{2+}$] is ~9% at the peak value of Ca$^{2+}$ during normal systolic Ca$^{2+}$ transients in the rat heart (6). The nonlinearity results in larger underestimation when the Ca$^{2+}$ transients are bigger. Thus when the Ca$^{2+}$ transients are observed to get bigger by some intervention, the true effect is always larger.

In addition, other possible sources of errors also exist. First, a change in the autofluorescence on stretch and/or acidification may cause an error in the measured fluorescence. To evaluate this possibility, autofluorescence changes of nonloaded atrial preparation were measured during stretch (1–4 mmHg intra-atrial pressure) and during acidosis (15 min in the presence of 20 mM propionic acid). The stretch did not affect the autofluorescence change (2.5 ± 1% n = 4, not significant (NS)) or the fluorescence at 405 nm (0% n = 4) or at 495 nm (0.25 ± 1% n = 4, NS). Similarly, acidosis did not have a significant effect on the autofluorescence change (1.75 ± 0.01% n = 4, NS) on fluorescence at 405 nm (0% n = 4) or at 495 nm (1.75 ± 0.01% n = 4, NS). Because the autofluorescence is maximally ~20% compared with the fluorescence in an indo 1-AM-loaded atrium, these changes are truly negligible. Second, the pH, reportedly has an effect on the Ca$^{2+}$ binding to the fluorescent indicators, among them indo 1-AM (27). Therefore, we tested the effects of pH and the effects of propionate on the Ca$^{2+}$-binding properties of indo 1-AM in our setup in vitro using calibration solutions with varying pH (7.0 and 7.2), different [Ca$^{2+}$] (0, 100 mM, 1 mM, and 10 mM), and with or without propionate (8 mM) at 37°C. The intracellular propionate concentration ([Prop]i) was estimated, as previously described (46), by using the equation [Prop]i = [Prop]o × 10$^{\text{[Fluo-}}$mu], where [Prop]o, is the extracellular propionate concentration (20 mM). On the basis of the pH measurements, after propionate application (see results), we estimated the [Prop]i to be ~8 mM, which was used in the calibration solutions. The free [Ca$^{2+}$] was calculated by Eqcalwin software (Biosoft) in the presence of 10 mM EGTA and 0.5 mM Mg$^{2+}$ at two given pH values (7.2 and 7.0) corresponding to the estimated pH before and after propionate application in rat atrial myocytes. From the in vitro calibrations, the changes in the indo 1-AM dissociation constant (Kd) and minimum and maximum ratios (Rmin and Rmax, respectively) were analyzed. Kd was determined by the

$\text{corr} = \frac{1}{[\text{H}^{+}]}$
equation derived previously (13). The indo 1-AM $K_a$ for Ca$^{2+}$ in pH 7.2 was 268 $\pm$ 58 nM ($n$ = 8). Both pH and propionate increased the $K_a$ significantly. The $K_a$ was significantly higher at pH 7.0 ($463 \pm 42$ nM, $P < 0.001$) and also higher in the presence of 8 mM (pH 7.2) propionic acid (417 $\pm$ 45 nM, $P < 0.01$). The changes of pH and propionate on $K_a$ were found to be additive, because $K_a$ in the solution of pH 7.0 and 8 mM propionic acid was 590 $\pm$ 57 nM ($P < 0.001$). However, there was no interaction of propionic acid and pH ($P = 0.02$ ($P$ = 0.05).)

Effect of acidosis on stretch-induced ANP secretion. Application of 20 mM propionate 16 min before the onset of stretch potentiated the stretch-induced ANP secretion into the perfusate when intra-atrial pressure was raised from 1 to 4 mmHg (Fig. 1A). The propionate-induced acid load caused a twofold increase in cumulative ANP secretion (over 32 min) from 8.0 $\pm$ 1.4 to 16.8 $\pm$ 3.0 ng (P < 0.05, $n$ = 7, Fig. 1C), without significant effect on the basal level of ANP secretion (Fig. 1D) before the onset of the stretch. The molecular form of the secreted ANP was the processed form of ANP, ANP-(99–126), and virtually no pro-ANP-(1–126) (the storage form) was found (Fig. 1B), as determined by HPLC. This indicates that also in acidosis ANP was secreted via the normal physiological exocytotic cascade (49).

Effect of acidosis on stretch-induced changes in [Ca$^{2+}$i]. It has been suggested that intracellular Ca$^{2+}$ could have a prominent role in the ANP secretion (23, 37, 38). Therefore, we then studied whether changes in ANP secretion are accompanied by changes in [Ca$^{2+}$i]. To accomplish this, the atria were stretched by increasing the intra-atrial pressure from 1 to 4 mmHg to see observe stretch-induced changes in nonacidotic atria. After these control measurements were made, the same atria were exposed to 20 mM propionate and the same stretch was applied. This experimental design allowed us to compare the effects of acidosis and stretch in the same atria. Because of slow indo 1-AM leakage from the cells and also light-induced quenching of indo 1-AM fluorescence, we could not measure the indo 1-AM fluorescence as long as we measured the ANP secretion. The peak ANP secretion can be detected from the perfusate samples collected between 4 and 8 min after the onset of the stretch. If we also take into account that the ANP secretion profile includes a perfusion delay of 2 min, we can estimate that the maximum ANP

![Figure 1](http://ajpheart.physiology.org/)
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secretion occurs within 2 and 6 min after the onset of the stretch. Thus we measured the indo 1-AM fluorescence up to 4 min after the onset of the stretch, which was sufficient to see whether the increase of ANP secretion is accompanied by the changes in the [Ca$^{2+}$]$. The recording example in Fig. 2A shows the effect of stretch on the diastolic and systolic intracellular Ca$^{2+}$ of the rat atrium (systolic maximum being the peak of the transients and diastolic being the value between the transients). At 1 mmHg the amplitude of the indo 1-AM transients was 0.26 ± 0.09 (n = 6). After 1.5 min of continuous stretch (4 mmHg), the amplitude increased to 0.36 ± 0.13 (P < 0.05, n = 6), and after 4 min of continuous stretch the amplitude increased to 0.37 ± 0.15 (P < 0.05, n = 6). The diastolic indo 1-AM fluorescence ratio was not significantly altered by stretch (NS, n = 6). When the same atria were preexposed to 20 mM propionate for 15 min in the low pressure, the amplitude of the Ca$^{2+}$ transients did not change significantly (Fig. 2B, 0.3 ± 0.08, NS, n = 6), but stretch increased the transients amplitude similarly as in the control. Ninety seconds after the onset of the stretch, the amplitude of the Ca$^{2+}$ transient was 0.38 ± 0.1 (P < 0.05, n = 6), and after 4 min it was 0.4 ± 0.13 (P < 0.05, n = 6). More interestingly, application of the stretch during acidosis increased the diastolic Ca$^{2+}$ levels compared with the nonacidotic control (P < 0.05, n = 6). The diastolic Ca$^{2+}$ level after 4 min of stretch was 0.16 ± 0.06 (n = 6) in control and 1.16 ± 0.07 (n = 6) during propionate load. Although no exact data on the actual Ca$^{2+}$ concentrations can be given, our preliminary attempts at calibration indicate that we are operating near the linear region of the indicator and that a change of the transient from 0.26 to (maximally) 0.37 in stretch would mean a similar increase in Ca$^{2+}$ (e.g., if the diastolic level were near 100 nM and would rise during systole 500 nM, the change would mean a systolic level near 680 nM). The exact calibration of multicellular cardiac preparations are notoriously difficult, because the intracellular Ca$^{2+}$ cannot be exactly controlled at low concentrations, but the use of ratios gives a semiquantitative result.

Effect of acidosis on contraction development during stretch. On the premise that acidosis has a prominent effect on the contraction force of the heart muscle, it should also modulate the stretch-induced changes in contraction force. It has also been suggested that the contraction force regulates the ANP secretion (37). Therefore, alterations in the contraction force could correlate with the ANP secretion changes seen during acidosis. To study this, we recorded the contraction force throughout the ANP experiments (Fig. 1). Control measurements were performed from seven atria with similar preincubation periods without propionate. The response of the contraction force to the step increase of intra-atrial pressure contained two distinct components. Immediately after the stretch increase the contraction force was increased but was followed by a secondary, slower augmentation of the force. The speed of this time-dependent development of the contraction force was analyzed by measuring the times to reach one-half of the maximum value of the contraction curves (the half-times). In control measurements the half-times were 1.17 ± 0.14 min, and in the propionate group the half-times were 2.25 ± 0.73 min (P < 0.01, n = 7, Fig. 3A), without a change in the contraction in low pressure (NS, n = 7). The normalized data in Fig. 3B further show that acidosis suppresses the stretch-induced increase in contraction force by inhibiting the fast component of the force development (1 min, n = 7, P < 0.05). Interestingly, there was no difference in the developed pressure between the two groups either after 10 min of continuous stretch (NS, n = 7) or thereafter up to 30 min. 

Relationship between ANP secretion and contraction development. The relationship between ANP secretion and the development of the contraction force was further analyzed by plotting the means of the contraction force and the concomitant ANP release from seven atrial preparations in the same figure, with the time of the last values before stretch set to 0 min, and thereafter showing the contraction at each subsequent time when the ANP secretion was sampled. To facilitate comparison, sigmoidal fits were made to each group of points. As can be seen from Fig. 4A, the ANP secretion slowly follows the increase of the contraction force after stretch. When the atria were preexposed to propionate, the force development was significantly slower (see also Fig. 3A) at the same time when the ANP secretion was significantly augmented and still rising. This shows that acidosis dissociates the correlation of ANP secretion and contraction force during stretch.

Fig. 2. Example recording of effect of intracellular acidification on diastolic and systolic intracellular Ca$^{2+}$ of rat atria during stretch. A: effect of increasing intra-atrial pressure from 1 to 4 mmHg on Ca$^{2+}$ (indo 1-AM fluorescence ratio) transient before stretch and 1, 5, and 4 min after onset of stretch. B: atrial intracellular Ca$^{2+}$ concentration ([Ca$^{2+}$]) response to same stretch after 15 min exposure to 20 mM propionate with Ca$^{2+}$ transients before stretch and 1, 5, and 4 min after onset of stretch.
DISCUSSION

We demonstrate that atrial stretch augments ANP secretion (Fig. 1), accompanied by an increase of the contraction force (Fig. 3) and an increase of the systolic Ca\(^{2+}\) transients (Fig. 2). The changes in ANP secretion and systolic Ca\(^{2+}\) transients suggest that the stretch-induced ANP secretion may be causally related to the change in the [Ca\(^{2+}\)]\(_i\), as suggested previously (23, 37). We also show that this complex process is modulated by intracellular acidosis. A small drop in pH\(_i\) slows down the contractile response to stretch (Fig. 3) but significantly increases the ANP secretion during stretch (Fig. 1). These changes are probably due to impaired Ca\(^{2+}\) handling during acidosis, which leads to an increase in diastolic [Ca\(^{2+}\)]\(_i\), when the muscle is stretched (Fig. 2).

Effects of simulated ischemia on function of atrial myocardium. The intracellular acidosis during ischemia has previously been simulated by applying lactic acid in the perfusion medium (7, 46). Lactic acid produces a decrease of the pH\(_i\), similarly as the propionic acid used in this study, and this leads to similar changes in the Ca\(^{2+}\) balance as we have shown here. According to our results, protons are likely to interact with several Ca\(^{2+}\) binding sites in the cytosol. First, modulation of the development of the stretch-induced increase in contraction force (Fig. 2, A and B) suggests the binding of H\(^+\) to Ca\(^{2+}\) sites in the contractile element, as previously suggested (5). Second, the rise of the diastolic [Ca\(^{2+}\)]\(_i\) in stretched acidic tissue can be interpreted to be caused by inhibition of the Ca\(^{2+}\)-extrusion mechanisms by elevated [H\(^+\)], for example by the inhibition of the Na\(^+\)/Ca\(^{2+}\) exchanger (10). When Ca\(^{2+}\) transients are augmented during stretch, acidotic cells are unable to remove the additional Ca\(^{2+}\) from the cytosol efficiently. This leads to the accumulation of Ca\(^{2+}\) as manifested as an increase of the diastolic [Ca\(^{2+}\)]\(_i\) (Fig. 2).

Mechanism of potentiation of stretch-induced ANP secretion by acidosis. ANP secretion is regulated by factors increasing either atrial stretch or the rate of contraction (37). Each of these factors can be linked to the changes in the [Ca\(^{2+}\)]\(_i\). The role of Ca\(^{2+}\) in ANP secretion has been studied in different animal models by measuring ANP secretion and simultaneously manipulating [Ca\(^{2+}\)]. In isolated, spontaneously beating rat hearts the Ca\(^{2+}\) ionophore A-23187 induces ANP secretion (40). ANP secretion can also be induced by BAY K 8644, a substance that can directly activate L-type Ca\(^{2+}\) channels in isolated beating hearts (39), paced atria (42), and isolated myocytes (31). Supporting this, acute elevation of the extracellular Ca\(^{2+}\)
concentration alone is able to induce ANP secretion (50) and potentiate the stretch-induced ANP secretion (23). Inhibition of the voltage-activated Ca\(^{2+}\) channels with nifedipine or verapamil inhibits ANP secretion (17). Ryanodine reduces the basal ANP secretion (20) and inhibits the stretch-induced ANP secretion (24), indicating that sarcoplasmic reticulum may have a role in the secretion process. In addition to this, several Ca\(^{2+}\)-dependent enzymatic cascades are thought to be involved in the ANP secretion. The possible role of protein kinase C has been studied by activating protein kinase C by phorbol esters. It has been shown that phorbol esters stimulate ANP release from cultured neonatal atrial (19, 31, 44) or adult (16) myocytes. In neonatal atrial myocytes, phorbol esters have a synergistic effect with ionomycin (31) and BAY K 8644 (19). This suggests that a Ca\(^{2+}\)-activated protein kinase C activation is involved in ANP secretion.

Stretch of the cardiac muscle as such increases the amplitude of the Ca\(^{2+}\) transients (1). From our results the stretch-induced ANP secretion may be sensitive to the increase of the systolic [Ca\(^{2+}\)], because Ca\(^{2+}\) transients were increased on stretch at the same time when the ANP secretion was augmented. During the cooccurrence of stretch and acidosis, the diastolic [Ca\(^{2+}\)] was also increased, in addition to augmentation of Ca\(^{2+}\) transients. The most evident mechanism for this would be the inhibition of the Ca\(^{2+}\) binding sites by H\(^+\) decreasing the Ca\(^{2+}\)-buffering power, leading to elevated systolic and diastolic [Ca\(^{2+}\)]. Potentiation of the stretch-induced ANP secretion by acidosis is likely to be caused by the increase of diastolic [Ca\(^{2+}\)], indicating that the effects of diastolic and systolic [Ca\(^{2+}\)] on the ANP secretion are additive. This kind of modulation of the exocytotic processes is typical of the described low-affinity Ca\(^{2+}\)-dependent mechanisms, where exocytosis is controlled by the time integral of [Ca\(^{2+}\)] (47). It is, however, interesting to note that among the Ca\(^{2+}\)-dependent processes of the cardiac myocytes, ANP secretion is far more tolerant of acidic shifts of pH than the contractile element, suggesting a different pH optimum of the exocytotic process.

Effect of acidosis and stretch on contraction of rat atria. The stretch-induced contractile changes in the heart muscle are manifested as an increase in the contraction force, the well-known Frank-Starling mechanism. The Ca\(^{2+}\) binding part of the contractile machinery, troponin C (TnC) (34), is known to be sensitive to muscle length (4, 14). Mechanical stimulation can also influence the Ca\(^{2+}\) balance of the myocytes. The length increase produces a gradual increase in Ca\(^{2+}\) transient amplitude (1) as also shown in this study. The mechanisms of these changes have not been described in detail. It is known that the stretch-dependent change in the cardiac contraction force has two components. Immediately after stretch, the contraction force is increased. This is followed by the additional slow increase in force. The stretch sensitivity of the TnC can explain the fast increase in contraction force, causing additional buffering of Ca\(^{2+}\) by the contractile element (22, 41) and the subsequent, rapid increase in contraction force. Previously, it has been suggested that myofilament sensitivity does not change during the slow increase of the contraction force after stretch (15). This leads to a conclusion that the slow part of the contraction development is due to augmentation of the Ca\(^{2+}\) transients.

The reduction of the contraction force by acidosis (33) is caused by competition between H\(^+\) and Ca\(^{2+}\) of Ca\(^{2+}\) binding sites in the contractile element (5, 36). It is thus not surprising that acidosis inhibited the fast part of contraction development during stretch (Fig. 3B) but had much less effect on the slow development of force (Fig. 3A). Therefore, the effects of acidosis on the stretch-induced contraction changes are mostly caused by H\(^+\) interactions with the contractile element with little or no effect on the mechanisms that cause the increase in the Ca\(^{2+}\) transients and the time-dependent changes in the contraction during stretch.

Possible pathological and clinical implications. Considering that control of the myocardium by stretch is a fundamental principle of heart function, the finding that even small changes in pH, modify this has wide implications. The intracellular acidification causes partial uncoupling of the control exercised by stretch, as evidenced by the slow development of the increase of the force, but the stretch-induced secretion of ANP is retained and even increased. Thus increased ANP secretion is an indication of dysfunction of the EC coupling. The uncoupling of the mechanotransduction and the concomitant increase in ANP secretion during intracellular acidosis emphasize the role of the natriuretic peptides secreted from the heart as markers of contractile dysfunction as suggested previously (30, 32). In addition to the atrial ANP secretion, this mechanism may also contribute to the ventricular ANP secretion during cardiac dysfunction (37) and thus the high plasma levels of these hormones seen after cardiac infarction (21, 35) and in heart failure (8).

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