Atrial Natriuretic Peptide Has Different Effects on Contractility and Intracellular pH in Normal and Hypertrophied Myocytes From Pressure-Overloaded Hearts

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Background—Atrial natriuretic peptide (ANP) depresses contractility in left ventricular myocytes. Its expression is upregulated in pressure-overloaded hypertrophied hearts; however, the effects of ANP on contractility in hypertrophied myocytes are not known. Our aims were (1) to examine the cellular mechanisms of this depression in contractility in normal myocytes and (2) to test the hypothesis that the effects of ANP on contractility differ in hypertrophied myocytes from rats with ascending aortic stenosis.

Methods and Results—We measured the myocyte shortening as an index of contractility, [Ca\(^{2+}\)]\(_i\), with fluo 3, and pH\(_i\) with seminaphthorhodafluor-1 (SNARF-1). In normal control myocytes (n=26), ANP caused a concentration-dependent depression of contractility and reduction in pH\(_i\). In the presence of 10\(^{-6}\) mol/L ANP, fractional cell shortening was 78±5% of baseline (P<0.05) and pH\(_i\) was reduced by 0.16±0.04 U from baseline (P<0.01) without changes in [Ca\(^{2+}\)]\(_i\). The magnitude of the depression of contractation caused by ANP was similar to that caused by intracellular acidification induced by an NH\(_4\)Cl pulse. The effects of ANP on contractility and pH\(_i\) were prevented in the presence of 5-(N-ethyl-N-isopropyl)-amiloride (EIPA), which inhibits the Na\(^+/H^+\) exchanger. In hypertrophied myocytes (n=23), ANP did not depress either myocyte contractility or pH\(_i\) at concentrations of either 10\(^{-8}\), 10\(^{-7}\), or 10\(^{-6}\) mol/L. ANP caused no change in pH\(_i\) or the [Ca\(^{2+}\)]\(_i\), transient in hypertrophied myocytes. The cGMP level was increased and Na\(^+/H^+\) exchanger mRNA levels were normal in left ventricles from aortic stenosis rats compared with controls.

Conclusions—ANP directly depresses contractility in normal myocytes via intracellular acidification, which decreases myofilament [Ca\(^{2+}\)]\(_i\) sensitivity. In contrast, ANP causes no effects on contractility and pH\(_i\) in hypertrophied myocytes, suggesting a suppression in the coupling of the ANP-cGMP intracellular signaling pathway to the Na\(^+/H^+\) exchanger. (Circulation. 1998;98:2760-2764.)

Key Words: atrial natriuretic factor hypertension myocytes contractility calcium

Atrial natriuretic peptide (ANP) maintains circulatory homeostasis and changes myocardial performance by modulating cardiac preload and afterload via diuresis or natriuresis, vasodilatation, and suppression of the autonomic pressure response.\(^1\) In addition to these indirect effects, ANP directly depresses myocardial contraction, which is mediated by the second messenger cGMP.\(^2,3\) However, the underlying mechanism of modification of myocyte excitation-contraction coupling is controversial.

ANP is expressed in the myocardium during fetal heart development\(^4\) and reexpressed in the left ventricle (LV) in response to pressure overload in adult hypertrophied hearts.\(^5,6\) However, the effect of ANP on myocardial function in hypertrophied myocytes characterized by chronic basal up-regulation of ANP synthesis has not yet been examined. Our purposes in this study were (1) to identify the mechanism of depression of contraction by ANP in normal myocytes and (2) to examine the effects of ANP on hypertrophied myocytes from rats with chronic aortic stenosis, in which we have demonstrated upregulation of LV ANP in the development of hypertrophy.\(^6\) Because ANP could potentially affect contractility by changing [Ca\(^{2+}\)]\(_i\), homeostasis or by changing intracellular pH to alter the myofilament sensitivity to [Ca\(^{2+}\)].\(^7,8\) we measured pH\(_i\) and [Ca\(^{2+}\)]\(_i\), in isolated control and hypertrophied myocytes challenged by ANP. In this study, we determined that ANP depressed myocyte contractility by promoting intracellular acidification in normal myocytes; in contrast, ANP failed to depress either contractility or pH\(_i\) in hypertrophied myocytes.

Methods

Weanling male Wistar rats (Charles River Breeding Laboratories, Raleigh, NC) underwent ascending aortic banding (n=12) to make...
an aortic stenosis or a sham operation (n=24) at the age of 3 to 4 weeks. We have previously shown that this aortic stenosis rat model is characterized by concentric LV hypertrophy, the absence of chamber dilatation, and preserved ejection fraction.8,9,10 At 9 to 11 weeks after the surgery, after closed-chest LV pressure was recorded, the animals were euthanized and LV myocytes were prepared with collagenase perfusion by a method described previously.11-14 Myocyte [Ca2+]i was measured with fluo 3 (Molecular Probes, Inc) with an excitation wavelength of 540 nm and emission signal detected at 580 and 640 nm.12,13 The pH i for each cell was calibrated in situ by exposing cells to solutions of varying pH elsewhere6 and used a probe of cDNA fragment encoding the Na+/H+ exchanger provided by Dr John Orlowski. The message levels were normalized to those of GAPDH.

To identify potential biochemical changes in ANP signaling, cGMP levels and Na+/H+ exchanger mRNA levels were measured in LV’s from additional control (n=12) and aortic stenosis rats (n=14). The LV tissues were frozen in liquid nitrogen and homogenized, and cGMP was measured by enzyme immunoassay with a commercially available kit (Amersham Life Science). The methods of total RNA extraction and Northern blot analysis were described in detail elsewhere6 and used a probe of cDNA fragment encoding the Na+/H+ exchanger provided by Dr John Orlowski. The message levels were normalized to those of GAPDH.

Two-way ANOVA with repeated measures was used to compare the values measured in response to exposure to ANP for control and hypertrophied myocytes. An unpaired Student’s t test was used for comparisons of body and LV weights, in vivo LV pressure, and baseline myocyte characteristics between the groups. A probability value of P<0.05 was considered significant. Results are expressed as mean±SEM.

Results

The in vivo parameters of LV function and baseline function of LV myocytes are shown in the Table. The LV weight and ratio of LV weight to body weight were significantly increased in aortic stenosis rats. LV systolic pressure and LV developed pressure per unit mass were significantly increased in aortic stenosis rats at this stage of compensatory hypertrophy compared with controls. Diastolic myocyte length was slightly increased in hypertrophied myocytes compared with controls. Consistent with the in vivo LV performance indices, fractional cell shortening was increased in the hypertrophied myocytes. The systolic and diastolic [Ca2+]i, values and pH i at baseline were similar in hypertrophied and control myocytes. In response to 10-6 mol/L ANP, myocyte fractional shorte-
ing was 78±5% of baseline (P<0.05). The effects of ANP on pH<i> were shown at right, demonstrating a concentration-dependent reduction in pH<i>. In the presence of 10⁻⁶ mol/L ANP, pH<i> was reduced by −0.16±0.04 U compared with baseline (P<0.01). There was no effect of 10⁻⁶ mol/L ANP on peak systolic [Ca²⁺], (393±24 versus 346±15 nmol/L, P=NS) or diastolic [Ca²⁺], (112±11 versus 137±14 nmol/L, P=NS) compared with baseline. To determine whether the acute intracellular acidification was sufficient to explain the magnitude of depression of contraction by ANP, we examined the relationship between pH<i> and fractional shortening in comparison with this relationship in myocytes in response to an NH₄Cl pulse, as we recently reported. Our data with 10⁻⁶ mol/L ANP challenge were similar to the relationships between pH<i> and fractional shortening during acidification caused by NH₄Cl in normal myocytes (Figure 2). This indicates that the depression of contraction by ANP is similar in magnitude to the depression of contractility when acute intracellular acidification is induced by the differing mechanism of an NH₄Cl challenge. In the presence of blockade of Na⁺/H⁺ exchange by 10⁻⁵ mol/L EIPA, 10⁻⁶ mol/L ANP failed to cause either depression of contraction (94±1% of baseline, P=NS) or intracellular acidification (Δ0.01±0.02 U, P=NS) in control myocytes.

In contrast with the effects of ANP in normal myocytes, ANP failed to cause either a depression of myocyte fractional shortening or intracellular acidification in hypertrophied myocytes at any concentration examined (fractional shortening, % of baseline: 10⁻⁸ mol/L, 96±5%, P=NS; 10⁻⁷ mol/L, 97±9%, P=NS; and 10⁻⁶ mol/L, 101±10%, P=NS). As summarized in Figure 3, with ANP 10⁻⁶ mol/L challenge, intracellular pH was also not depressed in hypertrophied myocytes. There was no effect of 10⁻⁶ mol/L ANP on either peak systolic or diastolic [Ca²⁺], in the hypertrophied myocytes.

cGMP level was significantly higher in LVs from aortic stenosis rats (n=9) than in control rats (n=7) (411.9±86.4 versus 763.1±115.1 pmol/g protein, P<0.05), indicating enhanced steady-state production of LV cGMP, at least partly via upregulated ANP. The mRNA level of Na⁺/H⁺ exchanger was similar in LVs of rats from the control (n=5) and the aortic stenosis (n=5) rats (1.0±0.3 versus 0.5±0.1 densitometric units, P=NS).

Discussion

The immediate administration of ANP has been reported to depress contractility of freshly isolated myocytes and ven-
tricular papillary muscle. Our finding of the concentration-dependent negative inotropic effects of ANP examined in normal myocytes is consistent with these studies. The concentration of ANP examined in our study was higher than the steady-state concentration in normal rat and human plasma. In some previous studies of isolated myocytes and cultured myocytes, the ANP concentrations that displayed negative inotropic effects and induced maximum cGMP levels in myocyte suspension were also higher and similar to the ANP levels found in our study. In addition, ANP concentration adjacent to myocytes in vivo may be markedly higher in the presence of local tissue autocrine and paracrine release.

The effects of ANP on intracellular Ca\(^{2+}\) are controversial. We observed that ANP has no effect on net Ca\(^{2+}\) homeostasis, as reported by the [Ca\(^{2+}\)]\(_i\) transient in contracting normal adult myocytes. In contrast, we found that the acute depression of contractility by ANP is mediated in part by intracellular acidification. Intracellular acidification is well established as a mechanism that depresses myofilament sensitivity to [Ca\(^{2+}\)]\(_i\), and causes reductions in tension development and contraction amplitude. We previously reported that nitric oxide donors and 8-bromo-cGMP (cGMP analogue) cause both depression of myocyte contractility and intracellular acidification by disabling forward Na\(^+/H^+\) exchange. Because cGMP has been shown to be the major second messenger of ANP, our data are consistent with the notion that ANP directly causes intracellular acidification via cGMP signaling by disabling forward Na\(^+/H^+\) exchange. Our observations of the acute effects of ANP on contractility and pH do not exclude the potential of other intracellular effects mediating changes in contractility, particularly during long-term exposure.

In this study, we also make the novel observation that ANP does not modify myocyte contractility or promote intracellular acidification in hypertrophied myocytes, in contrast to normal myocytes. Our data and findings of others in the kidney and vasculature suggest that expected physiological responses to ANP are blunted or abolished in the presence of chronic upregulation of local tissue or plasma ANP. In this study, we observed that the cGMP levels are upregulated in hypertrophied LV, which is consistent with previous observations in mechanically overloaded hypertrophied heart models. In our previous study, we showed in hypertrophied myocytes that the direct challenge of 8-bromo-cGMP did not cause either the depression of contraction or intracellular acidification that was observed in normal myocytes. Conversely, we have shown that the basal Na\(^+/H^+\) exchanger activity measured by the recovery from NH\(_4\)Cl-induced intracellular acidosis is similar in control and hypertrophied myocytes. In the present study, we showed that expression of Na\(^+/H^+\) exchanger expression and activity are preserved in compensated hypertrophy, whereas the downstream coupling of cGMP to Na\(^+/H^+\) exchanger is altered in hypertrophied myocytes in the presence of chronic elevation of LV cGMP levels. The mechanism of absence of ANP effects on hypertrophied myocytes might be the inhibition of Na\(^+/H^+\) exchanger activity by phosphorylation of its associated protein by cGMP-activated protein kinase G or suppressed activity of protein kinase G by its translocation. Further studies are needed to clarify the downstream exchange regulation by cGMP using in vitro cell models and to examine the interplay with other signaling pathways.

Both ANP and brain natriuretic peptide (BNP) are currently under investigation as therapeutic agents for the treatment of patients with heart failure. A major concern is the potential for stimulation of the ANP-cGMP pathway to further depress LV contractility. Our data suggest that the capacity for ANP to acutely depress contractility is blunted or absent in myocytes with pathological hypertrophy. In addition, both ANP and nitric oxide have the potential to negatively modulate pathological growth of myocardi and smooth muscle cells and to compete with the effects of angiotensin II and other growth factors whose actions are mediated in part by stimulation of Na\(^+/H^+\) exchange and intracellular alkalinization. The present study suggests that this physiological pH-mediated regulation of growth by ANP and other cGMP signaling molecules may be altered in pathological hypertrophy.

In summary, we report that intracellular acidification contributes to the immediate negative inotropic effects of ANP in normal adult myocytes. This effect of ANP on intracellular pH regulation and contractility is absent in hypertrophied myocytes. Further study is needed to elucidate the molecular regulation of ANP induction and its signaling in pathological hypertrophy.

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References


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