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cAMP- and cGMP-independent stretch-induced changes in the contraction of rat atrium

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Abstract The stretch-induced changes in contraction force, cAMP and cGMP in isolated rat left atrium were studied. Increasing the diastolic intra-atrial pressure from 1 cmH₂O to 8 cmH₂O caused an immediate (<500 ms) increase in contraction force, the magnitude of which was 2.24 ± 0.29 ($n=6$) times the force elicited by 1 cmH₂O. This was followed by a slower, gradual increase of the force, which was maximal 8 min after the stretch (4.33 ± 0.31 , $n=6$). These phenomena were not accompanied by changes in the cAMP ($n=24$) or cGMP ($n=24$) concentrations within the tissue at any duration of stretch tested (2, 8, 20 and 36 min, $n=6$ at each time point). Furthermore, it was estimated that if the β -adrenergic receptor agonist isoprenaline (100 nM) was used to produce an increase of the contraction force of the same magnitude as that induced by stretch, the cAMP concentration was greater (4.20 ± 0.29 pmol/mg, $n=5$, $P < 0.001$) when compared to that produced after 20 min of stretch (2.69 ± 0.12 pmol/mg, $n=6$). Even without significantly changing the cGMP concentration, isoprenaline significantly increased the [cAMP]/[cGMP] ratio (3.4 ± 0.36 , $n=5$, $P < 0.01$) compared to stretch (1.95 ± 0.14 , $n=6$). This result shows that in the rat atrium stretch does not regulate the production or breakdown of cyclic nucleotides (cAMP or cGMP). Thus it seems very unlikely that the effects of stretch on rat atrium function are caused by cAMP or cGMP.

Keywords Atrium · cAMP · Cardiac · cGMP · Contraction · Stretch

Introduction

Cardiac tissue responds to acute stretch by rapidly increasing its contraction force, after which there is a secondary slow increase [6]. The rapid increase appears to be brought about through increased affinity of the contractile element for calcium. The mechanisms responsible for the later, slow increase of contractile force have yet to be clarified, but involve the augmentation of systolic calcium transients [5, 8]. Underlying phenomena could involve the activation of second messenger cascades. One obvious possibility is that stretch stimulates adenylyl cyclase. The resulting liberation of cAMP would in turn activate protein kinase A (PKA), which could both increase the influx of calcium through L-type calcium channels and accelerate calcium pumping into the sarcoplasmic reticulum (SR) by phosphorylating phospholamban. This would also account for the fact that the SR calcium content increases upon stretch [1]. Stretch increases the amount of cAMP in blood-perfused canine hearts [9] and isolated ferret papillary muscle [2]. The co-occurrence of slowly developing force and accumulation of cAMP is evident in some cardiac preparations, but it remains unclear whether there is a causal link between the two. This study was designed to investigate whether the cAMP causes the slow increase of force after stretch and whether modulation of the cGMP concentration interacts with cAMP during a stretch.

Materials and methods

Male Sprague-Dawley rats weighing 290–400 g were used. The rats were decapitated, and the hearts rapidly removed and placed in oxygenated (approx. 25°C) buffer solution (113.8 mM NaCl, 17.6 mM NaHCO₃, 4.7 mM KCl, 1.2 mM KH₂PO₄, 1.1 mM MgSO₄·6H₂O, 2.5 mM CaCl₂, 11.0 glucose, pH 7.4), which was also used at 37°C for superfusing the atrium. The experimental

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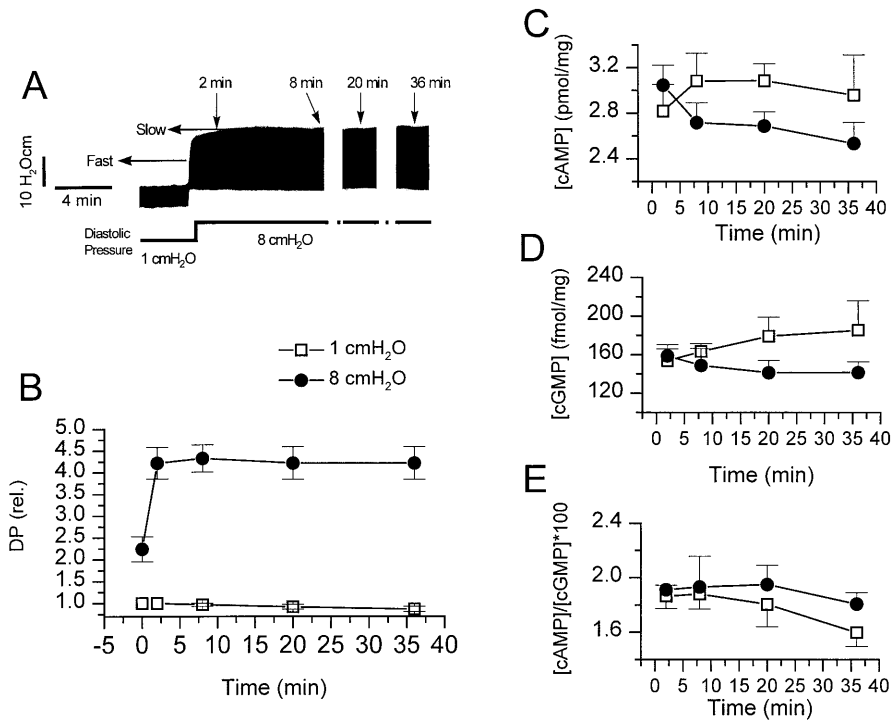


Fig. 1A–E Experimental design and stretch-induced changes in the rat atrium. **A** Intra-atrial pressure trace showing the typical development of contraction when intra-atrial diastolic pressure is increased from 1 cmH₂O to 8 cmH₂O. Arrows indicate the corresponding fast (first contraction pulse after onset of the stretch) and slow (increase of the force thereafter) phases of contraction development. The atria were detached and frozen for cAMP and cGMP content assay at different times indicated by arrows (2, 8, 20 and 36 min after onset of stretch). **B** Relative stretch-induced changes in the contraction force of rat atrium. The developed pressure (*DP*, as an indicator of the force) is expressed relative to the force measured prior to stretch. The *first point* represents the fast part of contraction development (i.e. 2.25 times the contraction prior to stretch). *Open squares* indicate the non-stretched control. **C** cAMP content at different times during a sustained stretch compared to control. **D** cGMP content at different times during a sustained stretch compared to control. **E** Ratio [cAMP]/[cGMP]·100 calculated from the data in **C** and **D**

model used in this study was the isolated rat atrial appendix, a thin-walled (wall thickness <0.35 mm) sack-like structure, prepared as described previously [8]. In brief, an X-branch polyethylene adapter was inserted into the lumen of the left atrium appendix, and the tissue was placed in a thermoregulated (37°C) organ bath. Another tube with a smaller diameter was inserted inside the adapter, in order to carry the perfusate inflow into the lumen of the atrium. The outflow from the lumen comes from one cross-branch of the X-cannula. The tissue was stretched by increasing the intra-atrial pressure. The other cross-branch of the X-cannula was connected to a pressure transducer (MP-15, Micron Instruments), so that the pressure in the lumen of the atrium could be recorded. Inflow and outflow (3 ml/min) both to the atrial lumen and to the organ bath were controlled by a peristaltic pump (7553–85, Cole-Parmer Instrument, USA) and the temperature was regulated. The contraction force (pressure generated by the contraction) was recorded throughout all experiments. The pacing frequency was 2 Hz throughout.

Before each experiment the atria were perfused in 1 cmH₂O for 30 min to equalize the conditions for individual tissues. To study the effects of stretch on contraction, as well as cAMP and

cGMP concentrations, the atria were stretched by increasing the diastolic intra-atrial pressure from 1 cmH₂O to 8 cmH₂O. This increase of diastolic pressure corresponds to a change from 10% to 80% of the length–force relationship of the rat atrium. The atria were then rapidly (<5 s) removed from the perfusion tube and frozen with liquid nitrogen at 2, 8, 20 or 36 min after the onset of the stretch. In control measurements the atria were perfused at 1 cmH₂O throughout the experiments and the tissues were frozen at identical times. The isoprenaline concentration was adjusted to produce an inotropic response corresponding to the slow development of force after stretch. In these experiments the atria were collected for the assay of cAMP and cGMP contents 15 min after isoprenaline application.

All tissue samples obtained from the experiments were stored at –70°C until assayed. The atria were homogenized with 1 ml of 6% trichloroacetic acid at 4°C, followed by centrifugation at 2000 g for 15 min. The supernatants obtained were collected and washed with 1 ml of water-saturated diethyl ether four times. The extracts were lyophilized and processed for the measurement of cAMP and cGMP content using a standard radioimmunoassay kit supplied by Amersham International. The quantities of cAMP and cGMP within the atria were quantified from a standardization curve generated using cAMP and cGMP standards supplied with the kit. Sample dilution was adjusted to obtain values within the linear scale of the standard curve. The sensitivity of the cAMP kit, defined as the amount of cAMP needed to reduce the zero dose binding by two SDs, is 1 fmole and the sensitivity of the cGMP kit is 0.5 fmole. In both cases the ratio between sensitivity and total concentration in the sample was more than 10,000. The cross-reactivity of the cAMP assay for cGMP is 0.0004%, and that of the cGMP assay for cAMP <0.001%. According to the manufacturer the reproducibility expressed as a coefficient of variation for these kits is approximately 6%. The cAMP concentration is expressed as pmol/mg of wet tissue and that of cGMP as fmol/mg wet tissue.

The values obtained from measurements of contraction force, cAMP and cGMP were tested using two-way analysis of variance followed by pairwise multiple comparison procedures (Student–Newman–Keuls method). *P*-values below 0.05 were regarded as statistically significant. All values are expressed as means ±SEM.

Results

Figure 1A shows typical stretch-induced changes in the contraction force of rat atria when the intra-atrial diastolic pressure was increased from 1 cmH₂O to 8 cmH₂O. Also shown are the normalized developed pressure (Fig. 1B), [cAMP] (Fig. 1C), [cGMP] (Fig. 1D) and the ratio [cAMP]/[cGMP] in stretched and non-stretched atria (control) (Fig. 1E). From the intra-atrial pressure curve two phases of contraction development can be elucidated. The fast increase is the change in contraction prior to the stretch to the first contraction pulse after the stretch. The slow development of contraction can be seen thereafter. The atria were able to maintain the contraction force unaltered throughout the 36 min of continuous stretch, since the contraction force did not reduce in a time-dependent way (Fig. 1B, $n=6$). Similarly, the contraction of control atria (non-stretched) did not change for 36 min (Fig. 1B, $n=6$). For determination of [cAMP] and [cGMP], different atria were frozen at different times during the stretch (2, 8, 20 and 36 min). Stretch did not alter [cAMP] at any time during stretch when compared to non-stretched tissue (Fig. 1C). The overall changes in [cAMP] during 36 min of stretch were not statistically significant ($n=24+24$). The functional modulation of cardiac function by cyclic nucleotides does not rely solely on cAMP, since most of cAMP's effects are antagonized by cGMP. In theory, cGMP could mask the stretch-induced changes in [cAMP] by increasing cAMP degradation through stimulation of cGMP-stimulated phosphodiesterase (PDE-II) [3]. Because of this, we also measured [cGMP] in the same atria as used in [cAMP] determination, and calculated the corresponding [cAMP]/[cGMP] ratio. As can be seen from Fig. 1D, [cGMP] is not significantly different in stretched tissues compared to control ($n=6$). When the overall changes in [cGMP] were compared, no significant changes between stretched and non-stretched tissues were observed ($n=24+24$). The changes in [cGMP] were not sufficient to change the [cAMP]/[cGMP] ratio at any given time. In addition, there was no change in the overall ratio during stretch.

To validate the methods used to measure cyclic nucleotide concentrations, we performed a "biological calibration" with isoprenaline. We also wanted to find out the concentration of intracellular cAMP that would be sufficient to cause a similar augmentation of contraction as seen during the slow development of force following rat atrial stretch. In preliminary experiments it was first found that 100 nM of the specific β -adrenergic receptor agonist isoprenaline increased contraction to the same extent as during the slow phase of the stretch response (Fig. 2A). The isoprenaline-treated atria were frozen after 15 min of exposure and used for [cAMP] and [cGMP] measurements. The isoprenaline-induced changes were compared with changes caused by 20 min of continuous stretch. As expected, β -adrenoceptor stimulation substantially increased the rat atrial cAMP content (4.20 ± 0.29 pmol/mg, $n=5$, $P < 0.001$). This is much great-

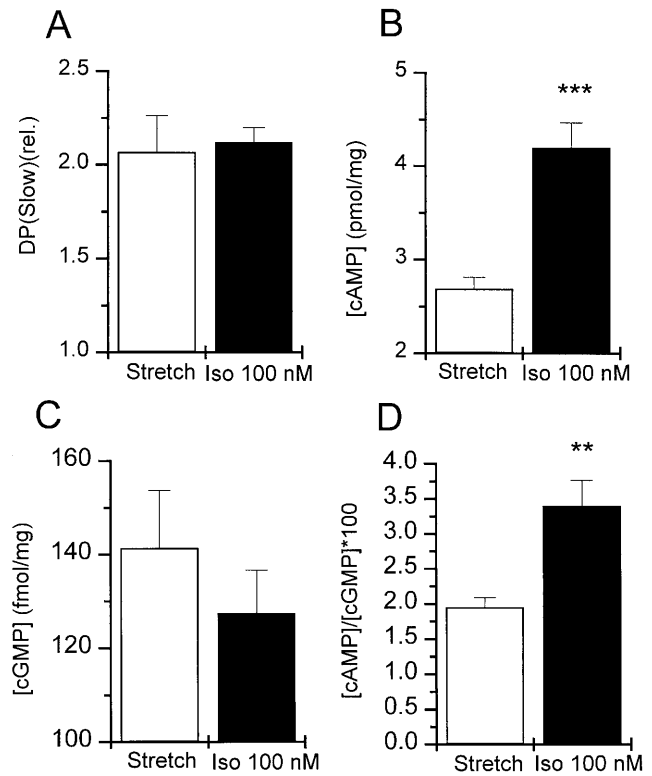


Fig. 2A–D Comparison of the inotropic effect of stretch and isoprenaline (100 nM) on the rat atrium. **A** Effect of 20 min of sustained stretch (intra-atrial pressure 8 cmH₂O, $n=6$) and 15 min of perfusion with isoprenaline-containing medium (100 nM, $n=5$) on the contraction force of rat atrium. **B** cAMP content in the rat atria exposed to either stretch ($n=6$) or isoprenaline ($n=5$). **C** cGMP content in the rat atria exposed to either stretch ($n=6$) or isoprenaline ($n=5$). **D** [cAMP]/[cGMP] ratio calculated from the data in **B** and **C**

er than that elicited by 20 min of stretch (2.69 ± 0.12 pmol/mg, $n=6$, Fig. 2B). Even without a significant change in [cGMP] (Fig. 2C), isoprenaline application also caused a significant increase in the [cAMP]/[cGMP] ratio (3.4 ± 0.36 , $n=5$, $P < 0.01$) when compared to the effect of stretch alone (1.95 ± 0.14 , $n=6$, Fig. 2D).

Discussion

The fundamental changes in cardiac muscle contraction induced by stretch include a biphasic increase in force of contraction, as demonstrated by this study and others [5, 8]. The stretch-induced sensitization of the contractile elements to calcium appears to be responsible for the fast part of the contraction development induced by stretch [4, 5]. This idea is supported by the fact that during the fast part of this phenomenon, the contraction force is augmented without a detectable change in the amplitude of the calcium transient [5]. In contrast, during the slow phase the contraction is enhanced solely by a gradual increase in the amplitude of the calcium transient [4, 5, 8]. Several hypotheses have been formulated to explain this

change in systolic calcium. One of the most appealing is the concept whereby stretch directly or indirectly generates the formation of cAMP. To support this, stretch has been reported to increase the cAMP content in blood-perfused canine heart [9], ferret papillary muscle [2] and in frog ventricle [7]. Accumulation of cAMP leads to the activation of protein kinase A (PKA), which in turn phosphorylates some essential proteins involved in calcium handling. The PKA-induced phosphorylation of L-type calcium channels would increase the channel open probability, thus increasing calcium influx during action potentials. By phosphorylating the phospholamban that regulates the SR calcium pump, calcium pumping into the SR is accelerated, leading to an increased SR calcium load, which is manifested as bigger calcium transients. It has been shown that the calcium content increases slowly after stretch in rabbit papillary muscle, but that this change in SR calcium content does not mediate the slow response phase [1]. In support of this, it was shown recently that the stretch-induced changes in cAMP content are not large enough to enhance phospholamban phosphorylation in ferret papillary muscle [2]. This means that the remaining potential source of additional calcium during stretch may be L-type calcium channels. If stretch causes cAMP accumulation and subsequent calcium channel phosphorylation, the increase in L-type channel current would precede the slow changes in calcium. Challenging this idea, it has been shown that stretch does not induce any L-type calcium current changes in isolated rat ventricular myocytes [4]. It is also noteworthy that one of the phosphorylation targets of PKA is the troponin I molecule of the thin filament in the contractile element [10]. This phosphorylation decreases the calcium sensitivity of the myofilaments, suppressing the contraction force. Thus, when stretch increases the cAMP content during the slow phase of force development, a reduction of calcium sensitivity following force development would ensue. This argues against the finding that stretch increases the calcium sensitivity of the contractile elements at the beginning of stretch without changing the sensitivity during the slow development of force in rat ventricular trabeculae [5].

In the present study we found that in the rat atria [cAMP] did not change during the slow changes in contraction induced by stretch. This finding excludes the possibility that cAMP is responsible for these slow changes in the rat atrium. In addition, we evaluated that the isoprenaline concentration required to increase contraction to a similar extent as stretch increased the tissue [cAMP] by 61%. This shows that the method used in this study is able and sensitive enough to detect physiological changes in [cAMP]. As cAMP accumulation during stretch is evident in other studies [2,9], we have to consider possible reasons for the conflicting results of the present study. One possibility is the difference between atrial and ventricular tissue. Interestingly, all reports of stretch-induced changes in [cAMP] have been carried out on ventricular tissue: blood-perfused canine heart [9], ferret papillary muscle [2] and frog ventricle [7].

This suggests that the cAMP content of atrial tissue responds to stretch differently from ventricular tissue. The [cAMP] values obtained in the present study (approx. 2.5–3.0 pmol/mg) are slightly greater than those measured in rat ventricle ([9], approx. 1.8–2.6 pmol/mg). The values are not strictly comparable, since atrial and ventricular tissues contain different amounts of non-myocyte cells (endothelial, smooth muscle), which may contain different baseline [cAMP] levels. On the basis of a recent study by Zhang et al. [11], rat atrial myocytes have an abundance of non-selective cation channels, which are sensitive to longitudinal distension of the cell. These channels could serve as a mechanism for generating typical stretch-activated slow contraction changes in rat atrium [8]. If these channels are atrial-specific, the differences in the transduction of stretch between atrial and ventricular myocytes would be obvious. Clearly, more research exploring this question is required.

In conclusion, the stretch-induced slow increase in the force of contraction of rat atrium is not caused by changes in the cyclic nucleotide content in the tissue. In addition, neither cAMP nor cGMP production is sensitive to stretch in rat atrial tissue. This result suggests that control of cyclic nucleotide production in cardiac muscle during stretch is very different in ventricular and atrial tissues.

References

1. Bluhm WF, Lew WYW (1995) Sarcoplasmic reticulum in cardiac length-dependent activation in rabbits. *Am J Physiol* 269:H965–H972
2. Calaghan SC, Colyer J, White E (1999) Cyclic AMP but not phosphorylation of phospholamban contributes to the slow inotropic response to stretch in ferret papillary muscle. *Pflügers Arch* 437:780–782
3. Hartzell HC, Fischmeister R (1986) Opposite effects of cyclic GMP and cyclic AMP on Ca^{2+} current in single heart cells. *Nature* 323:273–275
4. Hongo K, White E, LeGuennec J-Y, Orchard CH (1996) Changes in $[Ca^{2+}]_i$, $[Na^+]_i$ and Ca^{2+} current in isolated rat ventricular myocytes following an increase in cell length. *J Physiol (Lond)* 491:609–619
5. 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
6. Parmley WW, Chuck L (1973) Length-dependent changes in myocardial contractile state. *Am J Physiol* 224:1195–1199
7. Singh J (1982) Stretch stimulates cyclic nucleotide metabolism in the isolated frog ventricle. *Pflügers Arch* 395:162–164
8. Tavi P, Han C, Weckström M (1998) Mechanisms of stretch-induced changes in $[Ca^{2+}]_i$ in rat atrial myocytes: role of increased troponin C affinity and stretch-activated ion channels. *Circ Res* 83:1165–1177
9. Todaka K, Ogino K, Gu A, Burkhoff D (1998) Effects of ventricular stretch on the contractile strength, calcium transient, and cAMP in intact canine hearts. *Am J Physiol* 274:H990–H1000
10. Winegrad S (1984) Regulation of the cardiac contractile proteins. Correlation between physiology and biochemistry. *Circ Res* 55:565–574
11. Zhang YH, Youm JB, Sung HK, Lee SH, Ryu SY, Lee S-H, Ho W-K, Earm YE (2000) Stretch-activated and background non-selective cation channels in rat atrial myocytes. *J Physiol (Lond)* 523:607–619