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Short communication

Stretch-induced increase in atrial natriuretic peptide secretion is blocked by thapsigargin

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Abstract

The cellular mechanisms by which mechanical forces regulate myocardial function such as secretion of atrial natriuretic peptide (ANP), are uncertain. We studied the effects of thapsigargin, a specific inhibitor of sarcoplasmic reticulum Ca^{2+} adenosine triphosphatase, that depletes intracellular Ca^{2+} stores, on basal and atrial stretch-induced ANP secretion in the isolated, perfused, paced rat heart preparation. Addition of 300 nM thapsigargin into the perfusate caused gradual increase in perfusion pressure, contractile force and ANP release ($P < 0.001$). Thapsigargin pretreatment at concentrations (30 and 100 nM) that did not affect baseline cardiac function or hormone secretion blocked mechanical stretch-induced increase in ANP secretion. These results suggest that thapsigargin-sensitive intracellular Ca^{2+} pools serve as mechanotransducers in the mechanical loading-induced changes in cardiac myocytes.

Keywords: Atrial natriuretic peptide; Thapsigargin; Hormone secretion; Mechanical stretch

1. Introduction

A number of different cell types have transduction systems that convert externally applied mechanical forces to signals that regulate cellular function (Watson, 1991). For example, during every myocardial contraction, each cardiac cell shortens against the load imposed by the adjoining cells and develops force. Mechanical loading of myocytes also increases protein synthesis and induces the expression of specific genes (Komuro and Yazaki, 1993) as well as being a potent stimulus for secretion of atrial natriuretic peptide (ANP), a cardiac hormone involved in the regulation of blood pressure and salt and water balance (Ruskoaho, 1992). The cellular mechanisms by which mechanical forces regulate myocardial function are, however, uncertain, although there is evidence that several factors may be involved including stretch-activated ion channels and several protein kinases (Ruskoaho, 1992; Komuro and Yazaki, 1993; Sadoshima and Izumo, 1993).

The possibility that intracellular Ca^{2+} pools play an essential role in mechanotransduction is particularly attractive because Ca^{2+} is fundamentally important for the normal cardiac function. Ca^{2+} is accumulated within pools via members of the intracellular sarcoplasmic/endoplasmic reticulum Ca^{2+} adenosine triphosphatase (ATPase) family of Ca^{2+} pump proteins (Clapham, 1995). These pumps have been shown to be highly sensitive to blockade by the plant sesquiterpene lactone, thapsigargin (Thastrup et al., 1990). In the present study we used stretch-induced ANP secretion as a myocyte-specific marker to examine the role of intracellular Ca^{2+} pools in mechanotransduction in the heart. The modified perfused rat heart preparation (Ruskoaho et al., 1990) that enabled the stepwise distension of the atrial myocytes was used as an experimental model for mechanical load-stimulated ANP secretion.

2. Materials and methods

2.1. Isolated, perfused, rat heart preparation

Male Sprague-Dawley rats (weighing 250–350 g) from the Center for Experimental Animals at the University of Oulu were used. The isolated, perfused, heart preparations used in this study were similar to those previously described (Ruskoaho et al., 1990; Taskinen et al., 1994). The aorta was cannulated superior to the aortic valve and retrograde perfusion was begun with modified Krebs-Henseleit bicarbonate buffer, pH 7.40, equilibrated with

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95% O_2-5% CO_2 at 37°C. Variations in perfusion pressure arising from changes in coronary vascular resistance were recorded on a Grass polygraph (model 7 D; Grass Instruments, Quincy, MA, USA) with pressure transducer (model MP-15; Micron Instruments, Los Angeles, CA, USA) situated on a side arm of the aortic cannula. The isometric force of contraction was recorded by a strain gauge transducer (model FT03; Grass Instruments) connected to the Grass polygraph. The hearts were submitted to a resting tension of 2 g. Heart rate was counted from the contractions by the Grass tachograph and increased 15–20% above the spontaneous beating rate by using a Grass stimulator (model S88, 10 V, 0.5 ms).

The right atrial pressure was recorded on a Grass polygraph via a cannula (PE-60) in the inferior vena cava connected to a pressure transducer (model MP-15). A glass cannula was inserted into the pulmonary artery for the collection of perfusate. The right atrial pressure was kept constant at the desired level by adjusting the level of the pulmonary artery cannula tip (Ruskoaho et al., 1990). During the equilibration period (60 min), the hearts were perfused with a peristaltic pump (Miniplus 3, model 312; Gilson, Villiers, France) at a flow rate of 7 ml/min. In order to confirm the possible vasoconstrictor effects of infused drugs, the vasculature was dilated by decreasing the perfusion rate to 5 ml/min before experiments began (Ruskoaho et al., 1990).

2.2. Experimental design

In the first series of experiments a 10 min control period was followed by the addition of vehicle (less than 0.03% dimethylsulfoxide) or thapsigargin (Calbiochem, San Diego, CA, USA) into the aortic perfusion cannula as a continuous infusion via an infusion pump (Secan PSA 55; Skyelectronics, Grenoble, France) at a rate of 0.5 ml/min for 30 min. In the second series of experiments after a 10 min control period, a continuous infusion of vehicle or thapsigargin was made for 41 min and atrial stretch was superimposed for 10 min after 25 min drug infusion by elevating the level of the pulmonary artery cannula tip. The coronary venous effluents were collected at 1 or 2 min intervals, placed immediately on dry ice and stored at −20°C until assayed. Hearts were only used for one experiment. The experimental protocols were approved by the Committee for Animal Experimentation of the University of Oulu.

2.3. Assay of immunoreactive ANP in perfusate

Immunoreactive ANP was measured from the unextracted perfusate samples by the RIA as described earlier (Vuolteenaho et al., 1985; Ruskoaho et al., 1990). The sensitivity of the assay was 0.3 fmol/tube. The 50% displacement of the standard curve were at 6 fmol/tube. The intra-assay and inter-assay variations were less than 10 and 15%, respectively. Serial dilutions of the perfusate showed parallelism to the synthetic ANP standard.

2.4. Data analysis

The results are expressed as mean ± S.E.M. The data were analyzed with one- or two-way analysis of variance (ANOVA). The statistical significance of the difference between two groups was determined with Student's *t*-test for paired data.

3. Results

3.1. Effects of thapsigargin on ANP secretion and cardiac function in isolated, perfused, paced rat hearts

We first studied the effect of thapsigargin alone on cardiac function to find out the concentration of the drug which would minimally influence myocardial contractility and coronary vasculature. The mean baseline contractile

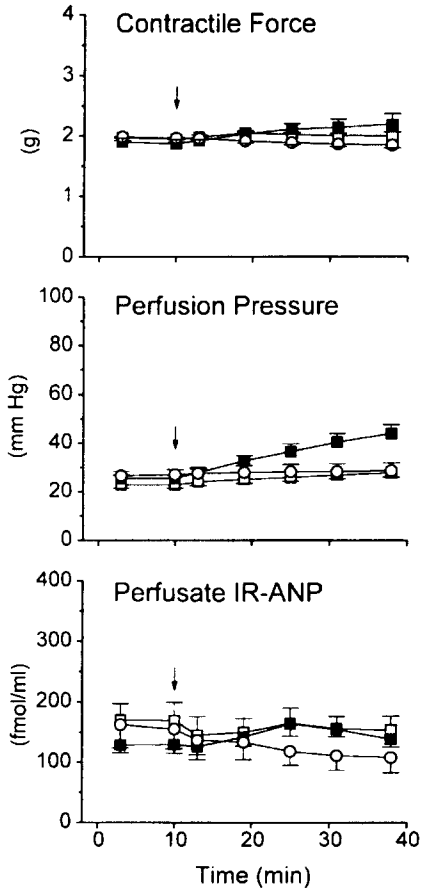

Fig. 1. Effect of thapsigargin on haemodynamics and immunoreactive atrial natriuretic peptide (IR-ANP) release in the isolated, perfused, paced rat hearts. At the time 10 min, as indicated by the arrows, vehicle (○, $n = 8$), thapsigargin 100 (□, $n = 7$) or 300 nM (■, $n = 6$) were added into the perfusion fluid for 30 min. Values are expressed as mean ± S.E.M.

force was 2.0 ± 0.1 g, heart rate was 330 ± 5 beats/min and perfusion pressure 25 ± 1 mm Hg ($n = 21$) before vehicle or drug infusions. As shown in Fig. 1, during vehicle infusion haemodynamic variables remained constant. Addition of 300 nM thapsigargin to the perfusate caused a gradual increase in perfusion pressure (maximally 43 ± 4 mm Hg, $P < 0.001$) and contractile force ($P < 0.001$) when compared to the control group. There was a tendency toward higher perfusion pressure in response to infusion of 100 nM thapsigargin, but this difference was not significant ($F = 2.1$, N.S., Fig. 1). Furthermore, concentrations lower than 100 nM had no effect on haemodynamic variables (see below). At high concentrations (1–10 μM) thapsigargin induced a transient increase in contractile force followed later by myocardial depression (data not shown), as reported earlier by Vigne et al. (1992).

Next we determined whether the same concentrations of thapsigargin that increased contractile force and perfusion pressure, also affect ANP release. The baseline concentration of immonoreactive ANP in the perfusate was 146 ± 13 fmol/ml ($n = 21$) (Fig. 1). Addition of thapsigargin at the concentrations of 300 nM into the perfusate increased immonoreactive ANP release ($P < 0.001$). Instead no difference was observed in the baseline secretion when isolated rat hearts were perfused with 100 nM (Fig. 1) or 30 nM (see below) thapsigargin. The concentrations of 30 and 100 nM thapsigargin were thus chosen for further experiments.

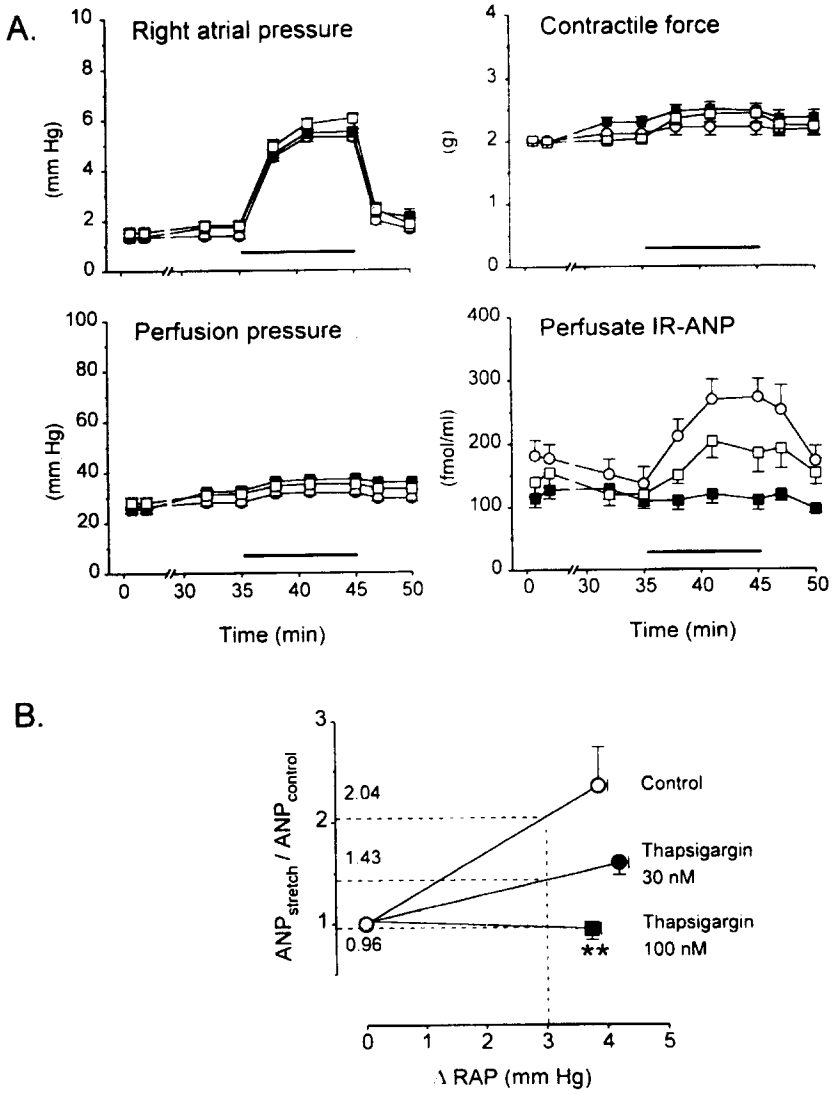

Fig. 2. A: Effect of thapsigargin on stretch-induced release of atrial natriuretic peptide (IR-ANP) in the isolated, perfused, paced rat hearts. At the time 10 min, vehicle or thapsigargin was added into the perfusion fluid for 41 min. The right atrium was distended for 10 min (horizontal lines) by elevating the pulmonary artery cannula tip 25 mm after the start of infusion. Control (○, $n = 10$), thapsigargin 30 nM (□, $n = 7$) and 100 nM (■, $n = 7$). Values are expressed as mean ± S.E.M. B: Effect of thapsigargin on the relation between the change of right atrial pressure (RAP) and changes in ANP secretion in the isolated, perfused, paced rat hearts. ** $P < 0.01$ (Student's t-test, unpaired).

3.2. Stretch-induced ANP secretion

Right atrial pressure was varied by manipulation of atrial afterload by means of adjustment of the cannula leading into the pulmonary artery. When the level of pulmonary artery cannula tip was elevated, right atrial pressure increased immediately (Fig. 2A). Ten minutes of continuous stretch resulted in a marked increase in immunoreactive ANP concentration ($P < 0.001$ between stretch and control group) in the coronary venous effluent of the perfused rat heart (Fig. 2A). To compare the amount of ANP release during stretch, the ratio of immunoreactive ANP released during the atrial stretch to the rate of release of immunoreactive ANP into the perfusate before atrial distension was calculated in each distension experiment. This change in immunoreactive ANP release into the perfusion fluid was then related to increase in right atrial pressure as shown in Fig. 2B. Thus, a 3-mm Hg increase in the right atrial pressure produced 2.05-fold increase in ANP secretion during the vehicle infusion under these experimental conditions. If the pulmonary artery cannula tip was not elevated, haemodynamic variables and perfusate immonoreactive ANP concentration remained stable throughout the experimental period (Fig. 2A).

3.3. Dependence of stretch-induced ANP secretion on thapsigargin-sensitive intracellular Ca^{2+} pools

To examine the role of intracellular Ca^{2+} pools in stretch-activated ANP release, we depleted Ca^{2+} stores by starting the infusion of thapsigargin 25 min prior the elevation of the right atrial pressure. As shown in Fig. 2, the changes in right atrial pressure were similar in vehicle- and thapsigargin-treated perfused hearts during the distension experiments ($n = 24$). On the other, a slight increase in contractile force and perfusion pressure was noted during atrial stretch (Fig. 2A). However, these increases in contractile force (0.1–0.4 g) and perfusion pressure (3–5 mm Hg) were similar in all experimental groups during the distension. Thus, it was possible to separate direct action of thapsigargin on immunoreactive ANP release at the level of the myocyte itself from potential indirect secretory effects caused by chemically induced changes in the rate of contraction frequency and contractile force.

Mechanical stretch during thapsigargin infusion resulted in a significantly smaller increase in the perfusate immunoreactive ANP concentration when compared to the infusion of vehicle alone (Fig. 2A). A significant decrease (32%, $P < 0.05$) in perfusate immunoreactive ANP levels was seen during infusion of 30 nM thapsigargin and addition of 100 nM completely blocked stretch-activated ANP secretion ($P < 0.001$). Furthermore, during thapsigargin infusion, the relationship between changes in immonoreactive ANP and in right atrial pressure shifted dose-dependently to the right. The calculated ANP increase corresponding to the 3-mm Hg increase in the right atrial pressure was 1.43-fold when 30 nM thapsigargin was infused and 0.96-fold when 100 nM thapsigargin was infused (Fig. 2B).

4. Discussion

Mechanical loading can evoke a variety of signals in cardiomyocytes, but the molecules converting the mechanical stress signal into biochemical events that regulate cellular function are unclear. One important signaling mechanism regulating contracting cardiac myocytes involves release of Ca^{2+} from thapsigargin-sensitive intracellular stores. Here we report that thapsigargin completely blocks mechanical stretch-activated secretion of ANP, a myocyte-specific marker of mechanotransduction in cardiac cells. These results also suggest that thapsigargin-sensitive intracellular Ca^{2+} stores may be important in the regulation of other mechanical stretch-induced physiological and pathophysiological alterations in cardiac myocytes.

Changes in cytosolic free Ca^{2+} concentration ($[Ca^{2+}]_i$) constitute an important element of signal transduction in various cells (Clapham, 1995). Sarcoplasmatic reticulum is the major intracellular Ca^{2+} store in cardiac cells. It is sensitive to caffeine and ryanodine and has an important role in excitation-contraction coupling. In addition, recent studies have provided evidence for the presence of an inositol 1,4,5-trisphosphate (IP_3)- and thapsigargin-sensitive intracellular Ca^{2+} pool in atrial cells (Vigne et al., 1992; Negretti et al., 1993; Lewartowski et al., 1994). Vigne et al. (1992) showed that in atrial cells thapsigargin at the concentrations of 0.1–10 μM increased $[Ca^{2+}]_i$ in a manner that was independent of the presence of external Ca^{2+} and of the production of inositol phosphates. In agreement with the observation that thapsigargin rapidly raises $[Ca^{2+}]_i$ in cardiac cells (Vigne et al., 1992) and vascular cells (Thastrup, 1990), we observed that addition of thapsigargin into perfusate at the concentrations above 100 nM increased the contractile force and produced coronary vasoconstriction in the perfused rat heart preparation.

Previous studies concerning cellular calcium concentration and ANP secretion by using spontaneously beating or paced isolated atria or hearts have shown that compounds such as calcium ionophore calcimycin (A23187), L-type calcium channel agonist Bay k8644 (1,4-dihydro-2,6-dimethyl-5-nitro-4-[2-(trifuoromethyl)-phenyl]-3-pyridine) and KCl, known to increase $[Ca^{2+}]_i$, also increase basal ANP secretion while L-type calcium channel blockers and ryanodine, an inhibitor of sarcoplasmatic reticulum calcium channels, decrease secretion (for review see Ruskoaho, 1992). In contrast, when either isolated cells that do not contract or arrested atria and hearts are examined, no effect or even a decrease in ANP secretion has been seen when procedures known to increase intracellular Ca^{2+} are performed (for a recent review, see De Bold et

al., 1996). Our present results showed that thapsigargin increases basal ANP secretion suggesting that mobilization of Ca^{2+} from intracellular stores may be involved in ANP secretory response in isolated paced perfused rat hearts.

Several studies have addressed also the importance of Ca^{2+} in stretch-induced ANP. Ca^{2+} has been shown to negatively modulate ANP secretion from the isolated osmotically stretched atrial myocytes (Greenwald et al., 1988). Stretch-induced ANP release in the isolated rat atrial preparations has been demonstrated to be independent of extracellular calcium and take place even in the presence of chelators of intracellular calcium such as EGTA (ethylene glycol-bis-β-aminoethyl ether)-*N,N,N,N*-tetraacetic acid) and BAPTA (chelator of intracellular calcium, 1,2-bis(2-aminophoxy)ethane-*N,N,N,N*-tetraacetic acid) (De Bold et al., 1996). Similarly, ANP release from isolated rat atria induced by stretching was not inhibited by depolarization with KCl or a low concentration of external Ca^{2+} (Agnoletti et al., 1992). On the other hand, ANP released by stretch has been shown to be partially suppressed in the calcium-depleted heart (Ito et al., 1988) and increased with increasing extracellular Ca^{2+} concentration in the isolated rat atria (Page et al., 1991a). In the isolated perfused heart, low concentrations of Ca^{2+} and nifedipine significantly inhibited left atrial pressure-induced ANP secretion (Katoh et al., 1990). Furthermore, ryanodine inhibits stretch-induced ANP release in isolated rat atria preparations (Kuroski-De Bold and De Bold, 1991; Laine et al., 1994). Thus, experiments by using various Ca^{2+} modulating agents suggest both negative and positive modulation of stretch-stimulated ANP release by $[Ca^{2+}]_i$. Furthermore, even under identical experimental conditions Ca^{2+} may have a dual effect on cellular ANP release by stretch; an initial transient stimulation followed by more marked inhibition was noted as intracellular Ca^{2+} was increased (Page et al., 1991b).

In order to examine the role of intracellular Ca^{2+} pools in mechanotransduction of cardiac myocytes in the present study, we infused low doses of thapsigargin (30 and 100 nM) for 25 min into perfused heart to deplete the thapsigargin-sensitive Ca^{2+} stores. The results show that thapsigargin completely blocked stretch-activated ANP exocytosis from right atrial myocytes. This finding suggests that the stretch-induced increase in ANP secretion is linked to its capacity to mobilize a thapsigargin-sensitive intracellular Ca^{2+} pool. Our present data, together with those from previous studies, also suggest that there is a principal difference between the mechanical stretch- and agonist-induced ANP secretion, because thapsigargin has no influence on ANP secretion stimulated by endothelin-1 in cardiac cell cultures (Doubell and Thibault, 1994). Our data, however, do not exclude the possibility that thapsigargin interferes with ANP secretion through a still unknown mechanism not related to the IP_3-sensitive intracellular Ca^{2+} stores. The observations that both endothelin-1 and mechanical stretch have been shown to increase phosphatidylinositol turnover and IP_3 levels in cardiac myocytes (Von Harsdorf et al., 1989; Vigne et al., 1992; Sadoshima and Izumo, 1993), and that stretch-induced IP_3 elevations may be secondary to the release of substances such as endothelin, would be consistent with that possibility.

In conclusion, thapsigargin at concentrations that did not affect baseline cardiac function or hormone secretion completely blocked mechanical stretch-induced increase in ANP secretion suggesting that loading by a thapsigargin-sensitive Ca^{2+} ATPase may be a primary event to directly stimulate exocytosis from atrial granules. Because of this unique property, thapsigargin may be a very useful tool to further analyze whether thapsigargin-sensitive intracellular Ca^{2+} stores are also important for other mechanical stretch-induced events in the heart including regulation of cardiac gene expression and heart rate as well as the development of cardiac arrythmias and hypertrophy.

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