Block of stretch-activated atrial natriuretic peptide secretion by gadolinium in isolated rat atrium

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1. Isolated superfused rat atrial preparations were used to study the mechanism of stretch-induced atrial natriuretic peptide (ANP) secretion. The stretch of the atrial myocytes was induced by raising the intra-atrial pressure. The secretion rates were analysed by measuring ANP concentrations from the superfusate fractions by radioimmunoassay.
2. The effect of gadolinium, a blocker of stretch-activated ion channels, on stretch-induced and basal ANP secretion was investigated by superfusing the atrial preparation with 5, 20 or 80 μM $GdCl_3$. Gadolinium decreased stretch-induced ANP secretion in a dose-dependent manner, but did not affect basal secretion.
3. Because high concentrations of gadolinium may block voltage-gated calcium channels, we tested whether the selective blockers of L-type (diltiazem) and T-type ($NiCl_2$) calcium channels affect the stretch-stimulated ANP release. Neither diltiazem at 3 μM nor $NiCl_2$ at 50 μM affected stretch-induced ANP release in paced atrial preparation.
4. Gadolinium, but not diltiazem, also inhibited stretch-stimulated ANP secretion in non-paced, quiescent atria.
5. The findings that ANP release is inhibited by Gd^{3+}, but not by diltiazem or $NiCl_2$, and that the stretch-induced secretion in quiescent atria is also inhibited by Gd^{3+}, suggest that stretch-activated ion channels are involved in the regulation of stretch-induced ANP release.

Atrial natriuretic peptide (ANP) is a natriuretic and diuretic peptide hormone secreted by heart atria (DeBold, Borenstein, Veress & Sonnenberg, 1981; for review, see Ruskoaho, 1992). There is much evidence that increased blood pressure or volume loading increases ANP secretion (Lang, Thölken, Ganten, Luft, Ruskoaho & Unger, 1985; Ruskoaho, 1992), and it is known that stretch of myocytes and not pressure *per se* is a direct stimulator of ANP release (Ruskoaho, Thölken & Lang, 1986; Edwards, Zimmerman, Schwab, Heublein & Burnett, 1988). However, the precise cellular mechanisms linking stretch and hormone release are not known. Recently published observations of stretch-activated (SA) ion channels in rat cardiac myocytes (Kim, 1992, 1993) offer an interesting hypothesis for studying stretch–secretion coupling. The activation of these channels in response to an increase in atrial wall tension could transduce pressure signals into intracellular biochemical events that induce exocytosis of ANP.

The stretch-activated ion channels are mechanosensitive in the sense that their open probability depends on mechanical stress at the membrane. The major SA channels are of a type permeable to both monovalent and divalent cations (French, 1992). Following their discovery (Guharay & Sachs, 1984), SA channels have been described in a wide range of animals, plants and prokaryotes. SA channels appear to be a part of the standard membrane machinery of almost every cell, rather than being confined to a few specialized mechanoreceptor cells (Sachs, 1988). SA channels regulate many important physiological mechanisms, including fluid balance and control of membrane potential (Morris, 1990).

A major difficulty in physiological work on mechanotransduction has been the lack of a specific inhibitor of SA channels. Recently, a new promising blocker, gadolinium (Gd^{3+}), has been found. Gadolinium is a trivalent lanthanide that reversibly blocks SA channels at micromolar concentrations in frog oocytes (Yang & Sachs, 1989). It produces a concentration-dependent decrease in open channel current, and a reversible inhibition of channel opening (Yang & Sachs, 1989). Three types of mechanosensitive non-selective cation channels and two types of K^+ channels have been reported in chick cardiomyocytes, and all these five different types of

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stretch-activated channels were blocked by 20 μM gadolinium (Ruknudin, Sachs & Bustamente, 1993). Thus, it is possible to block stretch-activated ion channels by gadolinium in heart muscle.

Although the inhibition of SA channels by gadolinium is relatively specific at low concentrations (Yang & Sachs, 1989), trivalent lanthanides have also been reported to inhibit Ca^{2+} influx through voltage-gated Ca^{2+} channels at millimolar concentrations (Lansman, 1990). On the other hand, an agonist of the voltage-gated L-type Ca^{2+} channel, Bay K 8644, is known to enhance ANP release (Ruskoaho, Toth, Ganten, Unger & Lang, 1986). When studying the effect of Gd^{3+} on ANP release, it is thus important to ensure that the effect of gadolinium is not mediated by inhibition of L-type or T-type voltage-gated Ca^{2+} channels, present in heart atrial muscle cells (Bean, 1985).

In the present study we used a superfused rat atrial *in vitro* preparation to investigate the mechanism of ANP secretion. Our hypothesis was that, if stretch–secretion coupling of ANP release is mediated by gadolinium-sensitive SA channels, gadolinium should inhibit stretch-stimulated ANP release. We show here that Gd^{3+} decreases stretch-induced ANP secretion, but does not affect basal secretion. In addition, we demonstrate that the inhibition is not mediated by an unspecific block of T-type or L-type calcium channels or by an inhibitory effect on contractility.

METHODS

Animals and atrial preparation

Male Sprague–Dawley rats weighing between 300 and 400 g were used. They were bred at 20–22 °C and had free access to tap water and standard food. The animals were decapitated, and their hearts were rapidly removed and placed in the following oxygenated warm (37 °C) buffer solution at pH 7·4 (mM): 137 NaCl, 5·6 KCl, 2·2 $CaCl_2$, 5·0 Hepes, 0·12 $MgCl_2$ and 2·5 glucose. This solution was also used for superfusion of the atrial preparation. The experimental set-up was constructed as previously described (Laine, Weckström, Vuolteenaho & Arjamaa, 1993). First a polyethylene cannula (2·0 mm diameter) was then inserted into the left atrial auricle. The tissue was attached to the cannula with one to two lengths of silk suture and the remaining portions were removed, so that the preparation contained primarily the left atrial appendage and a small part of the body of the atrium. Another tube with a smaller diameter was inserted inside the cannula and it carried the perfusate inflow to the lumen of the atrium. The fluid flowed out through a T-branch of the larger cannula. The flow (2 ml min^{-1}) was controlled by a peristaltic pump (P-3, Pharmacia, Uppsala, Sweden). The preparation was inserted in a constant-temperature (37 °C) organ bath (2 ml) that was filled with the fluid coming from inside the atrium. Intra-atrial pressure was controlled by the height of the tip of the outflow catheter. The perfusate was collected every 2 min by a fraction collector (Golden Retriever, ISCO, Lincoln, NE, USA) into plastic tubes. The atrium was paced by a field stimulus using platinum electrodes (3 Hz).

The influence of gadolinium on resting tension and developed tension was investigated by atrial strip recordings. The left auricle preparation was placed between two hooks and the initial resting tension was adjusted to 4·2 mN. The auricle was paced by a supramaximal field stimulus (3 Hz). The changes in tension were measured on a Grass polygraph (model 7DA, Grass Instruments, Quincy, MA, USA).

Experimental design

To stabilize the secretion rate of ANP, the atrium was perfused for 60 min at baseline distension before starting to collect samples. Pacing was started immediately after inserting the preparation in the organ bath. The tissue was stretched by increasing the intra-atrial pressure. Furthermore, stretch-stimulated experiments were preceded by a 20 min period of superfusion with vehicle or the appropriate drug until termination of the experiment. The effect of gadolinium on basal ANP secretion was investigated by superfusing the tissue with vehicle first for 4 min, then with vehicle or 80μM $GdCl_3$ for 20 min. The intra-atrial pressure was kept at basal level (1·5 mmHg) throughout the experiment.

To investigate the effect of gadolinium on SA secretion of ANP, the superfusion with vehicle or gadolinium (5, 20 or 80 μM) was started 20 min before starting to collect samples. The pressure was raised to 8 cmH_2O (6·2 mmHg) after two perfusate samples from the basal level of 2 cmH_2O (1·5 mmHg). Experiments with calcium channel blockers were performed by superfusion with 3 μM diltiazem or 50 μM $NiCl_2$ with a similar protocol to the gadolinium experiments. In non-paced experiments pacing was stopped 10 min before starting to collect samples. The effects of gadolinium and diltiazem on ANP secretion in quiescent atria were tested by superfusing with 80 μM gadolinium or 30 μM diltiazem. Apart from the removal of pacing, the protocol was similar to paced experiments.

Three concentrations of $GdCl_3$ (5, 20 and 80 μM) were used to study the effects of gadolinium on contractility in the atrial strip preparation. The change in resting tension and developed tension was measured 20 min after applying the inhibitor. The effects of $NiCl_2$ (50 μM) and diltiazem (3 μM) on contractility were recorded similarly.

Radioimmunoassay

ANP concentration in perfusate samples was measured by radioimmunoassay (Vuolteenaho, Arjamaa & Ling, 1985). Briefly, duplicate samples (100 μl) of each 2 min perfusate fraction were incubated with the specific rat ANP antiserum (100 μl) at a final dilution of 1/200000. Synthetic rat ANP_{99-126} in the range 0–500 pg $tube^{-1}$ was used as a standard. ANP_{99-126} was radioiodinated by the chloramine-T technique followed by desalting on a Sephadex G25 column (Pharmacia) and final purification by reverse phase HPLC using a Vydak C18 column (Separations Group, Hesperia, CA, USA). The tracer was added after 18–24 h incubation. After further incubation at +4 °C overnight, immunocomplexes were precipitated by double antibody in the presence of polyethyleneglycol followed by centrifugation (4000 *g*, 20 min). The radioactivity in the precipitates was counted in a 12-channel gamma-counter (LKB-W, Turku, Finland). The sensitivity of the assay was 2 pg $tube^{-1}$ and the intra- and interassay coefficients of variation were $< 10\%$ and $< 15\%$, respectively. TheANP antiserum cross-reacts 100 % with rat proANP but it does not recognize the *N*-terminal fragment of

proANP, brain natriuretic peptide, C-type natriuretic peptide, vasopressin, angiotensin or endothelins (cross-reactivity < 0·01%).

Molecular form of secreted ANP

ANP is stored in atrial granules as a pro-hormone and it is processed into active form during the exocytotic process (Vuolteenaho *et al.* 1985). In some situations this process is disturbed and the hormone is secreted in an unprocessed form. To ensure that gadolinium does not influence the processing of the pro-hormone of ANP we performed HPLC analysis of the superfusates. ANP was first extracted from perfusion fluid with Sep-Pak C18 cartridges (Waters, Milford, MA, USA). The Sep-Pak eluate was dried in a concentrator (Savant Instruments, Hicksville, NY, USA) and reconstituted in 0·3 ml of the HPLC mobile phase (see below). Samples corresponding to 3·5 ml of the superfusate originally collected were used for the gel filtration analysis. A 0·7 × 30 cm Protein-Pak I-125 HPLC gel filtration column (Waters) was eluted with 40% acetonitrile in 0·1% aqueous trifluoroacetic acid at 1 ml min^{-1}. Fractions of 0·5 ml were collected and dried. After reconstitution in radioimmunoassay (RIA) buffer, the ANP content was determined with RIA (see above). The column was calibrated with bovine serum albumin (void volume), proANP purified from rat atria, rat ANP_{99-126} and radioiodine (total volume). The major secreted form of ANP had the molecular size corresponding to ANP_{99-126} in stretched atria superfused with and without gadolinium.

Statistical analyses

The results are expressed as means ± S.E.M. The changes in secretion rate were analysed by analysis of variance for repeated measurements. The mean stretch-stimulated secretion was calculated by subtracting the mean basal secretion from the (mean) total secretion (see Laine *et al.* 1993). The differences between groups were tested by analysis of variance and followed by Student's *t* test. Differences at the 95% level were considered statistically significant.

Materials

Hepes was obtained from Sigma Chemical Co., St Louis, MO, USA; KCl, glucose, $CaCl_2$, $MgCl_2$ and $NiCl_2$ from Merck, Darmstadt, Germany; NaCl from Riedel-de-Haën, Seelze, Germany; $GdCl_3$ from Aldrich Chemical Company, Milwaukee, WI, USA and diltiazem from Orion-Farmos Pharmacological Company, Turku, Finland.

RESULTS

Effect of gadolinium on basal and stretch-dependent ANP secretion

To separate the effects of gadolinium on basal and stretch-stimulated secretion of ANP, the influence of gadolinium on basal ANP secretion was first examined using a superfused *in vitro* preparation with a constant pressure of 2 cmH_2O (Fig. 1). The concentration of gadolinium was increased from 0 to 80 μM after two superfusate samples. These results were compared with those obtained without the addition of gadolinium (Fig. 1). The mean secretion of the two control samples was 215 ± 66 pg min^{-1} (mean ± S.E.M.) in the experiments with gadolinium addition ($n = 6$) and 175 ± 52 pg min^{-1} in the experiments perfused without it ($n = 6$). Gadolinium did not affect ANP secretion within the 20 min control period and there was no statistically significant difference between the mean secretion rates of atria superfused with 0 or 80 μM gadolinium (140 ± 54 and 192 ± 54 pg min^{-1} respectively).

To test the hypothesis that stretch–secretion coupling of ANP release is mediated by stretch-activated ion channels, we examined the effect of gadolinium on stretch-

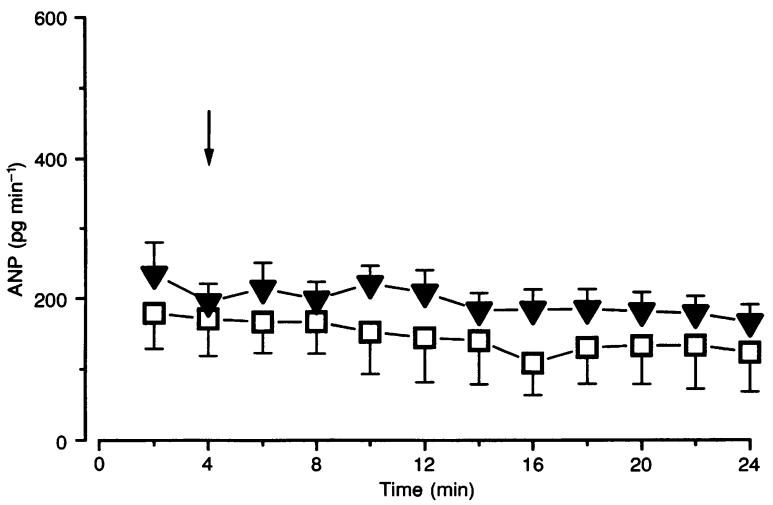

Figure 1. Effect of 80 μM gadolinium on basal ANP secretion in superfused rat atrial *in vitro* preparation

Gadolinium (▼) was added (arrow) after two superfusate samples. No effect on ANP secretion was observed within the 20 min superfusion period when compared with atria superfused with no gadolinium (□). Results are means ± S.E.M. of six experiments. For clarity, only the upper or lower error bars are shown.

stimulated ANP secretion (Fig. 2). Stretch was induced by increasing the intra-atrial pressure from 2 cmH_2O (1·5 mmHg) to 8 cmH_2O (6·2 mmHg). In the control atria with no gadolinium added, the ANP secretion rate increased rapidly after the pressure was raised, and the mean secretion was 815 ± 236 pg min^{-1} (Fig. 2*A*). Three concentrations of gadolinium (5, 20 and 80 μM) were used to study the effect of gadolinium on stretch-induced ANP release. Gadolinium inhibited the total ANP secretion in a dose-dependent manner. The mean ANP secretion rate was 550 ± 269 pg min^{-1} with 5 μM $GdCl_3$ (Fig. 2*A*), 408 ± 174 pg min^{-1} with 20 μM $GdCl_3$ (Fig. 2*B*) and 220 ± 174 pg min^{-1} with 80 μM $GdCl_3$ (Fig. 2*C*) after the pressure was raised.

As shown above, gadolinium does not decrease the basal secretion. Therefore we were able to separate the stretch-regulated and the basal secretion by subtracting basal secretion from the total secretion. Gadolinium decreased stretch-stimulated ANP secretion (Fig. 2*D*) by 46% in the atria superfused with 5 μM gadolinium, 71% with 20 μM gadolinium ($P < 0·05$) and 100% with 80 μM gadolinium ($P < 0·01$). These results show that gadolinium effectively inhibits stretch-stimulated ANP secretion.

Effect of diltiazem and $NiCl_2$ on ANP secretion

To test whether the inhibition of stretch-induced ANP secretion caused by gadolinium is mediated by voltage-activated calcium channels, we tested two different types of calcium channel blocker. Superfusion with 3 μM diltiazem, a blocker of L-type calcium channels, did not significantly affect ANP secretion (Fig. 3*B*). The mean stretch-stimulated secretion was 1224 ± 339 pg min^{-1} in control experiments (Fig. 3*A*) and 1224 ± 406 pg min^{-1} in diltiazem experiments (Fig. 3*B*). The biological activity of diltiazem was tested in strip recordings, where 3 μM

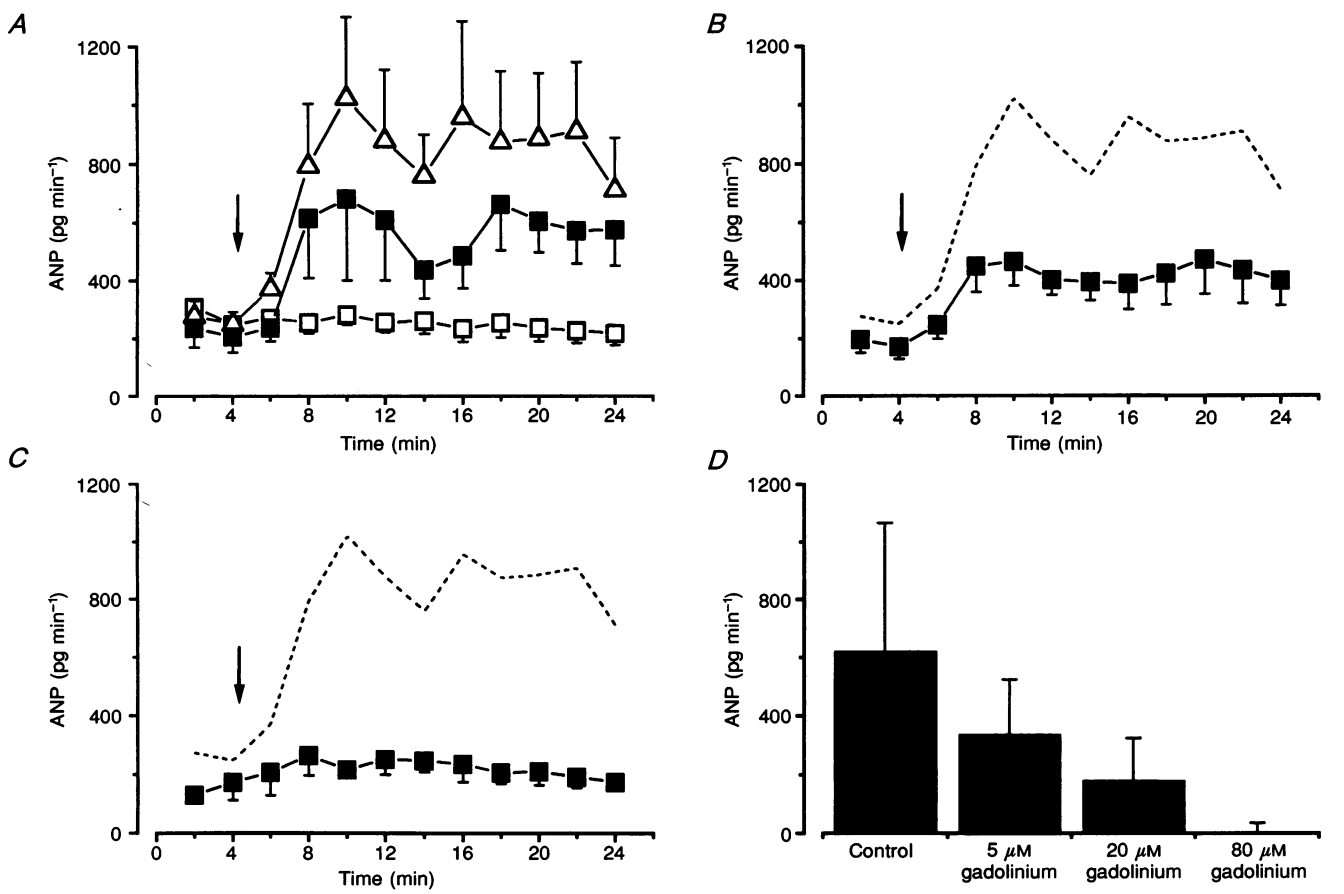

Figure 2. Effects of gadolinium (5, 20 and 80 μM) on ANP secretion in superfused rat atrial *in vitro* preparation

A, basal (□) control experiments with no gadolinium and stretch-stimulated ANP secretion in atria superfused with 5 μM gadolinium (■) or vehicle (△). *B*, ANP secretion in atria superfused with 20 μM gadolinium (■) or vehicle (dashed line). *C*, ANP secretion in atria superfused with 80 μM gadolinium (■) or vehicle (dashed line). *D*, mean stretch-stimulated ANP secretion with different concentrations of gadolinium. For each condition, the pressure was raised (arrows) after two samples from 2 cmH_2O (1·5 mmHg) to 8 cmH_2O (6·2 mmHg). Results are means ± S.E.M. of seven experiments. For clarity, only the upper or lower error bars are shown.

diltiazem decreased ($P < 0.05$) developed tension by 31% ($n = 4$). When the basal secretion was subtracted from the total secretion in the same way as in the gadolinium experiments, no statistically significant difference was found (Fig. 3*D*).

The other blocker used, $NiCl_2$, is quite a selective blocker of T-type plasmalemmal calcium channels in micromolar concentrations (Hirano, Fozzard & January, 1989) and it is an inhibitor of the Na^+–Ca^{2+} exchanger at millimolar concentrations (Varro, Negretti, Hester & Eisner, 1993). In the atria perfused by 50 μM $NiCl_2$, the stretch-activated secretion was 936 ± 409 pg min^{-1} (Fig. 3*C*). Again, when the basal secretion was subtracted from total secretion, there was no statistically significant difference between controls and the atria superfused with $NiCl_2$ (Fig. 3*D*). Instead, the dynamics in $NiCl_2$ experiments were different when compared to control atria. The secretion in atria treated with $NiCl_2$ decreased as a function of time ($P < 0.05$), whereas in the control experiments no statistically significant decline in the secretion profile was found. No change in resting or developed tension was observed after adding 50 μM $NiCl_2$ to the superfusate in strip recordings ($n = 4$).

Effect of gadolinium on atrial contractility

There is evidence that the frequency of contractions modulates the secretion rate of ANP (Schiebinger & Linden, 1986). It has also been demonstrated that the translocation of ANP to the lumen of the atrium depends on the contractile activity of the tissue (Cho, Kim, Hwang & Seul, 1993). Hence, gadolinium could decrease ANP secretion by inhibiting the atrial contractility. We found no significant change in the resting tension (4·2 mN) after gadolinium was added (Fig. 4). With lower concentrations (5 or 20 μM) no statistically significant decrease in developed tension was observed either. The developed

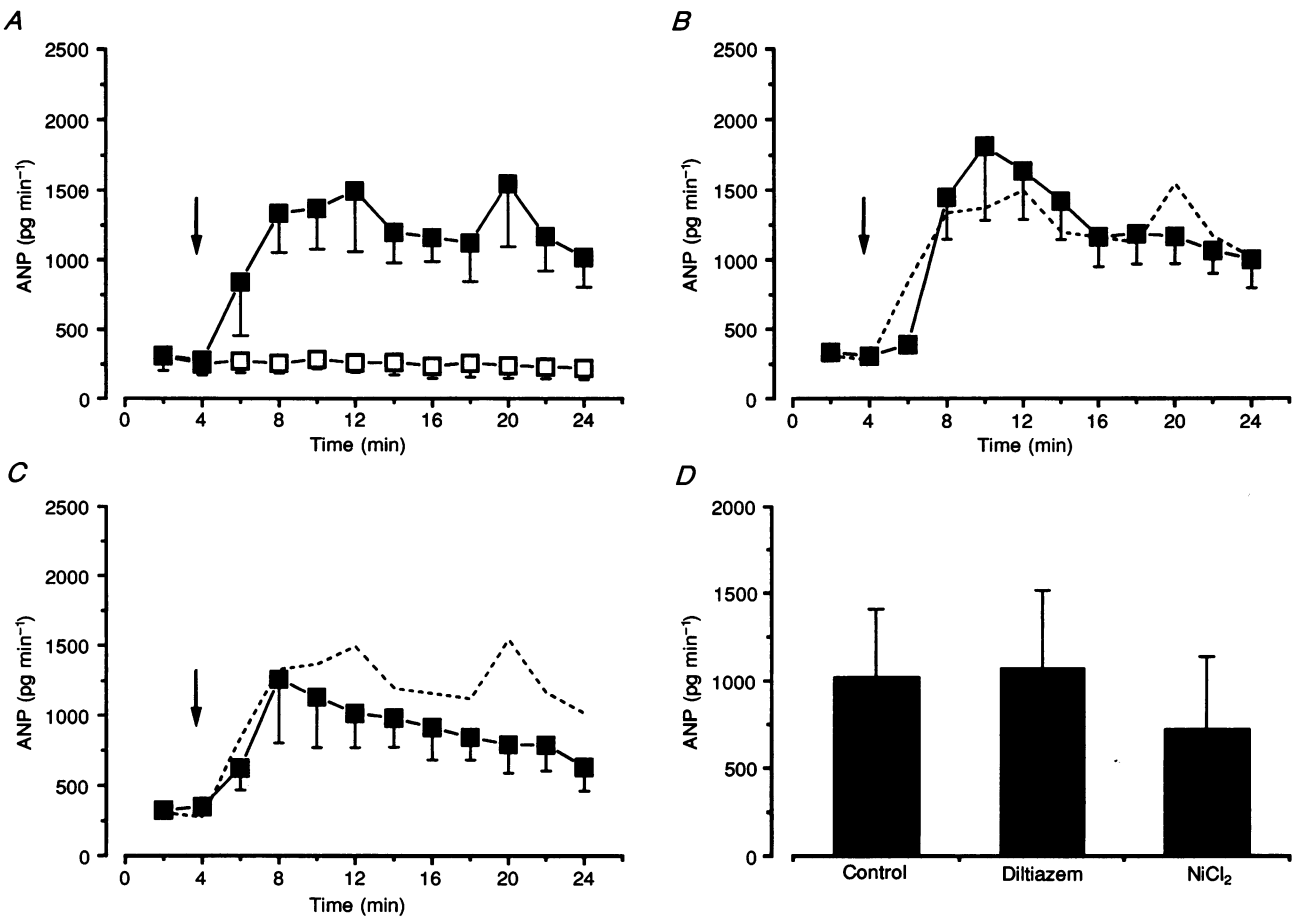

Figure 3. Effects of 3 μM diltiazem and 50 μM $NiCl_2$ (calcium channel blockers) on ANP secretion
A, basal (□) and stretch-stimulated (■) control experiments. *B*, total ANP secretion in atria superfused with 3 μM diltiazem (■) or vehicle (dashed line). *C*, total ANP secretion in atria superfused with 50 μM $NiCl_2$ or vehicle (dashed line). *D*, effects of diltiazem and $NiCl_2$ on mean stretch-stimulated ANP secretion. For each condition, the pressure was raised (arrows) after two samples from 2 cmH_2O (1·5 mmHg) to 8 cmH_2O (6·2 mmHg). Results are means ± S.E.M. of seven experiments. For clarity, only the upper or lower error bars are shown.

tension decreased by 22% during the 20 min period after 80 μM gadolinium was added ($P < 0.05$). Statistics were performed on seven independent experiments with atrial strip recordings.

Effect of gadolinium and diltiazem on ANP secretion in non-paced atria

The study of ANP secretion in paced atria is complicated by the fact that the secretion is influenced by action potentials and contractions. However, it has been shown previously that depolarizations and the muscle contractions which follow are not necessary for the secretory process (Laine *et al.* 1993). We were therefore able to investigate ANP secretion in electrically silent non-contracting left atrial tissue. This enabled us to separate the effects related to action potentials from the other stimulatory mechanisms. The mean secretion in non-paced control atria was 765 ± 213 pg min^{-1} (Fig. 5*A*) after the pressure was raised, and in the atria perfused by 80 μM gadolinium, the corresponding mean secretion was 438 ± 159 pg min^{-1} (Fig. 5*B*), with an inhibition of 71% ($P < 0.05$) in stretch-stimulated secretion (Fig. 5*D*). This indicates that gadolinium inhibits stretch-stimulated ANP secretion in quiescent atria also.

It was not possible to study the effect of $NiCl_2$ in the silent atria since it caused irregular spontaneous contractions in the tissue. Diltiazem (30 μM) did not cause any significant changes in ANP secretion rate in the non-paced atria (Fig. 5*C*). The mean secretion after the pressure was raised was 400 ± 336 pg min^{-1}. When the stretch-activated secretion was calculated no statistically significant difference was found (Fig. 5*D*).

DISCUSSION

The results of the present study using the isolated superfused rat atrial preparation show that gadolinium, a blocker of mechanosensitive channels, inhibits stretch-activated ANP secretion in a dose-dependent manner, whereas blockers of L-type or T-type calcium channels do not. We also demonstrated the inhibition of stretch-stimulated ANP secretion by gadolinium in quiescent, non-contracting atria.

Inhibition of stretch-stimulated ANP secretion by gadolinium

In the present study the highest concentration of gadolinium (80 μM) completely blocked the stretch response in ANP release but did not decrease basal secretion. Several types of mechanosensitive ion channels have been reported to occur in cardiac myocytes that can be modulated by gadolinium. Two stretch-activated K^+ channels and three non-selective cation channels were described in cultured chick myocytes (Ruknudin *et al.* 1993). Gadolinium at 20 μM blocked all these five types of stretch-activated channels. A mechanosensitive K^+ channel (Kim, 1992) and a cation-selective channel (Kim,

Figure 4. Effect of gadolinium on tension of atrial muscle
An example of the atrial strip recording of resting and developed tension of atrial muscle after the addition (arrows) of indicated concentrations of gadolinium.

1992) were demonstrated in cultured rat atrial myocytes. However, these channels were not sensitive to 100 μM gadolinium in isolated patches.

It has been reported that stretch increases the intracellular concentration of calcium in cardiac cells and that this response can be blocked by gadolinium (Sigurdson, Ruknudin & Sachs, 1993). Gadolinium is also known to inhibit, in a dose-dependent manner, the stretch-induced arrhythmias in isolated canine ventricles (Hansen, Borganelli, Stacy & Taylor, 1991). The mechanism that causes the activation of mechanosensitive ion channels to lead to ANP exocytosis is not known. We have previously shown that ryanodine, an inhibitor of sarcoplasmic reticulum calcium channels, inhibits stretch-induced ANP release (Laine *et al.* 1993). Thus, it is probable that the secretion mechanism of ANP involves calcium-induced calcium release, and that the primary calcium signal could be mediated by calcium-permeable mechanosensitive channels stimulated by stretch. However, there is also opposing evidence about the involvement of calcium in the secretory process of ANP (Page, Goings, Power & Upshaw- Earley, 1990; Ruskoaho, 1992).

Is the inhibition of ANP secretion by gadolinium mediated by voltage-gated calcium channels?

Because gadolinium blocks voltage-gated calcium channels at millimolar concentrations (Lansman, 1990), the inhibition in ANP secretion could be mediated by the block of T-type or L-type sarcolemmal calcium channels. To investigate this possibility we tested specific blockers of these channels. Diltiazem, a blocker of the L-type calcium channel (Zahradníková & Zahradník, 1992) failed to decrease the secretion rate, although the developed tension decreased in atrial strip recordings by 31%. This is

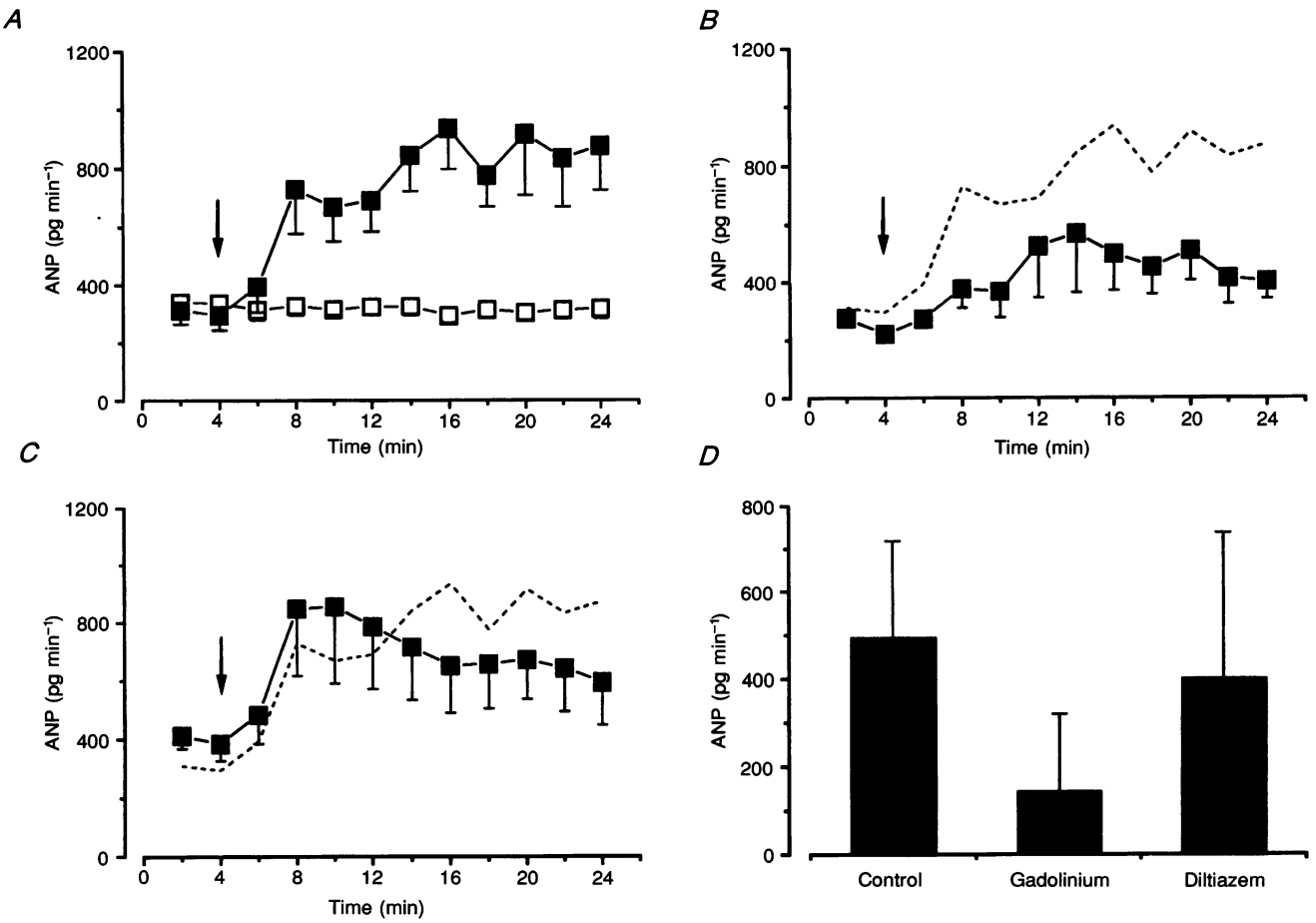

Figure 5. Effects of 80 μM gadolinium and 30 μM diltiazem on ANP secretion in non-paced, quiescent rat atria

A, basal (□) and stretch-stimulated (■) control experiments. *B*, total ANP secretion in atria superfused with 80 μM gadolinium (■) or vehicle (dashed line). *C*, total ANP secretion in atria superfused with 30 μM diltiazem (■) or vehicle (dashed line). *D*, effects of gadolinium and diltiazem on mean stretch-stimulated ANP secretion in non-paced atria. For each condition, the pressure was raised (arrows) after two samples from 2 cmH_2O (1·5 mmHg) to 8 cmH_2O (6·2 mmHg). Results are means ± S.E.M. of six experiments. For clarity, only the lower or upper error bars are shown.

consistent with the earlier experiments using nifedipine, another L-type calcium channel blocker (Deng & Lang, 1992).

Superfusion with $NiCl_2$ (50 μM), a blocker of the T-type calcium channel (Hirano *et al.* 1989), did not affect the mean stretch-stimulated ANP release. $NiCl_2$ caused spontaneous contractions in our left atrial preparation, which indicates that the inhibition of the Na^+–Ca^{2+} exchanger at 5 mM $NiCl_2$ reported earlier (Wolska & Lewartowski, 1993) is manifested already at this concentration. It can be observed from Fig. 3*C* that $NiCl_2$ decreased ANP release during the last minutes of the experiments. Some late inhibition of the secretion was seen also by Page *et al.* (1990) and it may be due to inhibition of the Na^+–Ca^{2+} exchanger leading to the depletion of calcium stores. Thus, we conclude that the blocking of ANP secretion by gadolinium is unlikely to be mediated by voltage-activated calcium channels.

Is the decrease in ANP secretion by gadolinium mediated by inhibition of contractility?

Atrial mechanical contractions interfere with the translocation of secreted ANP into the atrial lumen (Cho *et al.* 1993). It has also been demonstrated that the dynamics of ANP release are different in non-contracting and contracting atria (Laine *et al.* 1993). Thus, it is possible that the effects of gadolinium on the contractility of the atrium may modulate ANP secretion rate. No change was observed in resting tension after increasing doses of (5, 20 or 80 μM) gadolinium. There was also no change in developed tension when atria were superfused with 5 or 20 μM gadolinium. On the other hand, developed tension decreased by 22% from the control level when superfused with 80 μM. The slight decrease in contractility with this dose does not explain the inhibitory effect of gadolinium on ANP secretion, because the decrease in secretion was also seen with the lower doses (5 and 20 μM) of gadolinium. A larger decrease in developed tension was observed with 3 μM diltiazem (31%) which did not affect ANP release. Because the contractility was measured in an atrial strip preparation which is not fully comparable to the sac preparation used in ANP measurements, we also tested gadolinium in quiescent atria. The fact that gadolinium also decreased stretch-stimulated ANP secretion in non-contracting atria suggests that the inhibition is mediated by some mechanism other than a decrease in contractility.

What is the mechanism of the gadolinium block?

Several possible mechanisms must be considered regarding the stretch–secretion coupling of ANP release and hence the site of the action of gadolinium. The simplest assumption is that stretch is detected by the atrial muscle cells themselves. Stretch of heart muscle leads to a variety of changes in myocytes, including a rise in intracellular Ca^{2+} (Allen & Kurihara, 1982), activation of multiple biochemical signal transduction pathways (Sadoshima & Izumo, 1993) and alterations in action potentials (Franz, Burghoff, Yue & Sagawa, 1988). The activation of specific genes in response to chronic stretch causes hypertrophy of heart muscle (Morgan, Xenophon, Xenophontos, Haneda, McGlaughin & Watson, 1989). How the mechano-transduction is mediated into these signals is not known, but many of the effects could be explained by the presence of mechanosensitive ion channels (Morris, 1990). Such channels have been documented in the tissue-cultured chick heart (Sigurdson *et al.* 1992; Ruknudin *et al.* 1993), in rat heart atria (Kim, 1992, 1993) and in frog heart atria (Kohl, Kamkin, Kiseleva & Streubel, 1992).

Another possibility is that the primary stretch sensors are situated in cells other than the myocytes. Such cells could be endothelial or endocardial. Stretching endothelial cells cultured on an elastic membrane causes a production of endothelin (Sumpio & Widmann, 1990), a hormone that is known to enhance atrial stretch-induced ANP release (Mãntymaa, Leppäluoto & Ruskoaho, 1990). Furthermore, gadolinium-sensitive stretch-activated ion channels have recently been described in cultured endothelial cells (Naruse & Sokabe, 1993). The release of endothelial relaxing factor (nitric oxide, NO) is known to be stimulated by chronic increases in blood flow (Miller & Vanhoutte, 1988). NO stimulates ryanodine-sensitive calcium channels in the sarcoplasmic reticulum of myocytes via its effects on intracellular cyclic GMP and cyclic ADP ribose (Berridge, 1993). Interestingly, a block of these channels by ryanodine almost completely inhibits stretch-stimulated ANP release in the superfused *in vitro* preparation (Laine *et al.* 1993). Even if these paracrine mechanisms may not be the primary ones controlling stretch–secretion coupling of ANP release, they may have an important modulatory role in the secretory process.

In summary, the fact that stretch-activated ANP release is inhibited by gadolinium, but not by diltiazem or $NiCl_2$, and that secretion in quiescent atria is also inhibited by Gd^{3+} strongly suggests that the stretch-activated ion channels are involved in the regulation of stretch-induced ANP release.

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