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Ca^{2+}-induced Ca^{2+} Release from Sarcoplasmic Reticulum Negatively Regulates Myocytic ANP Release in Beating Rabbit Atria

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It is not clear whether Ca^{2+}-induced Ca^{2+} release from the sarcoplasmic reticulum (SR) is involved in the regulation of atrial natriuretic peptide (ANP) release. Previously, we have shown that nifedipine increased ANP release, indicating that Ca^{2+} entry via voltage-gated L-type Ca^{2+} channel activation decreases ANP release. The purpose of the present study was two-fold: to define the role of SR Ca^{2+} release in the regulation of ANP release and whether Ca^{2+} entry via L-type Ca^{2+} channel is prerequisite for the SR-related effect on ANP release. Experiments were performed in perfused beating rabbit atria. Ryanodine, an inhibitor of SR Ca^{2+} release, increased atrial myocytic ANP release (8.69 ± 3.05, 19.55 ± 1.09, 27.31 ± 3.51, and $18.91 \pm 4.76\%$ for 1, 2, 3, and 6 μM ryanodine, respectively; all $P<0.01$) with concomitant decrease in atrial stroke volume and pulse pressure in a dose-dependent manner. In the presence of thapsigargin, an inhibitor of SR Ca^{2+} pump, ryanodine-induced increase in ANP release was not observed. Thapsigargin attenuated ryanodine-induced decrease in atrial dynamic changes. Blockade of L-type Ca^{2+} channel with nifedipine abolished ryanodine-induced increase in ANP release ($0.69 \pm 5.58\%$ vs. $27.31 \pm 3.51\%$; $P<0.001$). In the presence of thapsigargin and ryanodine, nifedipine increased ANP release and decreased atrial dynamics. These data suggest that Ca^{2+}-induced Ca^{2+} release from the SR is inversely involved in the regulation of atrial myocytic ANP release.

Key Words: Atrial natriuretic peptide, L-type Ca^{2+} channels, Sarcoplasmic reticulum Ca^{2+} release

INTRODUCTION

Atrial volume change is the most prominent factor to regulate atrial natriuretic peptide (ANP) release (Dietz, 1984; Lang et al, 1985). However, the mechanism by which the volume change regulates ANP release is not well understood. Previously, we have proposed a two-steps sequential mechanism for the mechanically stimulated secretion of ANP (Cho et al, 1995). First, ANP is released from atrial myocytes into the surrounding extracellular space (ECS). Second, convective transendocardial translocation of the ANP-containing extracellular fluid (ECF) into the blood stream is induced by atrial contraction. The convective translocation of the ECF was shown to be dependent on the volume and rate changes of the atrium (Cho et al, 1995) and the size of the ECS (Cho et al, 2002). An increase in atrial workload by increasing atrial rate accentuated the secretion of ANP by increasing the translocation of the ECF, the final step of the two-steps sequential mechanism (Cho et al, 1995; 2002). On this occasion, atrial myocytic release of ANP, the first step, is accentuated by mechanical stimulation with simultaneous tonic negative modulation by Ca^{2+} (Cho et al, 1995; Wen et al, 2000). The latter is closely related to an increase in Ca^{2+} entry via voltage-gated L-type Ca^{2+} channel at higher atrial rate. Because Ca^{2+} entry via L-type Ca^{2+} channel activation decreases myocytic ANP release (Kim et al, 1997; Wen et al, 2000), these results suggest that mechanically stimulated ANP secretion is accompanied by a tonic inhibition by Ca^{2+} entry of atrial myocytic ANP release (Kim et al, 1995; Wen et al, 2002).

However, the roles of Ca^{2+} remain controversial: Ca^{2+} is a negative or positive regulator. Increases in Ca^{2+} entry via L-type Ca^{2+} channel activation increased ANP secretion (Ruskoaho et al, 1986; Saito et al, 1986; Schiebinger, 1989; McDonough et al, 1994; Schiebinger & Cragoe Jr, 1994). In contrast, Ca^{2+} has also been shown to be a negative regulator for ANP secretion (Ito et al, 1988; De Bold & De Bold, 1989; Ruskoaho et al, 1990; Deng & Lang, 1992; Kim et al, 1997; Lee et al, 2000; Wen et al, 2000). Stimulation-induced increase of ANP release has been shown to be dependent on the ryanodine-sensitive SR Ca^{2+} store in perfused beating atria and heart (Schiebinger, 1989; Katoh et al, 1990; Kuroski-De Bold & De Bold, 1991; Laine et al, 1994).

Recently, the importance of the spatial regulation of

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ABBREVIATIONS: ANP, atrial natriuretic peptide; ECS, extracellular space; ECF, extracellular fluid; CICR, Ca^{2+}-induced Ca^{2+} release; BAPTA-AM, 1,2-bis(2-aminophenoxy)ethane-N,N,N,N-tetraacetic acid tetrakis (acetoxymethyl ester).

intracellular second messengers is emerging in the regulation of atrial myocytic ANP release (Wen et al, 2000; 2004; Cui et al, 2002). Wen *et al.* (2000) have shown distinct roles of Ca^{2+} entry via L- and T-type Ca^{2+} channels: L-type Ca^{2+} channel is negatively and T-type is positively involved in the regulation of ANP release. Subtype-specific roles of cAMP phosphodiesterases in the regulation of ANP release are also considered as an example of compartmentalized function of intracellular cAMP in the regulation of atrial myocytic ANP release (Cui et al, 2002). Distinct roles for the particulate and soluble guanylyl cyclase systems have also been shown in the regulation of atrial ANP release (Wen et al, 2004).

Ca^{2+} entry via the voltage-gated L-type Ca^{2+} channel activation and intracellular Ca^{2+} release from the SR are the important factors for regulation of intracellular Ca^{2+} balance. Ca^{2+} entry via the L-type Ca^{2+} channel is involved in a variety of Ca^{2+} dependent processes, including excitation-contraction coupling in muscle cells and excitation-secretion coupling in endocrine and neuronal cells. In ventricular cardiomyocytes, excitation increases Ca^{2+} entry via L-type Ca^{2+} channel in the sarcolemma. This Ca^{2+} entry activates the release of Ca^{2+} from the SR through the Ca^{2+} release channel (ryanodine receptors) (Ca^{2+}-induced Ca^{2+} release, CICR; Fabiato, 1983). CICR elevates intracellular Ca^{2+}, resulting in myocardial contraction (excitation-contraction coupling, E-C coupling). Elevated intracellular Ca^{2+} concentration returns to the baseline levels via two pathways: extrusion from the cell by Na^{+}-Ca^{2+} exchange protein and sarcolemmal Ca^{2+} ATPase, and reuptake into the SR by SR Ca^{2+} ATPase. The purpose of the present study was to define the role of SR Ca^{2+} release and to examine whether CICR is involved in the regulation of ANP release in perfused beating rabbit atria.

METHODS

Beating perfused rabbit atrial preparation

New Zealand White rabbits were used. All experiments were carried out under approval of the Ethics Committee in the Institute for Medical Sciences of Jeonbug National University. An isolated perfused atrium was prepared by the method described previously (Cho et al, 1995; 2002), allowing atrial pacing and measurements of changes in atrial volume during contraction (stroke volume), pulse pressure, transmural extracellular fluid (ECF) translocation and ANP secretion. The atrium was perfused with HEPES buffer solution by means of a peristaltic pump (at 36.5°C, 1 ml/min). The composition of the buffer was as follows (mM): 118 NaCl, 4.7 KCl, 2.5 $CaCl_2$, 1.2 $MgCl_2$, 25 $NaHCO_3$, 10.0 glucose, and 10.0 HEPES (pH adjusted to 7.4 with NaOH).

Experimental protocols

The atria were perfused for 60 min to stabilize ANP secretion and atrial dynamics. [^{3}H]inulin was introduced to the pericardial fluid 20 min before the start of sample collection (Cho et al, 1995; Wen et al, 2000). The perfusate was collected at 2-min intervals at 4°C for analyses. The atria were paced at 1.3 Hz. The control period (48 min or 12 min) was followed by an infusion of ryanodine or tetracaine (Overend et al, 1998), inhibitors of SR Ca^{2+} release channel (for ryanodine; 0.3 μM, n=6; 1.0 μM, n=9; 2.0 μM, n=4; 3.0 μM, n=12; 6.0 μM, n=8; for tetracaine; 50 μM, n=7). To analyze the effects of ryanodine on ANP release and atrial dynamics, 36 min of thapsigargin treatment, an inhibitor of SR Ca^{2+} ATPase and uptake at μM range (Delgado et al, 1999), was followed by an infusion of ryanodine [thapsigargin (1 μM) plus ryanodine (3.0 μM), n=6; thapsigargin (30.0 μM) plus ryanodine (3.0 μM), n=8] or vehicle [thapsigargin (1.0 μM) alone, n=9; thapsigargin (30.0 μM) alone, n=7]. To analyze the effects of ryanodine, 36 min of treatment with an inhibitor of L-type Ca^{2+} channel, nifedipine (1.0 μM) was followed by an infusion of ryanodine (3.0 μM) (n=6) or vehicle (n=6). In another series of experiments, the control period (48 min) was followed by an infusion of nifedipine (1.0 μM, n=7). To analyze the effects of nifedipine on ANP release and atrial dynamics in the absence of SR Ca^{2+} release, 36 min of the combined presence of thapsigargin (1.0 μM) and ryanodine (3.0 μM) was followed by an infusion of nifedipine (n=7) or vehicle (n=7). In another series of experiments, the effects of intracellular chelator of Ca^{2+} were tested. The control period (12 min) was followed by an infusion of membrane-permeable BAPTA-AM [1,2-bis(2-aminophenoxy)ethane-N, N,N,N-tetraacetic acid tetrakis (acetoxymethyl ester), 30 μM, n=9] or membrane-impermeable BAPTA (30 μM, n=5). For the time-matched control experiments (n=9), vehicle was introduced and the values obtained during the periods corresponding to the control and experimental observations were compared.

Radioimmunoassay of ANP

Immunoreactive ANP in the perfusate was measured by a specific radioimmunoassay, as described previously (Cho et al, 1995). The amount of immunoreactive ANP secreted was expressed in nanograms of ANP per minute per gram of atrial tissue. The molar concentration of immunoreactive ANP, calculated in terms of ECF translocation, which reflects the concentration of extracellular ANP in the atrium, therefore, indicates the rate of myocytic release of ANP into the surrounding paracellular space (Cho et al, 1995), was calculated as ANP released (μM=immunoreactive ANP (in pg min^{-1} g^{-1})/ECF translocated (in μl min^{-1} g^{-1} 3063) [mol/wt., ANP-(1-28)]. Most of the ANP secreted is processed ANP (Cho et al, 1995).

Statistical analysis

Significant difference was compared using repeated measures ANOVA followed by Bonferronis multiple-comparison test (Figs. 1, 3, 5, 7 and 9). Students t-test for unpaired data (Figs. 2, 4, 6 and 8) was also applied. Statistical significance was defined as $P<0.05$. The results are given as means±S.E.M.

RESULTS

Ryanodine increases atrial myocytic ANP release with a decrease in atrial dynamics

ANP concentration, which reflects the rate of atrial myocytic ANP release into the surrounding paracellular space of the atrium, was stably maintained during the experiments (Fig. 1*A* and *B*). Atrial dynamic changes,

including stroke volume and pulse pressure, were also stable and repeatable.

Ryanodine (3 μM) significantly increased ANP concentration; i.e., atrial myocytic ANP release by 27.31±3.51% (Fig. 1*Aa*). Ryanodine decreased atrial dynamics (Fig. 1*Ab* and *Ac*). The responses to ryanodine were observed within 4 to 6 min and showed a peak at around 10 min of the administration. Ryanodine, in micromolar range (0.3−6.0 μM), decreased atrial dynamics and increased ANP release in a concentration-dependent manner, which means that Ca^{2+} release from the SR decreases atrial myocytic ANP release (Fig. 2). The change in atrial dynamics in response to ryanodine was more sensitive than that in ANP release. The minimal concentration to decrease atrial dynamics was 0.3 μM. In this concentration, ryanodine-induced increase in ANP release was not observed. The minimal concentration of ryanodine to increase atrial myocytic ANP release was 1.0 μM. Similarly, tetracaine (50 μM, Overend et al, 1998) increased atrial myocytic ANP release (23.02±2.09%, n=7, vs. −4.81±3.06%, n=6, control; $P<0.001$) concomitantly with a decrease in atrial stroke volume (−82.64±6.85%, n=7, vs. −0.78±0.29%, n=6, control; $P<0.001$).

Thapsigargin blocks ryanodine-induced increase in ANP release

To analyze the effect of ryanodine on the ANP release, thapsigargin was introduced prior to an infusion of ryanodine. Thapsigargin (1 μM) significantly decreased ANP concentration (Fig. 3*Aa* and *Ba*), and significantly increased atrial stroke volume up to third or fourth cycle of infusion (Fig. 3*Ab* and *Bb*) which then returned to the control level. As shown in Fig. 3*A*, thapsigargin attenuated the ryanodine-induced increase of ANP release (10.19±2.77%, n=6, vs. 27.31±3.51%, n=12; $P<0.01$; Figs. 3*Aa* and 4). At higher concentration of thapsigargin (30 μM), ryanodine-induced increase in ANP release was not observed (Fig. 4), suggesting that ryanodine-induced increase in ANP release is related to the function of SR Ca^{2+} release. In the presence of thapsigargin, ryanodine-induced decrease in

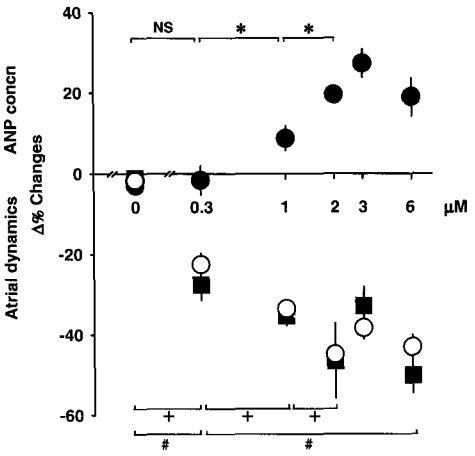

Fig. 2. Concentration-dependent effects of ryanodine on atrial secretion and dynamics. Concentration-dependent effects of ryanodine on the changes in ANP concentration (concn, •), atrial stroke volume (○) and pulse pressure (■). The responses were compared with differences of mean values of 2 fractions before (fraction number 23 and 24) and after 1 cycle (fraction number 29 and 30) of ryanodine or vehicle. Number of experiments: ryanodine (0.3 μM), n=6; ryanodine (1.0 μM), n=9; ryanodine (2.0 μM), n=4; ryanodine (3.0 μM), n=12, data derived from Fig. 1*A*; ryanodine (6.0 μM), n=8; control, n=9, data derived from Fig. 1*B*. Values are mean±SE. $^{*}P<0.05$, $^{+}P<0.01$ (stroke volume), $^{\#}P<0.01$ (pulse pressure), NS: not significant.

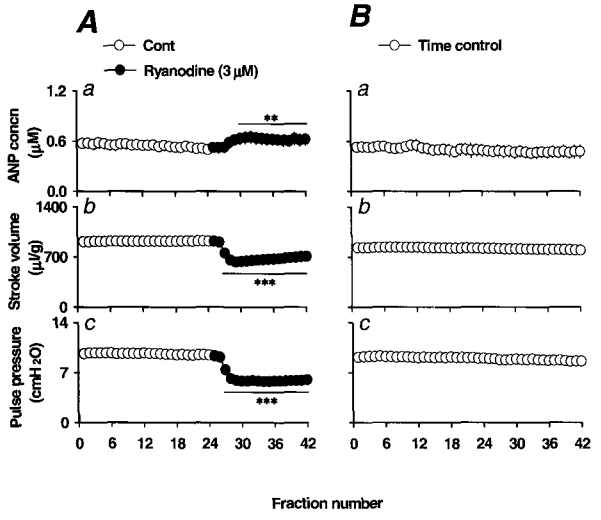

Fig. 1. Effects of ryanodine on atrial secretion and dynamics. (*A*) Effects of ryanodine (3 μM) on ANP concentration (*Aa*), atrial stroke volume (*Ab*) and pulse pressure (*Ac*) in perfused beating rabbit atria (1.3 Hz; n=12). (*B*) Time-matched controls for the same parameters (*Ba-Bc*) were stable during the period corresponding to ryanodine infusion (n=9). Values are means±SE. $^{**}P<0.01$, $^{***}P<0.001$ vs. control period (Cont).

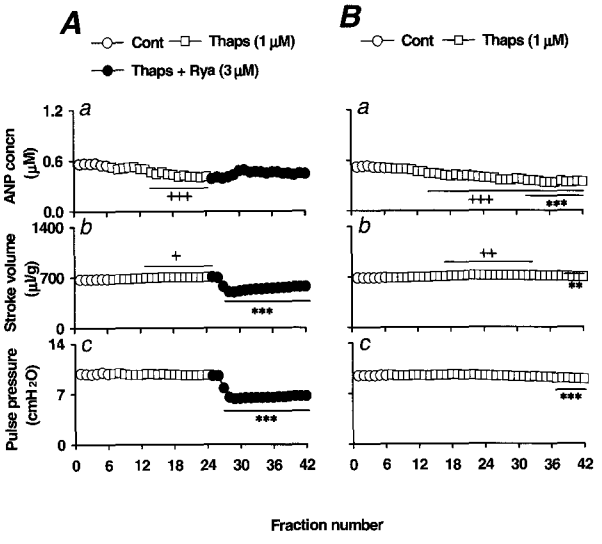

Fig. 3. Effects of thapsigargin on ryanodine-induced changes in atrial secretion and dynamics. (*A*) Effects of thapsigargin (1.0 μM) on ryanodine (3.0 μM)-induced changes in ANP concentration (*Aa*), atrial stroke volume (*Ab*) and pulse pressure (*Ac*) in perfused beating atria (n=6). (*B*) Effects of thapsigargin on the same parameters (n=9). $^{+}P<0.05$, $^{++}P<0.01$, $^{+++}P<0.001$ vs. control period; $^{**}P<0.01$, $^{***}P<0.001$ vs. thapsigargin.

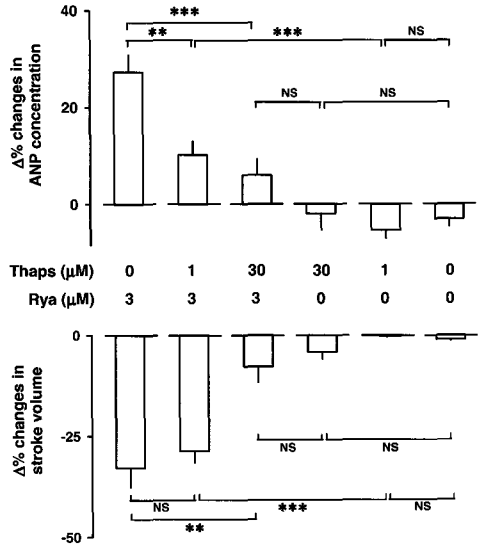

Fig. 4. Effects of thapsigargin on ryanodine-induced changes in ANP concentration and atrial stroke volume. The responses were compared with differences of mean values of 2 fractions before (fraction number 23 and 24) and after 1 cycle (fraction number 29 and 30) of ryanodine or vehicle. Values are mean ± SE. Ryanodine (3.0 μM) alone, n=12, data derived from Fig. 1*A*; thapsigargin (1.0 μM) plus ryanodine, n=6; thapsigargin (30.0 μM) plus ryanodine, n=8; thapsigargin (30.0 μM) alone, n=9; thapsigargin (1.0 μM) alone, n=7; control, n=9, data derived from Fig. 1*B*. $^{**}P<0.01$, $^{***}P<0.001$, NS: not significant.

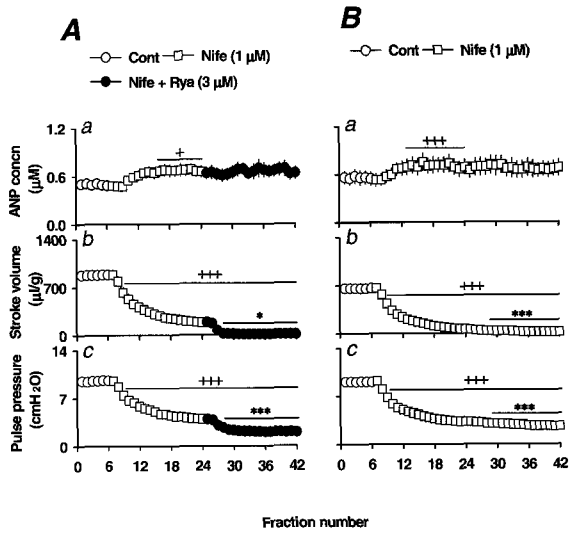

Fig. 5. Effects of nifedipine on ryanodine-induced changes in atrial secretion and dynamics. (*A*) Effects of nifedipine (1.0 μM) on ryanodine (3.0 μM)-induced changes in ANP concentration (*Aa*), atrial stroke volume (*Ab*) and pulse pressure (*Ac*) in perfused beating atria (n=6). (*B*) Effects of nifedipine on the same parameters (n=6). $^{+}P<0.05$, $^{+++}P<0.001$ vs. control period; $^{*}P<0.05$, $^{***}P<0.001$ vs. nifedipine.

atrial dynamics was attenuated (Figs. 3*Ab*, 3*Ac* and 4). As shown in Fig. 4, thapsigargin attenuated ryanodine-induced increase in ANP release and decrease in atrial stroke volume in a concentration-dependent manner, suggesting that Ca^{2+} release from the SR decreases myocytic ANP release.

Ca^{2+} entry via L-type Ca^{2+} channel is prerequisite for the ryanodine-induced increase in ANP release

To define the role of Ca^{2+} entry via voltage-gated L-type Ca^{2+} channel for the ryanodine-induced increase in ANP release, nifedipine was introduced prior to infusion of ryanodine. Nifedipine increased ANP release with concomitant decrease in atrial dynamics (Fig. 5). In the presence of nifedipine, ryanodine further decreased atrial dynamics (Fig. 5*Ab* and *Ac*). However, in the presence of nifedipine, ryanodine-induced increase of ANP release was not observed (0.69 ± 5.58%, n=6, vs. 27.31 ± 3.51%, n=12; $P<0.001$; Figs. 5*Aa* and 6). These results show that Ca^{2+} entry via L-type Ca^{2+} channel is prerequisite for the ryanodine-induced increase of ANP release. This means that Ca^{2+} entry via L-type Ca^{2+} channel is prerequisite for the SR-related decrease in myocytic ANP release.

Nifedipine increases ANP release in the presence and absence of SR Ca^{2+} release

To define the effect of nifedipine in the absence of SR Ca^{2+} release on atrial myocytic ANP release, both thapsigargin and ryanodine were simultaneously introduced prior to infusion of nifedipine. No significant changes in ANP

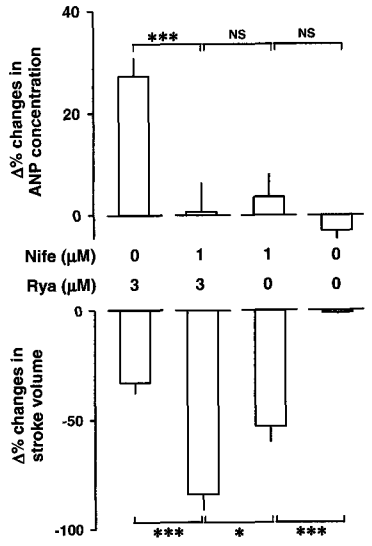

Fig. 6. Attenuation of ryanodine-induced increase in ANP concentration and atrial stroke volume by nifedipine. The responses were compared with differences of mean values of 2 fractions before (fraction number 23 and 24) and after 1 cycle (fraction number 29 and 30) of ryanodine or vehicle. Three cycles of nifedipine or vehicle were followed by ryanodine or vehicle. $^{*}P<0.05$, $^{***}P<0.001$, NS: not significant. Ryanodine (3.0 μM) alone, n =12, data derived from Fig. 1*A*; nifedipine (1.0 μM) plus ryanodine, n=6; nifedipine alone, n=6; control, n=9, data derived from Fig. 1*B*.

concentration were observed by combined infusion of thapsigargin and ryanodine (Fig. 7*Ba*). However, combined infusion of the agents significantly decreased atrial dynamics (Fig. 7*Bb* and *Bc*). In the presence of both

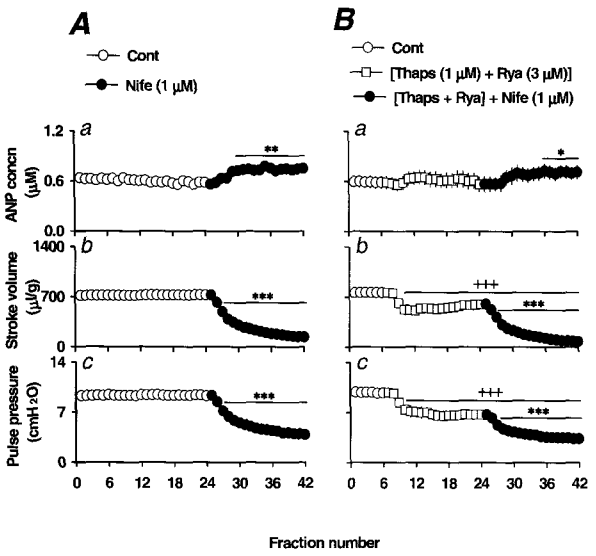

Fig. 7. Effects of nifedipine and modulation by combined treatment with thapsigargin and ryanodine. (*A*) Effects of nifedipine (1.0 μM) on the changes in ANP concentration (*Aa*), atrial stroke volume (*Ab*) and pulse pressure (*Ac*) in perfused beating atria (n = 7). (*B*) Effects of thapsigargin (1.0 μM) plus ryanodine (3.0 μM) on the nifedipine-induced changes in the same parameters (n=7). $^{+++}P<0.001$ vs. control period; $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$ vs. thapsigargin plus ryanodine or corresponding period.

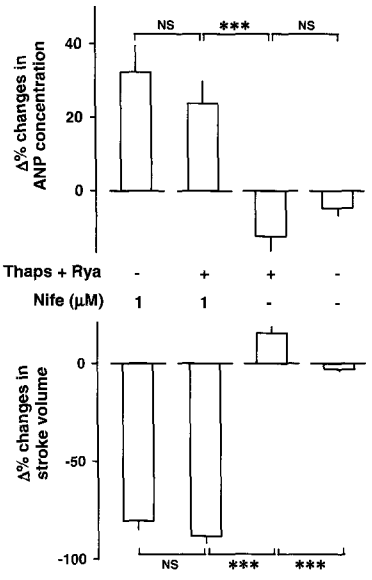

Fig. 8. Effects of thapsigargin plus ryanodine on the nifedipine-induced changes in ANP concentration and atrial stroke volume. The responses were compared with differences of mean values of 2 fractions before (fraction number 23 and 24) and after 3 cycles (fraction number 41 and 42) of nifedipine or vehicle. Thapsigargin plus ryanodine or vehicle was followed by nifedipine or vehicle. Nifedipine (1.0 μM) alone, n=7; thapsigargin plus ryanodine plus nifedipine, n = 7; thapsigargin plus ryanodine alone, n=7; control, n=9, data derived from Fig. 1*B*. $^{***}P<0.001$, NS: not significant.

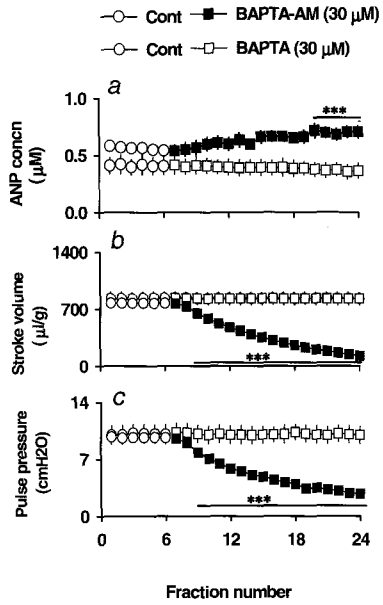

Fig. 9. Effects of BAPTA-AM (30 μM, n=9) and BAPTA (30 μM, n=5) on ANP concentration (*a*), atrial stroke volume (*b*) and pulse pressure (*c*) in perfused beating rabbit atria. $^{***}P<0.001$ vs. control period (Cont).

thapsigargin and ryanodine, nifedipine further decreased atrial dynamics. Nifedipine increased ANP concentration in the combined presence of thapsigargin and ryanodine (Fig. 7*Ba*). Nifedipine-induced increases of ANP concentration in the presence and absence of thapsigargin plus ryanodine were not significantly different (Figs. 7*Aa*, *Ba* and 8).

Intracellular Ca^{2+} chelation with BAPTA-AM increases ANP release

To define the effect of intracellular chelation of Ca^{2+} on ANP release, cell membrane-permeable BAPTA-AM was introduced. BAPTA-AM significantly increased ANP concentration (31.86±6.46%, n=9, BAPTA-AM, vs. −11.19±4.11%, n=5, BAPTA; $P<0.001$) with concomitant decrease of atrial dynamics (Fig. 9). In contrast, cell membrane-impermeable BAPTA had no effects on the ANP concentration and atrial dynamics. These findings indicate that intracellular Ca^{2+} is negatively involved in the regulation of atrial myocytic ANP release.

DISCUSSION

The present data show that ryanodine increases atrial myocytic ANP release and the effect is not observed in the presence of thapsigargin or nifedipine. Also it is shown that nifedipine increases ANP release in the presence or relative depletion of SR Ca^{2+} release. Therefore, we conclude that Ca^{2+} release from the SR decreases atrial myocytic ANP release, and that Ca^{2+} entry via L-type Ca^{2+} channel is prerequisite for the SR-related decrease in ANP release. The present findings, showing that Ca^{2+} entry via L-type Ca^{2+} channel is prerequisite for SR-induced decrease in myocytic ANP release and also decreases ANP release in

the absence of SR Ca^{2+} release, suggest that Ca^{2+} entry through L-type Ca^{2+} channel decreases atrial myocytic ANP release via two distinct pathways: one directly and the other indirectly through SR Ca^{2+} release. The present finding also indicates that depletion in intracellular Ca^{2+} with BAPTA-AM increases ANP release.

CICR is observed in the ventricular myocytes in which transverse-tubules are well developed. It has been shown in atrial myocytes, in which transverse-tubules are absent or sparse, that the peripheral SR is coupled to the sarcolemma (McNutt & Fawcett, 1969). Recently, it was further shown in the atrium that the subsarcolemmal SR ryanodine receptor is spatially related to the L-type Ca^{2+} channel (Lipp et al, 1990; Lewis Carl et al, 1995; Hueser et al, 1996; Woo et al, 2003), and that Ca^{2+} entry via L-type Ca^{2+} channel is temporally associated with SR Ca^{2+} release (Hueser et al, 1996; Sheehan & Blatter, 2003; Woo et al, 2003). These findings indicate that during excitation-contraction coupling in atrial myocytes, Ca^{2+} entry via L-type Ca^{2+} channel activates Ca^{2+} release from subsarcolemmal peripheral SR. Also, atrial myocytes contain the SR specific for ryanodine (Lewis Carl et al, 1995; Kockskaemper et al, 2001; Sheehan & Blatter, 2003; Woo et al, 2003). Taken together with present findings, it is possible to hypothesize that in atrial myocytes, Ca^{2+} release by CICR is inversely related to the regulation of cardiac hormone release.

The concentration (1 μM) of nifedipine might not be enough to completely block the L-type Ca^{2+} channels, while atrial stroke volume was almost completely suppressed. Because the gain of the SR Ca^{2+} release is a function of Ca^{2+} entry via L-type channels (Sheehan & Blatter, 2003) and ryanodine-induced changes in ANP release are concentration-dependent, as shown in the present data, the effects of ryanodine in the presence of nifedipine (1 μM) indicate the dependence of ryanodine on the Ca^{2+} entry via L-type Ca^{2+} channels. Because the SR Ca^{2+} load is positively related to the Ca^{2+} entry via L-type Ca^{2+} channels (Bers, 2000), the SR Ca^{2+} load should profoundly be decreased by channel blocker. Since the SR Ca^{2+} release is dependent on the SR Ca^{2+} load (Bers, 2000), a partial block of L-type Ca^{2+} channels may partially deplete the SR Ca^{2+} load, thus decreasing the SR Ca^{2+} release.

Also, the present finding suggests that atrial myocytic ANP release varies between beats, i.e., increase at relaxation and decrease at contraction. Therefore, it is likely that atrial myocytic ANP release into the ECS, which is the first step, occurs during diastole and then ECF translocation with released ANP, the second step, occurs during systole in terms of two-step sequential mechanism for ANP secretion. The present finding represents a homologous role for CICR in the excitation-contraction coupling of the myocardium. Therefore, it is possible that CICR is positively and negatively involved in the regulation of myocardial contraction and secretion, respectively.

Previously, we have shown that Ca^{2+} entry via L-type Ca^{2+} channel decreases atrial myocytic ANP release (Kim et al, 1997; Wen et al, 2000). Also, we have suggested that mechanically stimulated ANP release is influenced by a tonic inhibition by simultaneous Ca^{2+} entry via L-type Ca^{2+} channel activation in the beating atria (Wen et al, 2000). Therefore, the present data led us to propose that CICR as well as Ca^{2+} entry via L-type Ca^{2+} channel activation are involved in the tonic inhibition of mechanically stimulated ANP release in the atria.

In the present study, combined treatment of thapsigargin (1 μM) plus ryanodine (3 μM) for more than 36 min might have resulted in relatively depleted SR Ca^{2+} load. Previously, it was shown that, at moderately low SR Ca^{2+} load, Ca^{2+} release by CICR appears to fail (Bers, 2000). In a condition of relatively depleted SR Ca^{2+} release, nifedipine still increased ANP release. The response was slightly suppressed, but not significantly different from the values obtained from the atria with intact SR Ca^{2+} release (23.71 $\pm$6.00% in the combined presence of thapsigargin and ryanodine, n=7, vs. 32.74$\pm$7.14%, n=7; $P>0.05$). This suggests that the machinery related to the inhibition by Ca^{2+} of ANP release is spatially very close to the sarcolemmal L-type Ca^{2+} channel. Alternatively, Ca^{2+} entry via L-type Ca^{2+} channel is more sensitive than Ca^{2+} release from the SR to inhibit ANP release. This notion is based on the reports which showed CICR in atrial myocytes (Hueser et al, 1996; Sheehan & Blatter, 2003; Woo et al, 2003). Subsarcolemmal junctional SR is spatially and temporally related to the L-type Ca^{2+} channel and is involved in CICR in the atrial myocytes which are similar to ventricular myocytes (Bers et al, 1990; Cleemann & Morad, 1991).

It is noteworthy that thapsigargin decreased ANP release with concomitant increase in atrial stroke volume. It has been reported that store depletion in cardiomyocytes with thapsigargin increased Ca^{2+} entry via store-operated channel (Hunton et al, 2002; Pang et al, 2002; Hunton et al, 2004). If this was the case in our atrial preparation, the present data suggest that Ca^{2+} entry via store-operated channel is involved in the regulation of atrial dynamics and myocytic ANP release. Alternatively, an increase in intracellular Ca^{2+} resulting from the blockade of SR Ca^{2+}-reuptake by thapsigargin might be related to the decrease of ANP release with an increase of atrial dynamics. The present finding, showing a transient increase in atrial stroke volume by thapsigargin, is in agreement with an increase in contractility in electrically paced adult rabbit ventricular myocytes (Bassani et al, 1993) and paced (1 Hz) guinea-pig papillary muscle (Nario & Satoh, 1996).

It was shown that stretch-induced increase in ANP secretion depends on the ryanodine-sensitive Ca^{2+} store in perfused beating atria (Kuroski-De Bold & De Bold, 1991; Laine et al, 1994) and isolated perfused heart (Katoh et al, 1990). Isoproterenol-induced increase in ANP release is also attenuated by ryanodine in perfused beating atria (Schiebinger, 1989). Thapsigargin also blocked stretch-induced increase in ANP secretion in isolated perfused heart (Taskinen & Ruskoaho, 1996). In cultured ventricular or atrial myocytes, ryanodine blocked cAMP- or pacing-induced increase in ANP release (McDonough et al, 1994; Church et al, 2000). In contrast, it was also shown that ryanodine has no effect on stretch- or pacing-induced increase in ANP secretion in isolated atria (Page et al, 1990; Schiebinger et al, 1994). Similarly, in cultured atrial myocytes, ryanodine or thapsigargin has no effect on basal or pacing-induced ANP release (Iida & Page, 1989; Doubell & Thibault, 1994). Our data are in contrast with these reports. The difference may be due to the methodology used. We have proposed a two-steps sequential mechanism for the regulation of ANP release: myocytic ANP release and then convective translocation of ECF with released ANP (Cho et al, 1995). Although the myocytic release of ANP is a function of the atrial workload, the response is also inversely modulated by myocardial workload through an

increase in intracellular Ca^{2+} (Kim et al, 1997; Wen et al, 2000 and present data; Cho et al, 2002). Therefore, variable responses of the secretion of ANP to inotropic and chronotropic stimulations are expected.

In summary, Ca^{2+} entry through L-type Ca^{2+} channel decreases atrial myocytic ANP release via two distinct pathways: one directly and the other indirectly through SR Ca^{2+} release. The present data suggest that CICR is involved in the Ca^{2+}-induced negative regulation of atrial myocytic ANP release in coordination of atrial cycles.

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