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Subtype-specific roles of cAMP phosphodiesterases in regulation of atrial natriuretic peptide release

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Abstract

cAMP is known to control the release of atrial natriuretic peptide. To define the roles of cyclic nucleotide phosphodiesterase subtypes in the regulation of atrial natriuretic peptide (ANP) release, experiments were done with perfused beating rabbit atria. Phosphodiesterase 3 subtype-specific inhibitors, milrinone and cilostamide, inhibited myocytic ANP release with a concomitant increase in cAMP efflux. Similarly, trequinsin, another phosphodiesterase 3 inhibitor, decreased ANP release. A phosphodiesterase 4 subtype-specific inhibitor, rolipram, did not significantly change ANP release but increased AMP efflux. Also, 4-[(3-butoxy-4-methoxyphenyl)methyl]-2-imidazolidinone (Ro 20-1724), another phosphodiesterase 4 inhibitor, did not significantly change ANP release. The cAMP efflux was higher in the atrium treated with rolipram than in the atrium treated with milrinone or cilostamide. The data show that the cAMP pool, which is metabolized by phosphodiesterase 3, but not phosphodiesterase 4, is closely related to the basal regulation of atrial ANP release. The results suggest that intracellular cAMP is compartmentalized in the regulation of atrial ANP release, and that the release is controlled by a phosphodiesterase subtype-specific mechanism.

Keywords: ANP (atrial natriuretic peptide); Atrium; cAMP; Phosphodiesterase; ANP (atrial natriuretic peptide) secretion; ANP (atrial natriuretic peptide) release

1. Introduction

The cardiac atrium synthesizes and stores atrial natriuretic peptide (ANP) in myocytes (De Bold, 1985). Changes in atrial dynamics are closely related to the regulation of atrial ANP secretion (Dietz, 1984; Lang et al., 1985; Cho et al., 1993). The mechanism by which atrial stretch increases myocytic ANP release is not clearly understood.

There have been reports of variable modulators for the control of ANP secretion (Ruskoaho, 1992). The role of cyclic nucleotides in the regulation of ANP secretion has been of great interest. Recently, we have found that cGMP is a negative regulator of atrial myocytic ANP release (Lee et al., 2000). There are diverse reports on the effect of cAMP in the regulation of ANP secretion. Forskolin, an activator of adenylyl cyclase, has been shown to decrease ANP secretion from cultured atrial myocytes (Iida and Page, 1988; Shields and Glembotski, 1989; Muir et al., 1993) and perfused rat heart (Ruskoaho et al., 1990). 3-Isobutyl-1-methylxanthine (IBMX), a nonselective inhibitor of cyclic nucleotide phosphodiesterase (Iida and Page, 1988), and 8-bromoadenosine 3′,5′-cyclic monophosphate (Iida and Page, 1988; Shields and Glembotski, 1989) have also been shown to inhibit ANP secretion. Recently, it was shown that forskolin and IBMX inhibited ANP release via cAMP-protein kinase signaling in which forskolin-induced inhibition was a function of an accentuation of cAMP production (Cui et al., 2002). In contrast, it has also been shown that cAMP-elevating agents (Ruskoaho et al., 1986; Church et al., 1994; Azizi et al., 1995) and a cell membrane-permeant cAMP analogue (Schiebinger, 1988; Church et al., 1994; Azizi et al.,

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1995) increase ANP secretion. Hence, the current understanding of the cAMP-dependent regulation of ANP secretion is controversial.

The intracellular level of cAMP is determined by the rate of cAMP generation by adenylyl cyclase and its degradation by phosphodiesterases. It is likely that control of the degradation of cAMP is of fundamental importance in the regulation of cellular responses. At least four families of phosphodiesterases, phosphodiesterase 1, phosphodiesterase 2, phosphodiesterase 3, and phosphodiesterase 4, have been identified in the heart (Shahid and Nicholson, 1990; Fischmeister and Hartzell, 1991; Beavo, 1995). Phosphodiesterase 3 and phosphodiesterase 4, which have high affinity for cAMP, are the focus of interest. However, their respective roles in the cardiac atrium are not well understood. The cellular localization of cAMP and its hydrolyzing phosphodiesterase subtype isozymes are known to be compartmentalized (Buxton and Brunton, 1983; Fischmeister and Hartzell, 1991; Houslay and Milligan, 1997; Shakur et al., 2000; Houslay, 2001; Zaccolo and Pozzan, 2002). Especially, phosphodiesterase 4 isozymes are known to be compartmentalized and to be subjected to subtype-specific changes under certain circumstances (Kostic et al., 1997). Therefore, phosphodiesterase isozymes are expected to have distinct roles in intracellular signal processing. However, the regulation of ANP secretion has not been clarified. The purpose of the present study was to define the roles of phosphodiesterase subtypes, phosphodiesterase 3 and phosphodiesterase 4, in the regulation of ANP release. Experiments were carried out to test the effects of subtype-specific inhibitors of phosphodiesterase 3 and phosphodiesterase 4 on atrial myocytic ANP release in perfused beating rabbit atria.

2. Materials and methods

2.1. 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 atrial preparation was prepared by the method described previously (Cho et al., 1995; Cui et al., 2000), allowing atrial pacing and measurements of changes in atrial volume during contraction (stroke volume), transmural extracellular fluid (ECF) translocation, cAMP efflux and ANP secretion. The atrium was perfused with HEPES buffer solution by means of a peristaltic pump (1 ml/min).

2.2. Experimental protocols

The atria were perfused for 60 min to stabilize ANP secretion. [^{3}H]Inulin was introduced into the pericardial fluid 20 min before the start of sample collection (Cho et al., 1995; Wen et al., 2000). We collected the perfusate for analysis at 4 °C and at 2-min intervals. Atrial pacing at 0.8, 1, 1.3, 1.6 and 2 Hz was performed consecutively for 2 min at each frequency and repetitive frequency change. Repetitive frequency changes were separated by 2 min of 0.8-Hz pacing. Experiments were carried out using seven groups of atria. Milrinone (100 μM, $n=9$, group 1, Figs. 1, 6 and 7; 10 μM, $n=6$, group 2, Fig. 7; also cilostamide, 1 μM, $n=6$, group 3, Figs. 3 and 7, and trequinsin, 10 μM, $n=6$, group 4, Fig. 4) or rolipram {100 μM, $n=7$, group 5, Figs. 5–7; 10 μM, $n=6$, group 6, Fig. 7; also 4-[(3-butoxy-4-methoxyphenyl)methyl]-2-imidazolidinone (Ro 20-1724), 100 μM, $n=6$, group 7, Fig. 4}, phosphodiesterase 3- or phosphodiesterase 4-specific inhibitor, respectively was introduced

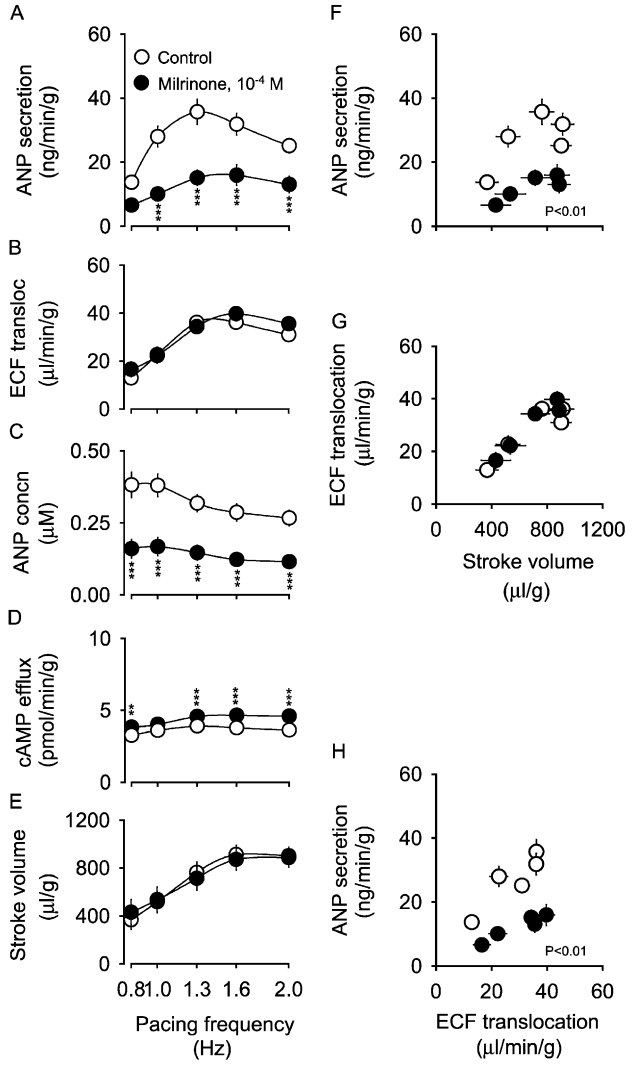

Fig. 1. Effects of milrinone (100 μM) on ANP secretion (A), extracellular fluid (ECF) translocation (transloc) (B), ANP concentration (concn) (C), cAMP efflux (D) and atrial stroke volume (E) in perfused beating rabbit atria (0.8, 1.0, 1.3, 1.6, 2.0 Hz) ($n=9$). Relationships between ANP secretion and atrial stroke volume (F), ECF translocation and atrial stroke volume (G), and ANP secretion and ECF translocation (H) were examined. Values are means ± S.E., $^{**}P<0.01$, $^{***}P<0.001$ vs. control.

into the perfusate just after the control cycle. One control cycle of 12 min was followed by three cycles of phosphodiesterase 3 or phosphodiesterase 4 inhibitor. The effects of agents were evaluated after two cycles of administration of the agent. For the time-matched control experiments, the atrium was stimulated with repetitive frequency change, and vehicle only was introduced (group 8, $n=11$; Fig. 2). Milrinone and rolipram were obtained from Sigma (St. Louis, MO). Cilostamide, trequinsin and Ro 20-1724 were from Biomol (Plymouth Meeting, PA). The concentrations of phophodiesterase inhibitors milrinone (10 and 100 μM), cilostamide (1 μM), trequinsin (10 μM) and rolipram (10 and 100 μM) were in the range of doses used previously (Floreani et al., 1997; Kajimoto et al., 1997; Bian et al., 2000; Friis et al., 2002).

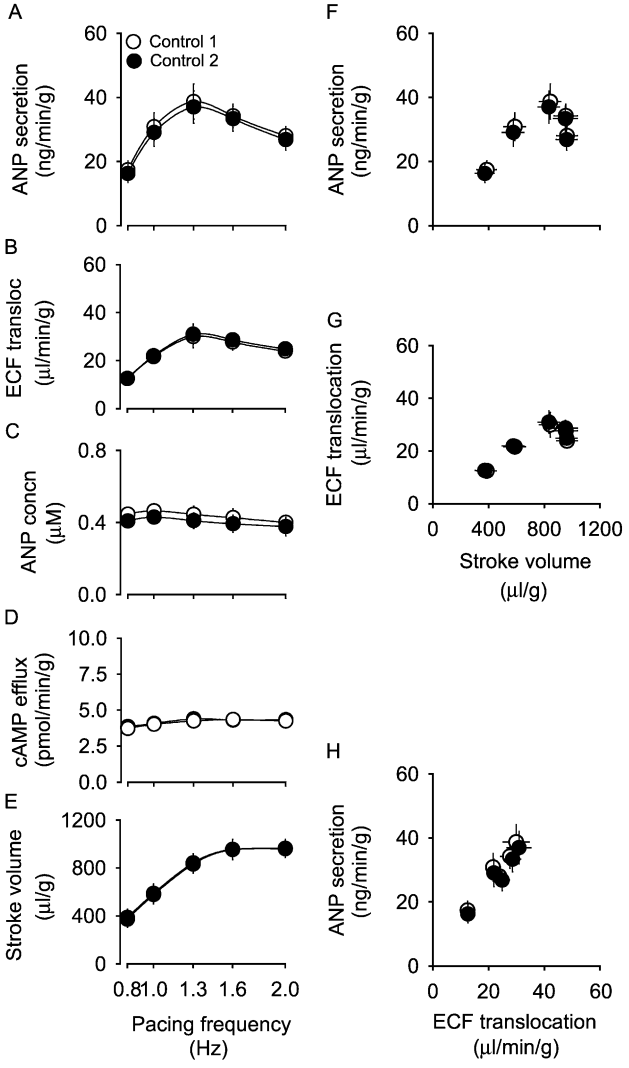

Fig. 2. Time-matched control levels of ANP secretion (A), ECF translocation (transloc) (B), ANP concentration (concn) in terms of ECF translocation (C), cAMP efflux (D) and atrial dynamics (E) ($n=11$). Relationships between ANP secretion and atrial stroke volume (F), ECF translocation and atrial stroke volume (G), and ANP secretion and ECF translocation (H) were examined. ○ and ● correspond to the control (Control 1) and experimental (Control 2) periods, respectively.

2.3. 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 is 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 and, therefore, indicates the rate of myocytic release of ANP into the surrounding paracellular space (Cho et al., 1993, 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., 1990).

2.4. Radioimmunoassay of cAMP

cAMP was measured with an equilibrated radioimmunoassay (Cui et al., 2000). Briefly, standards and samples were taken up in a final volume of 100 μl of 50 mM sodium acetate buffer (pH 4.8) containing theophylline (8 mM), and then 100 μl of diluted cAMP antiserum (Calbiochem-Novabiochem, San Diego, CA) and iodinated 2′-*O*-monosuccinyl-adenosine 3′,5′-cyclic monophosphate tyrosyl methyl ester {[^{125}I]ScAMP-TME, 10,000 cpm/100 μl} were added and incubated for 24 h at 4 °C. For the acetylation reaction, 5 μl of a mixture of acetic anhydride and triethylamine (1:2) was added to the assay tube before the addition of antiserum and tracer. The bound form was separated from the free form by charcoal suspension. [^{125}I]ScAMP-TME was prepared as described previously (Steiner et al., 1972). Immediately before use, [^{125}I]ScAMP-TME was repurified by high-performance liquid chromatography on a reversed phase μBondapak column (Waters Associates, Milford, MA) with a linear gradient (0–60% acetonitrile in 0.1% trifluoroacetic acid) elution. Radioimmunoassay for cAMP was done on the day of the experiments, and all samples from one experiment were analyzed in a single assay. Nonspecific binding was <2.0%. The 50% intercept was at 16.5 ± 0.79 fmol/tube ($n=10$). The intra- and interassay coefficients of variation were 5.0% ($n=10$) and 9.6% ($n=10$), respectively. The amount of cAMP efflux is expressed as pmol cAMP min^{-1} g^{-1} atrial tissue.

For the preparation of perfusates, 100 μl of the perfusate was treated with trichloroacetic acid (900 μl) to a final concentration of 6% for 15 min at room temperature and centrifuged at 4 °C. The supernatant (500 μl) was transferred to a polypropylene tube and extracted with water-saturated ether (1 ml) three times, and then dried using a SpeedVac concentrator (Savant, Farmingdale, NY). The

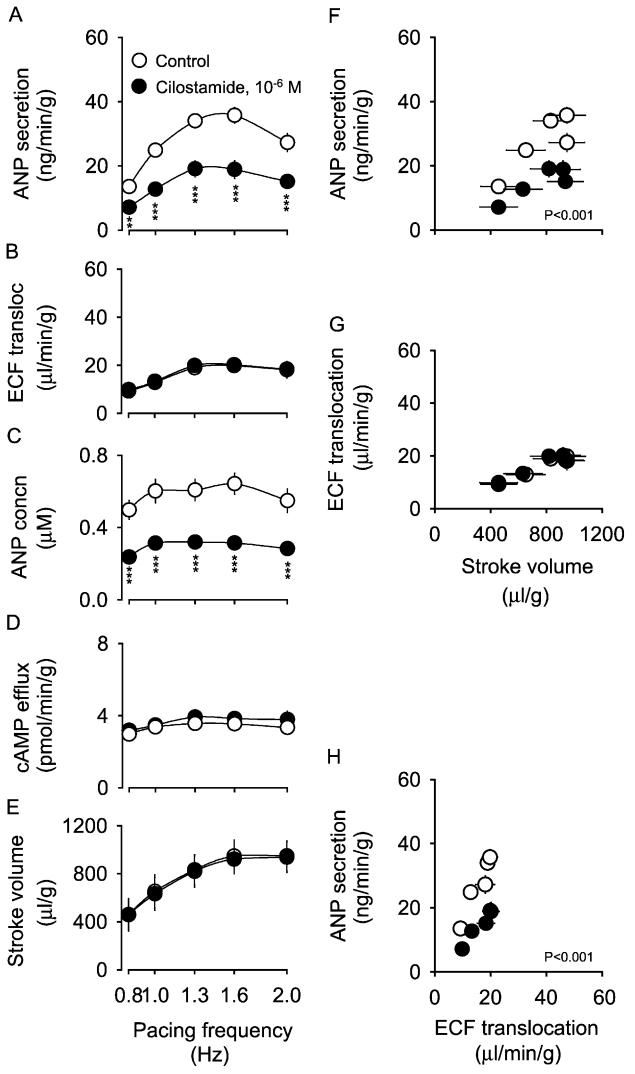

Fig. 3. Effects of cilostamide (1 µM) on ANP secretion (A), extracellular fluid (ECF) translocation (transloc) (B), ANP concentration (concn) (C), cAMP efflux (D) and atrial stroke volume (E) in perfused beating atria (0.8, 1.0, 1.3, 1.6, 2.0 Hz) ($n=6$). Relationships between ANP secretion and atrial stroke volume (F), and ECF translocation and atrial stroke volume (G), and ANP secretion and ECF translocation (H) were examined. Values are means ± S.E.

dried samples were resuspended with sodium acetate buffer.

2.5. Statistical analysis

Differences were compared with the use of a two-way analysis of variance for repeated measures (Figs. 1–3 and 5F–H; and 4B and D). Significant differences between paired data for a given pacing frequency (Figs. 1–3 and 5A–E; and 4A and C) were analyzed by repeated measures analysis of variance (ANOVA), followed by Bonferroni's multiple-comparison test. Student's *t*-test for unpaired data (Figs. 6 and 7) was also applied. Statistical significance was defined as $P<0.05$. The results are given as means ± S.E.

3. Results

3.1. Phosphodiesterase 3 inhibitor decreases ANP secretion with a slight increase in cAMP efflux

An increase in pacing frequency resulted in an increase in ANP secretion and ECF translocation with waning of the responses at a higher atrial rate (Fig. 1A and B). The concentration of ANP in the perfusate in terms of ECF translocation, which reflects the concentration of ANP released into the paracellular space of the atrium (Cho et al., 1995; Lee et al., 2000), was 0.25–0.40 µM (Fig. 1C). The rate of cAMP efflux into perfusate was 3.0–4.0 pmol min^{-1} g^{-1} (Fig. 1D). Atrial stroke volume increased in response to a stepwise increase in atrial rate (Fig. 1E). Both the changes in ANP secretion and ECF translocation were a function of the change in atrial stroke volume (Fig. 1F and G). The change in ANP secretion was well correlated with ECF translocation (Fig. 1H).

Milrinone, a specific inhibitor of phosphodiesterase 3, inhibited ANP secretion ($P<0.001$ at 1.0–2.0 Hz; Fig. 1A). Milrinone (100 µM; $P<0.001$; Fig. 1C) significantly decreased the concentration of ANP in the perfusate in terms of ECF translocation. Milrinone ($P<0.01$; Fig. 1D) slightly but significantly increased the level of cAMP in the perfusate. Milrinone did not change ECF translocation (Fig. 1B) and atrial stroke volume (Fig. 1E) in response to increased pacing frequency. Milrinone shifted the relationships between ANP secretion and atrial stroke volume or ECF translocation downward ($P<0.01$; Fig. 1F and H), which indicated inhibition of the myocytic release of ANP.

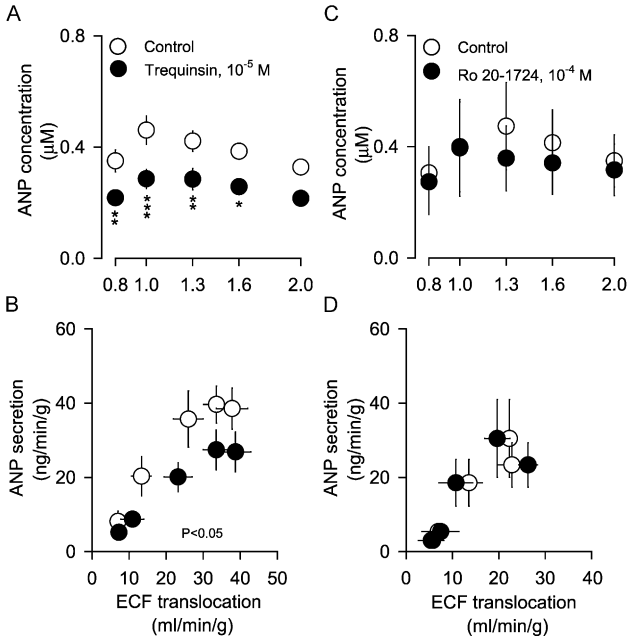

Fig. 4. Effects of trequinsin (10 µM; A and B, $n=6$) and Ro 20-1724 (100 µM; C and D, $n=6$) on ANP concentration in perfusate in terms of ECF translocation (A and C) and relationship between ANP secretion and ECF translocation (B and D). $*P<0.05$, $**P<0.01$, $***P<0.001$ vs. control.

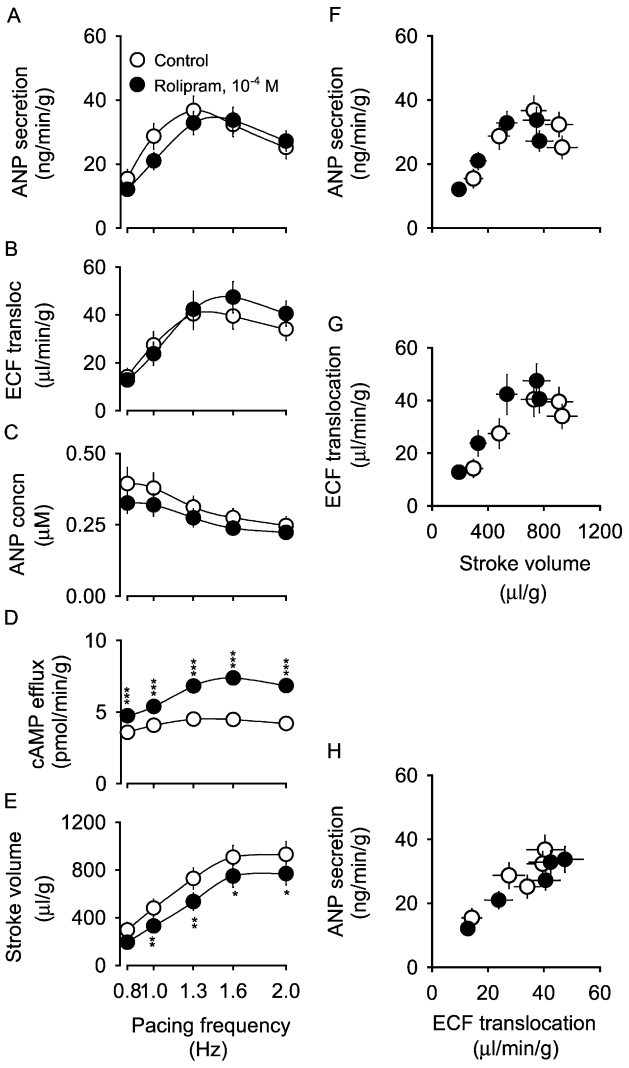

Fig. 5. Effects of rolipram (100 µM) on ANP secretion (A), extracellular fluid (ECF) translocation (transloc) (B), ANP concentration (concn) (C), cAMP efflux (D) and atrial stroke volume (E) in perfused beating rabbit atria (0.8, 1.0, 1.3, 1.6, 2.0 Hz) ($n=7$). Relationships between ANP secretion and atrial stroke volume (F), ECF translocation and atrial stroke volume (G), and ANP secretion and ECF translocation (H) were examined. * $P<0.05$, ** $P<0.01$, *** $P<0.001$ vs. control.

The relationship between ECF translocation and atrial stroke volume was not significantly changed by milrinone (Fig. 1G). A lower dose of milrinone (10 µM) had very similar effects (Fig. 7). For the time-matched control, changes in ANP secretion, ECF translocation, cAMP efflux and atrial stroke volume in response to repetitive changes in pacing frequency were constant and stable (Fig. 2). The responses were reproducible during the periods corresponding to the control and experimental observations. Differences between periods were not significant.

Cilostamide and trequinsin, and other phosphodiesterase 3 subtype-specific inhibitors, also showed similar effects. Cilostamide (1 µM) significantly decreased ANP secretion and its concentration (Fig. 3A and C). Cilostamide shifted the relationship between ANP secretion and atrial stroke volume or ECF translocation downward ($P<0.001$; Fig. 3F and H). A low dose of cilostamide increased cAMP efflux slightly but not significantly. At a pacing frequency of 1.6 Hz, the change was significant ($P<0.01$; Fig. 7). As shown in Fig. 4A and B, trequinsin (10 µM) significantly decreased ANP concentration ($P<0.05$ at 0.8–1.6 Hz) and shifted ($P<0.05$) the relationship between ANP secretion and ECF translocation downward, which indicated inhibition of the myocytic release of ANP.

3.2. Phosphodiesterase 4 inhibitor does not change ANP secretion but causes a large increase in cAMP efflux

In a separate series of experiments, the effects of rolipram, a specific inhibitor of phosphodiesterase 4, were tested. As shown in Fig. 5A and C, ANP secretion and ANP concentration after rolipram were not significantly different from those of the control period. Rolipram significantly increased the levels of cAMP in the perfusate (Fig. 5D). The increase in cAMP efflux induced by rolipram was larger than that induced by milrinone ($P<0.01$ at 0.8–2.0 Hz; Fig. 6). Rolipram decreased atrial stroke volume slightly but significantly ($P<0.05$ at 1.0–2.0 Hz; Fig. 5E). Changes in ECF translocation were not significant (Fig. 5B). The relationships between ANP secretion and atrial stroke volume or ECF translocation, and ECF translocation and atrial stroke volume were not changed by rolipram (Fig. 5F, G and H). This means that the myocytic release of ANP was not affected by rolipram.

Ro 20-1724, another phosphodiesterase 4 subtype-specific inhibitor, also showed similar effects. As shown in Fig. 4C and D, Ro 20-1724 did not significantly change ANP concentration and the relationship between ANP secretion

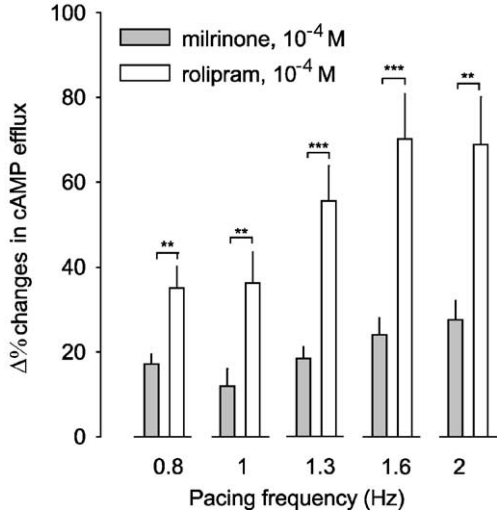

Fig. 6. Comparison of the effects of milrinone (100 µM) and rolipram (100 µM) on atrial cAMP efflux. Data were derived from Figs. 1D and 5D. Δ% changes are the difference in percent changes between experimental and control periods at a given pacing frequency. Values are means ± S.E.

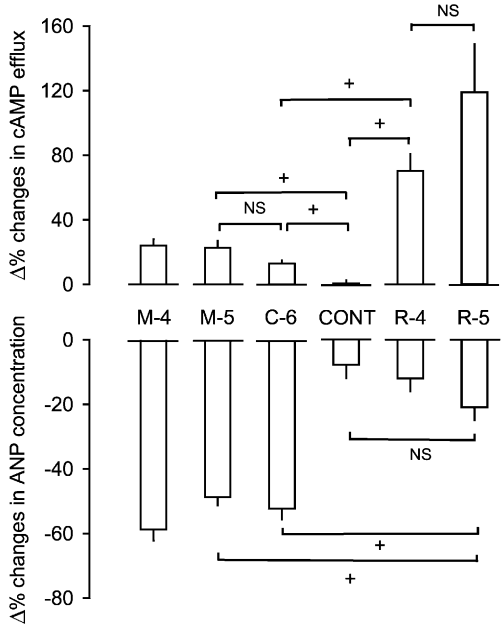

Fig. 7. Comparison of the effects of milrinone (100 μM, M-4, data derived from Fig. 1D; 10 μM, M-5, $n=6$), cilostamide (1 μM, C-6, data derived from Fig. 3D) and rolipram (100 μM, R-4, data derived from Fig. 5D; 10 μM, R-5, $n=6$) on atrial ANP release (ANP concentration) and cAMP efflux. Δ% changes are the difference in percent changes between experimental and control periods at 1.6 Hz. CONT, time-matched control experiments ($n=11$). Values are means ± S.E. $+P<0.01$; NS, not significant.

and ECF translocation. This means that the myocytic ANP release was not affected by Ro 20-1724.

As shown in Fig. 7, rolipram (10–100 μM) significantly increased cAMP efflux without changing ANP concentration, while milrinone (10–100 μM) and cilostamide (1 μM) significantly decreased ANP concentration with a slight but significant increase in cAMP efflux.

4. Discussion

The present study shows that phosphodiesterase inhibitors elicit distinct effects on atrial ANP release in a subtype-specific manner. Phosphodiesterase 3 but not phosphodiesterase 4 subtype inhibitors alone decreased myocytic ANP release. Both inhibitors increased cAMP production. These data suggest that phosphodiesterase 3 is closely related to the basal regulation of ANP release. In contrast, phosphodiesterase 4 may not be. Although the increase in atrial cAMP production was evident in both atria treated with milrinone, cilostamide or rolipram, inhibition of ANP release was observed in the atria treated with milrinone or cilostamide. Since the intracellular localization of phosphodiesterase 3 and phosphodiesterase 4 is particulate membrane and cytosolic, respectively, in the rabbit heart (Kithas et al., 1989; Shahid and Nicholson, 1990; Fischmeister and Hartzell, 1991) and the phosphodiesterases are compartmentalized (Shakur et al., 2000; Houslay, 2001), the data suggest that intracellular cAMP is compartmentalized and differently regulated by phosphodiesterase subtypes in the regulation of ANP release. In the cardiac atrium, the cAMP pool which is metabolized by phosphodiesterase 3 may be closely involved in the basal regulation of myocytic ANP release. It is possible that different cell types may be related to the distinct effects of milrinone and rolipram, but it is likely that two isozymes function within myocytes in which ANP is synthesized, stored and released. Since phosphodiesterase 3 and phosphodiesterase 4 are present and sensitive to milrinone and rolipram, respectively, in rabbit ventricular muscle (Shahid and Nicholson, 1990) and both inhibitors increase cAMP production in the rabbit atrium (present data), it is possible to interpret the results in terms of cAMP production. The distinct effects of phosphodiesterase 3 and phosphodiesterase 4 inhibitors on the regulation of ANP release observed under baseline conditions would not be observed in the cAMP-elevating condition, because the compartmentalization of cAMP may be disrupted when there is a global increase in intracellular cAMP (Houslay and Milligan, 1997). Or, under certain circumstances in which there are phosphodiesterase isozyme-specific changes, as shown in preconditioned heart (Kostic et al., 1997), phosphodiesterase 4 may also be involved in the basal regulation of ANP release. This notion is consistent with previous reports showing that cAMP is compartmentalized in the heart and differently regulated by phosphodiesterases (Buxton and Brunton, 1983; Zaccolo and Pozzan, 2002), and that phosphodiesterase 4 isozymes (Houslay, 2001) and phosphodiesterase 3 (Shakur et al., 2000) are differently targeted, implying distinct compartments.

The mechanism by which cAMP inhibits ANP release is not yet clearly defined. An increase in intracellular cAMP may affect several downstream signaling pathways for the regulation of ANP release, such as an increase in Ca^{2+} influx via L-type Ca^{2+} channells, protein phosphorylation via cAMP-dependent protein kinase activation and other pathways. It was shown in this laboratory that an increase in Ca^{2+} influx via L-type Ca^{2+} channells inhibits, whereas influx via T-type channells stimulates, mechanically stimulated atrial ANP release. Changes in intracellular Ca^{2+} metabolism of the sarcoplasmic reticulum were shown to be involved in both positive (Laine et al., 1994) and negative (Li et al., unpublished data) regulation of ANP release. Although it is known that protein kinase C activation increases ANP release (Ruskoaho et al., 1986; Shields and Glembotski, 1989; Ruskoaho, 1992), the roles of cAMP-dependent protein phosphorylation in the regulation of ANP release have still to be defined.

The present data showing an increase in cAMP production by phosphodiesterase 3 and phosphodiesterase 4 inhibitors are in contrast to the report by Kelso et al. (1995), in which cAMP was not accumulated in the presence of phosphodiesterase 3 or phosphodiesterase 4 inhibitors in isolated rat ventricular cardiomyocytes. The difference is

possibly related to the different methodology, species and tissues. It has been shown that the subcellular distribution of phosphodiesterase 3 and phosphodiesterase 4 in the myocardium is species dependent (Shahid et al., 1990).

cAMP-elevating agents, phosphodiesterase inhibitor and adenylyl cyclase activator, modulate ANP secretion. However, little information is available on the distinct roles of phosphodiesterase subtypes in the regulation of ANP release. The nonspecific phosphodiesterase inhibitor, IBMX, decreases ANP secretion (Iida and Page, 1988; Cui et al., 2002). Also, the adenylyl cyclase activator, forskolin, has been shown to increase (Ruskoaho et al., 1986; Azizi et al., 1995) or decrease (Iida and Page, 1988; Shields and Glembotski, 1989; Ruskoaho et al., 1990; Muir et al., 1993; Cui et al., 2002) ANP secretion.

Although phosphodiesterase 3 and phosphodiesterase 4 inhibitors increased atrial cAMP production, these agents failed to increase atrial dynamics. This is consistent with previous reports showing a lack of positive inotropic response to rolipram in guinea pig atria (Muller et al., 1990), and rabbit and rat ventricular papillary muscles (Shahid and Nicholson, 1990). Similarly, Frangakis et al. (1989) showed that milrinone did not significantly change the contractility of rat perfused heart. However, the lack of positive inotropic response to milrinone contrasts with a previous report showing a positive inotropic effect of milrinone in rabbit ventricular and atrial muscles (Alousi et al., 1983). These authors also presented data showing a much smaller response of the atrium compared to that of the ventricular papillary muscle. Even though the particulate phosphodiesterase activity associated with rabbit ventricular sarcoplasmic reticulum is very sensitive to milrinone (Kithas et al., 1988; Shahid and Nicholson, 1990), the inhibitor did not increase atrial dynamics. This may be related to the difference in the regulation of Ca^{2+} metabolism and myocardial dynamics between atrial and ventricular myocardium. These results suggest that the increase in cAMP levels produced by phosphodiesterase 3 or phosphodiesterase 4 inhibition is compartmentalized and unable to change rabbit atrial dynamics. It is known that the subcellular compartmentalization of cAMP is related to the functional diversity of Ca^{2+} transients and cardiac dynamics (Hohl and Li, 1991; Bers and Ziolo, 2001). In the present experiments, the phosphodiesterase 4 inhibitor, rolipram, inhibited atrial dynamics. This may be related to the intracellular acidosis caused by elevated cAMP (Shida et al., 1994; Vila Petroff et al., 2001). These authors showed that an increase in intracellular cAMP enhanced glycolysis and in turn caused a decrease in intracellular pH in cardiac myocytes. A decrease in intracellular pH is known to be related to reduced myofilament Ca^{2+} responsiveness (Fabiato and Fabiato, 1978).

In the past, phosphodiesterase 3 inhibitors were considered as potential therapeutics for the treatment of cardiovascular diseases. The decrease induced by phosphodiesterase 3 inhibitors in the intercellular ANP concentration may have some beneficial effect in cardiac remodeling because the natriuretic peptide receptor is down-regulated with accentuated ANP release in cardiac hypertrophy (Kim et al., 1999).

In summary, phosphodiesterase 3 but not phosphodiesterase 4 inhibitors alone decreased atrial myocytic ANP release with an increase in cAMP production. These data suggest that phosphodiesterase 3 but not phosphodiesterase 4 activity is closely related to the basal regulation of ANP release. This is the first report showing an involvement of phosphodiesterase isozymes in the compartmentalization of cAMP signaling in the regulation of atrial ANP release.

Acknowledgements

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