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Effects of pituitary adenylate cyclase activating polypeptide27 on cyclic AMP efflux and atrial dynamics in perfused beating atria

Xun Cui, Sook Jeong Lee, Sung Zoo Kim, Suhn Hee Kim, Kyung Woo Cho *

Department of Physiology, Medical School and Institute for Medical Sciences, Jeonbug National University, 2-20 Keum-Am-Dong-San, Jeonju 561-180, South Korea

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Abstract

Although pituitary adenylate cyclase activating polypeptide (PACAP) has been shown to increase cardiac force of contraction and to change the heart rate, the effect of PACAP on cyclic (c) AMP production in the atrium still has to be defined. In the present experiments, a simple protocol was developed for the evaluation of cAMP production in real-time base in the perfused beating left atria. The PACAP27-induced cAMP efflux in the atrial perfusate reflected changes in the production of cAMP in the atrial tissue. cAMP efflux was measured as an indicator of cAMP production in beating perfused rabbit atria. PACAP27 increased cAMP production in a dose- and time-dependent manner with a minor effect on atrial dynamics. These results suggest that PACAP27 has other roles besides control of force of contraction through cAMP production in the atrium.

Keywords: Atrium; cAMP; Pituitary adenylate cyclase activating polypeptide (PACAP)

1. Introduction

Pituitary adenylate cyclase activating polypeptide (PACAP) was first isolated by Miyata et al. (1989) from the bovine hypothalamus based on its property to stimulate adenylyl cyclase. PACAP is present in two forms, PACAP38 and PACAP27 (Miyata et al., 1990). PACAP38 has been shown to stimulate the release of growth hormone, prolactin, corticotropin and luteinizing hormone in pituitary cells (Miyata et al., 1989). PACAP has been identified in many discrete tissues including testis and pituitary gland as well as hypothalamus (Arimura et al., 1991). The atrium from rats has also been shown to contain PACAP38 and PACAP27 (Arimura et al., 1991). The PACAP receptor has been identified in the heart (Inagaki et al., 1994; Sreedharan et al., 1995; Wei and Mojsov, 1996). Cardiac effects of PACAP have been shown to increase the force of contraction (Ross-Ascuitto et al., 1993; Ascuitto et al., 1996; Yonezawa et al., 1996) and to alter the heart rate (Yonezawa et al., 1996; Hirose et al., 1997). Although PACAP has been shown to increase cyclic (c) AMP production in cultured neonatal cardiac myocytes (Suzuki et al., 1993) and the cardiac effect of PACAP was suggested to be related to cAMP production (Ross-Ascuitto et al., 1993; Ascuitto et al., 1996), the effect of PACAP on the cAMP production of the atrium has not been defined. In the present experiments, a simple protocol was developed for the evaluation of cAMP production in real-time base in beating atria. cAMP efflux in the atrial perfusate was positively related to the production of cAMP in atrial tissue. With this new protocol, we defined the effects of PACAP27 on atrial dynamics and changes in cAMP production in beating atria.

2. Materials and methods

2.1. Beating perfused rabbit atrial preparation

New Zealand white rabbits were used. An isolated perfused atrial preparation was prepared by a method described previously (Cho et al., 1995). All experiments were carried out under approval of the Ethics Committee in the Institute for Medical Sciences of Jeonbug National University. The hearts were removed and the left atrium was dissected. A calibrated transparent atrial cannula containing two small catheters was inserted into the left

* Corresponding author. Tel.: +82-652-274-9788; fax: +82-652-274-9892.

E-mail address: kwcho@moak.chonbuk.ac.kr (K.W. Cho).

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atrium. The cannulated atrium was transferred to an organ chamber and immediately perfused with HEPES buffer solution. Soon after setting up of the perfused atrium, transmural electrical field stimulation with a luminal electrode was started at 1.3 Hz (duration, 0.3 ms; voltage, 20–30 V).

The changes in atrial stroke volume were monitored by reading the lowest level of the water column in the calibrated atrial cannula during end diastole (Cho et al., 1995). Atrial pulse pressure was measured via a pressure transducer connected to the intra-atrial catheter and recorded on a physiograph. To estimate transendocardial extracellular fluid (ECF) translocation, transmural atrial clearance of [^{3}H]inulin was measured as described previously (Cho et al., 1993, 1995). Radioactivity in the atrial perfusate and pericardial buffer solution was measured, and the amount of ECF translocated through the atrial wall was calculated.

2.2. Experimental protocols

The atria were perfused for 60 min to stabilize cAMP efflux and atrial dynamics. [^{3}H]inulin was introduced to the pericardial fluid 20 min before the start of sample collection. The perfusate was collected at 2-min intervals at 4°C for analyses. The atria were paced at 1.3 Hz. The control period (12 min) was followed by an infusion of PACAP27 or 3-isobutyl-1-methylxanthine (IBMX), an inhibitor of phosphodiesterase, plus PACP27 (group 1; see Figs. 2 and 3). To analyze the effects of PACAP27 on cAMP production and atrial dynamics, the control period (12 min) was followed by an infusion of PACAP 27 for 36 min (group 2; see Fig. 4) or infusions of variable doses of PACAP27 or isoproterenol for 12 min, spaced by 24-min recovery periods (group 3; see Figs. 5 and 7). For the time-control (group 4), vehicle was introduced and the values obtained during the periods corresponding to the control and experimental observations were compared (Fig. 9). In another group of experiments, to define the relationship between cAMP efflux and ECF translocation, atrial pacing at 0.8, 1, 1.3, 1.6 and 2 Hz was performed consecutively for 2 min at each frequency with repetitive frequency changes (see Fig. 1). Repetitive frequency changes were spaced by 2 min of 0.8-Hz pacing. PACAP27 was introduced after the first control cycle (for 12 min) and continued for three cycles (for 36 min). For the evaluation of effects, values from a control period were compared to those from the third cycle of the experimental period.

2.3. Radioimmunoassay of cAMP

Production of cAMP was measured by equilibrated radioimmunoassay. Briefly, standards or 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). Briefly, 2 μg of ScAMP-TME (Sigma, St. Louis, MO) was introduced into a vial containing 100 mM phosphate buffer (pH 7.4) followed by addition of 1 mCi of [^{125}I]Na (Amersham International, Buckinghamshire). Chloramine-T (0.4 mg/ml) was added to the reaction vial (total reaction volume is 50 μl), mixed gently, and 1 min later, the reaction was terminated with sodium metabisulfite (0.2 mg/ml) and NaI (5 mM). The reaction mixture was immediately applied to a Sephadex G-10 column (1 × 20 cm) previously washed with 10 mM phosphate buffer. [^{125}I]ScAMP-TME was eluted with 10 mM phosphate buffer containing 150 mM NaCl (pH 7.4),

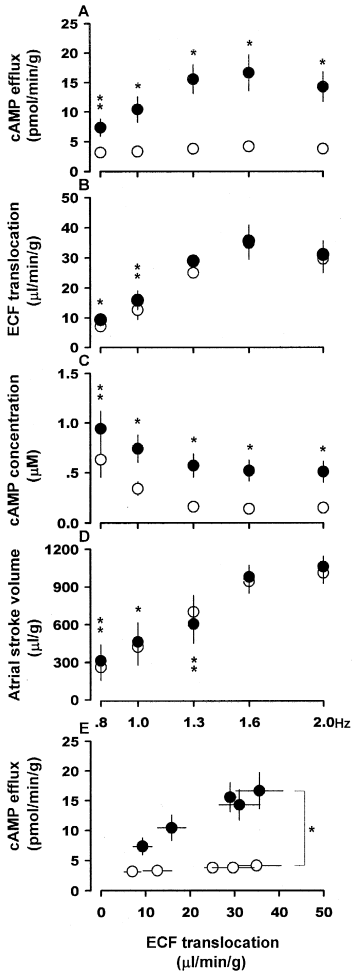

Fig. 1. Effects of PACAP27 on atrial efflux of cAMP (A), ECF translocation (B), cAMP concentration in terms of ECF translocation (C) and atrial stroke volume (D) in beating rabbit atria (0.8–2.0 Hz). The Relationship between cAMP efflux and ECF translocation is shown in panel E. ○ Control period, ● experimental period, third cycle of PACAP; PACAP27, 30 nM. $^{**}P < 0.05$; $^{*}P < 0.01$ experimental vs. control periods.

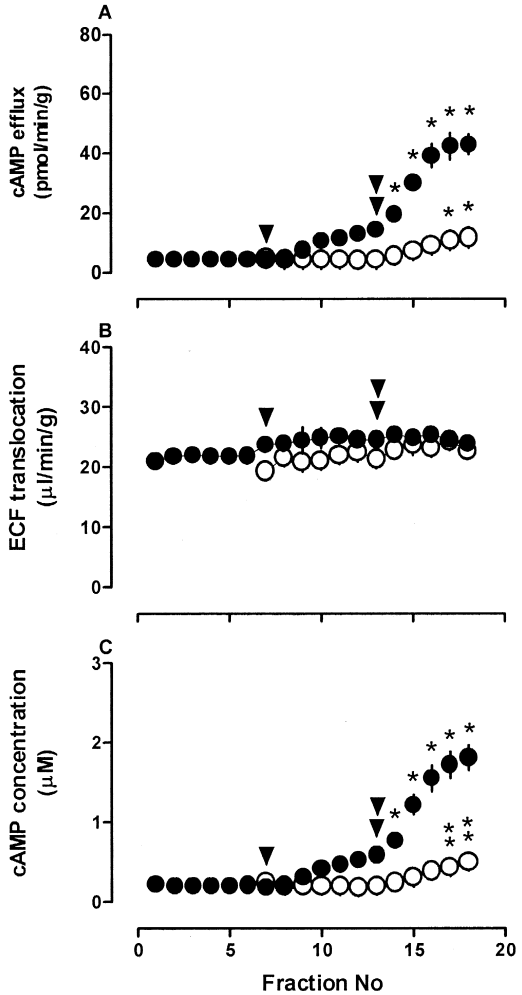

Fig. 2. Accentuation by IBMX (0.3 mM) of the effect of PACAP27 (100 nM) on cAMP efflux (A) and cAMP concentration (C) in terms of (B) ECF translocation in beating atria (1.3 Hz). ○ Control atria, six 2-min sample collections for control are followed by six 2-min sample collections for PACAP (double arrowhead); ● IBMX-treated atria, six 2-min sample collections for control are followed by IBMX-treated (single arrowhead) and IBMX + PACAP-treated sample collections. Fraction No, serial 2-min sample collections as in Section 2. $^{**}P < 0.05$; $^{*}P < 0.001$ experimental vs. control periods.

and stored at −20°C until use. Immediately before using, [^{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. Non-specific binding was $< 2.0\%$. The 50% intercept was at 16.50 ± 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 was expressed as pmol cAMP/min/g atrial tissue. The molar concentration of cAMP efflux in terms of ECF translocation, which may reflect the concentration of cAMP in the interstitial space fluid (Cho et al., 1993, 1995), was calculated as follows.

$$\text{cAMP efflux}\,(\mu\text{M}) = (\text{cAMP in pmol/min/g}) / (\text{ECF translocated in } \mu\text{l/min/g})$$

2.4. Preparation of samples

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 dried samples were resuspended with sodium acetate buffer. For preparation of the atrium, perfused atrial tissue was separated from the atrial cannula, lightly blotted and quickly frozen in liquid nitrogen. Atrial tissue was minced in 2 ml ice-cold trichloroacetic acid (6%) solution and homogenized at 4°C by three 30-s bursts in a Polytron homogenizer. The homogenates were centrifuged at 1000 $\times g$ for 10 min at 4°C, and the supernatant was subjected to ether extraction as described previously. The pellet was treated with NaOH (1 N) and ultrasonication and used for protein determination.

2.5. Statistical analyses

The significance of differences was determined with a two-way analysis of variance (ANOVA) for repeated measures (see Fig. 1E), one-way ANOVA followed by Bonferroni's multiple comparison (see Figs. 2, 4, 5, 7 and 9) and the Duncan multiple range test (see Figs. 6 and 8). Student's *t*-test for paired data from a given pacing frequency (see Fig. 1A–D) was also applied. Correlation coefficients were determined using least-squares linear regression anal-

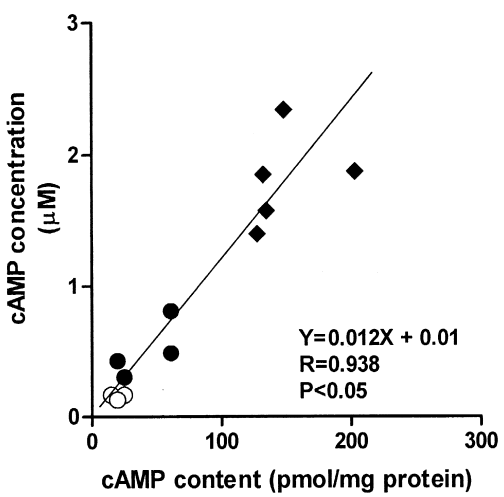

Fig. 3. The relationship between cAMP concentration in terms of ECF translocation and atrial cAMP content. ○ Control; ● PACAP27, 100 nM; ♦ IBMX, 0.3 mM, plus PACAP27, 100 nM.

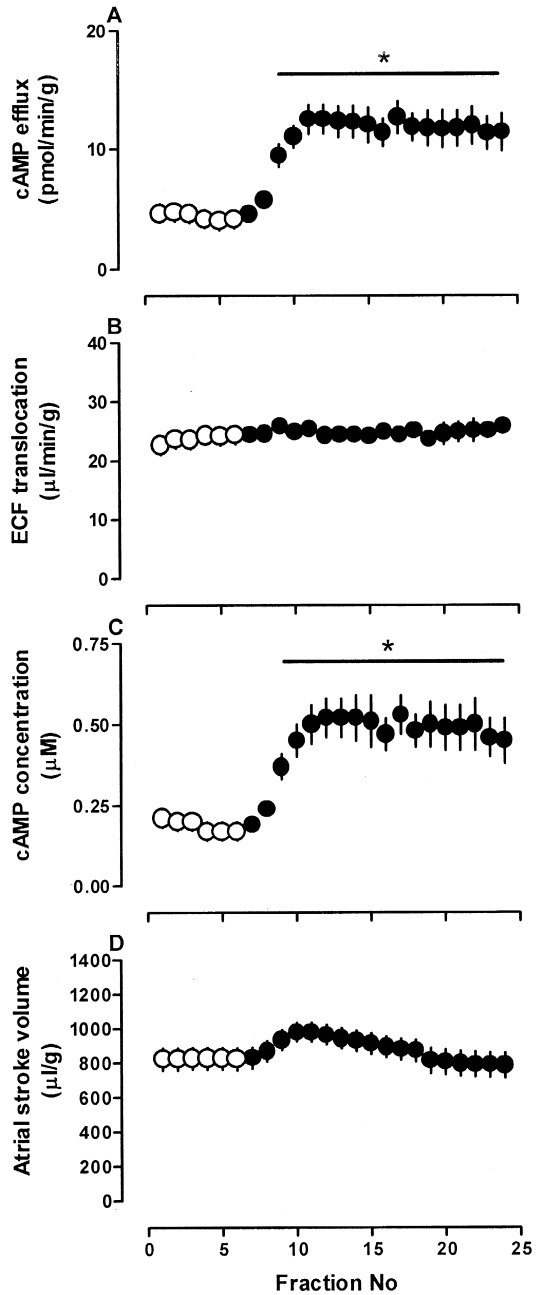

Fig. 4. Sustained effect of PACAP27, on cAMP efflux (A), (B) ECF tranlocation and cAMP concentration (C) with a minor effect on atrial stroke volume (D). ○ Control; ● PACAP27, 30 nM. Fraction No, serial 2-min sample collections. * Different from the control value at $P < 0.001$.

ysis (see Fig. 3). Statistical significance was defined as $P < 0.05$. The results are given as means ± S.E.M.

3. Results

3.1. Effects of PACAP27 on the atrial efflux of cAMP and tissue cAMP content: linear relationship between cAMP efflux and tissue cAMP

Because the amount of cAMP in the lumen appeared to be produced by atrial cells may be related with atrial dynamics and therefore ECF translocation (Cho et al., 1993, 1995), cAMP efflux was analyzed in terms of ECF translocation. The concentration of cAMP in terms of ECF

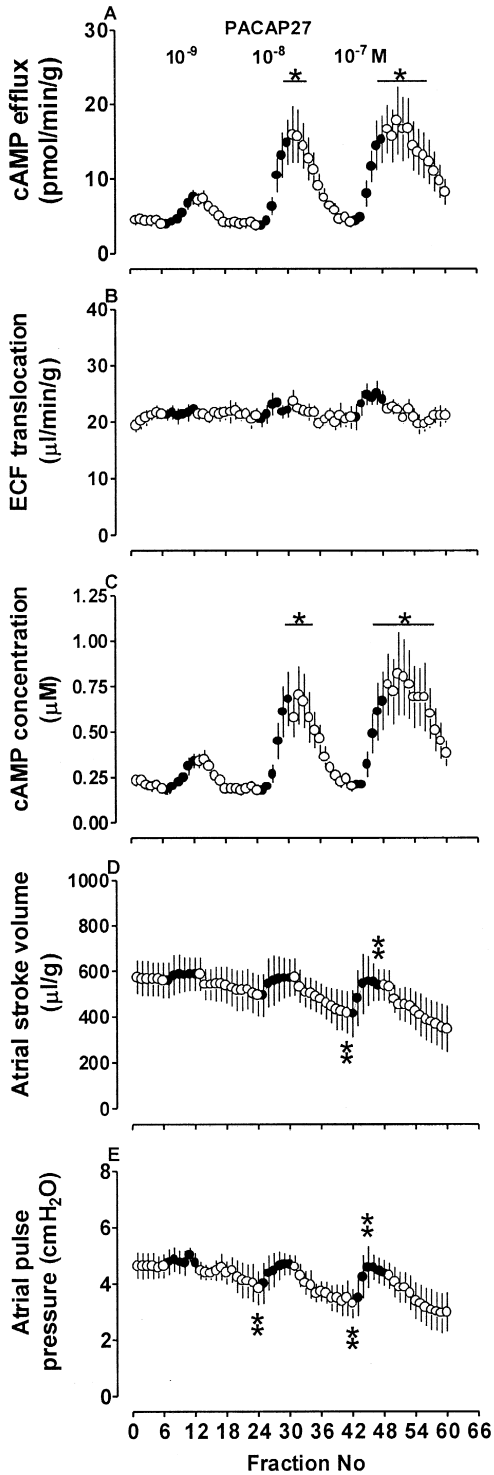

Fig. 5. Dose- and time-dependent effects of PACAP27 on cAMP efflux (A), (B) ECF translocation, cAMP concentration (C) and atrial dynamics (D and E). ○ Regular buffer; ● PACAP27 in a dose indicated. Fraction No, serial 2-min sample collections. ** $P < 0.05$; * $P < 0.01$ experimental vs. control periods.

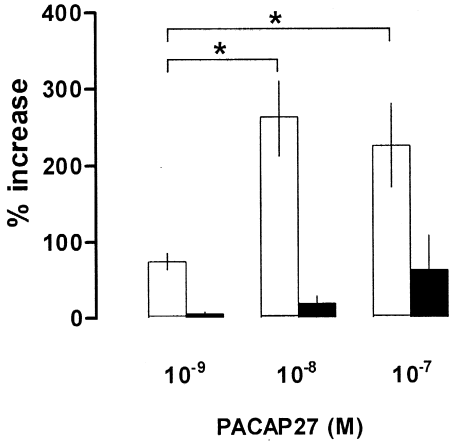

Fig. 6. Peak differences of changes induced by PACAP27 in atrial cAMP efflux and atrial stroke volume. □ Percent changes in cAMP efflux, ■ percent changes in atrial stroke volume. * $P < 0.05$.

translocation may reflect cAMP concentration in the interstitium and also the rate of cAMP efflux from atrial cells.

One series of experiments tested the function of ECF translocation on the cAMP efflux in the perfusate. In these experiments, the atria were paced at increasing pacing frequency (0.8–2 Hz) to change atrial dynamics and ECF translocation (Cho et al., 1993, 1995). As shown in Fig. 1, the increase in pacing frequency resulted in an increase in atrial stroke volume (D) and ECF translocation (B) ($n = 7$). cAMP efflux in the perfusate was fairly constant during the control period (Fig. 1A). PACAP27, 30 nM, increased cAMP efflux and cAMP concentration in terms of ECF translocation (Fig. 1A and C, $n = 7$). The change in ECF translocation after PACAP27 was slightly different from that in the control at a lower pacing frequency, which coincided with a slight increase in atrial stroke volume (Fig. 1D). After the treatment with PACAP27, the cAMP efflux increased concomitantly with ECF translocation, which was similar pattern as shown for the ANP secretion (Cho et al., 1993, 1995). Therefore, the change in cAMP efflux was plotted as a function of ECF translocation (Fig. 1E). The treatment with PACAP27 shifted the relationship between cAMP efflux and ECF translocation upward, showing cAMP efflux to be a function of ECF translocation (Fig. 1E). cAMP concentration was decreased by increasing pacing frequency in both control and experimental periods (Fig. 1C).

As shown in Fig. 2, PACAP27, 100 nM, increased cAMP efflux in the perfusate in beating atria (1.3 Hz) (A, $n = 5$). ECF translocation was not significantly changed by PACAP27 (Fig. 2B). PACAP27 increased the concentration of cAMP efflux in terms of ECF translocation significantly (Fig. 2C). IBMX, 0.3 mM, increased cAMP efflux slightly, but not significantly (Fig. 2A, $n = 5$). Also, the increase by IBMX of cAMP concentration was not significant (Fig. 2C). In the presence of IBMX, PACAP27 increased the cAMP efflux and also the cAMP concentration (Fig. 2A and C). At the end of the experiments, the atrial cAMP content was measured as in Section 2. To

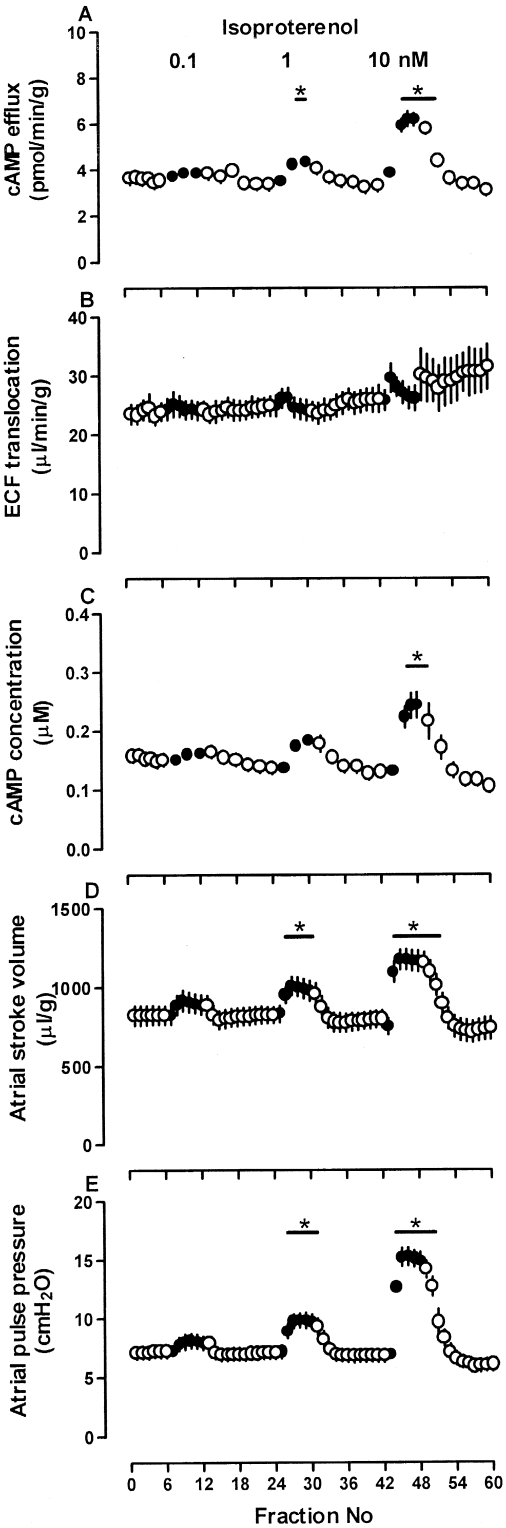

Fig. 7. Dose- and time-dependent effects of isoproterenol (ISO) on cAMP efflux (A), ECF translocation (B), cAMP concentration (C) and atrial dynamics (D and E). ○ Regular buffer; ● ISO in a dose indicated. Fraction No, serial 2-min sample collections. * $P < 0.01$ experimental vs. control periods. cAMP was measured in a sample from every other collection after the first control period.

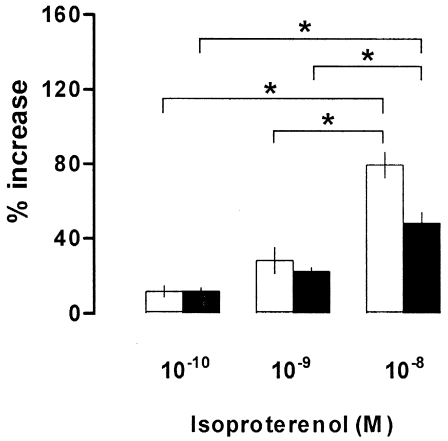

Fig. 8. Peak differences of changes by ISO in cAMP efflux and atrial stroke volume. □ Percent changes in cAMP efflux, ■ percent changes in atrial stroke volume. * $P < 0.05$.

determine whether a relationship exists between cAMP concentration in terms of ECF translocation and atrial cAMP content, correlation analysis was performed (Fig. 3). The change in cAMP concentration induced by PACAP27 was linearly correlated with atrial cAMP content. The change in cAMP efflux was also significantly related with changes in atrial cAMP content (data not shown). In another series of experiments for time-control, atrial dynamics, cAMP efflux, cAMP concentration and ECF translocation were stable and reproducible during the periods corresponding to the control and experimental observations ($n = 3$, some data not shown, cAMP concentration and cAMP content are shown in Fig. 3).

3.2. PACAP27 increases cAMP production in a dose- and time-dependent manner with minor changes in atrial dynamics

Fig. 4 shows the effects of PACAP27 on atrial efflux of cAMP and atrial stroke volume. PACAP27, 30 nM, induced a three-fold increase in cAMP efflux (12.49 ± 1.24 at peak response vs. 4.08 ± 0.15 pmol/min/g at basal level, Fig. 4A, $n = 9$). The changes in ECF translocation induced by PACAP27 were not significant (Fig 4B). PACAP27 increased cAMP concentration of the perfusate in terms of ECF translocation significantly (0.522 ± 0.057 at peak response vs. 0.171 ± 0.010 μM at basal level, Fig. 4C). Incremental responses of cAMP efflux and cAMP concentration to PACAP27 were significant at 6 min and persisted during the period of the administration without significant changes. PACAP27, 30 nM, resulted in a non-significant increase in atrial stroke volume followed by recovery to the basal level during the treatment (Fig. 4D).

Fig. 5 shows the PACAP27-induced dose-dependent responses of cAMP efflux and cAMP concentration. PACAP27, 1 nM, tended to increase cAMP efflux (Fig. 5A, $n = 4$) and cAMP concentration (Fig. 5C). PACAP27, 10 nM, increased cAMP efflux and cAMP concentration significantly after 8 min of administration and the increase persisted for up to 10 min after cessation of the administra-

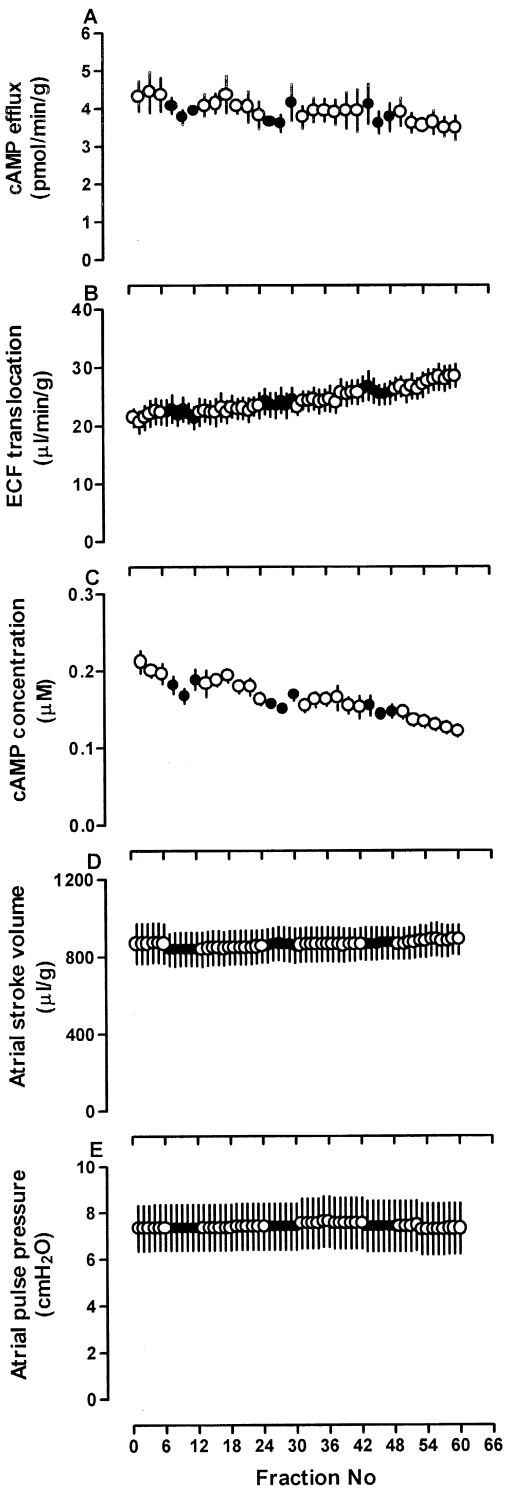

Fig. 9. Time-matched control levels in cAMP efflux (A), ECF translocation (B), cAMP concentration in terms of ECF translocation (C) and atrial dynamics (D and E). ○ and ● correspond to the control and experimental periods. Fraction No, serial 2-min sample collections.

tion (cAMP efflux: 14.87 ± 3.05 at peak response vs. 3.96 ± 0.45 pmol/min/g at basal level; cAMP concentration: 0.676 ± 0.146 at peak response vs. 0.190 ± 0.013 μM at basal level; Fig. 5A and C). At a higher concentration of PACAP27, 100 nM, the peak responses were similar to those with 10 nM (cAMP efflux: 15.24 ± 3.21 at peak response vs. 4.53 ± 0.45 pmol/min/g at basal level; cAMP concentration: 0.670 ± 0.165 at peak response vs. 0.221 ± 0.015 μM at basal level) but the duration was prolonged. The cAMP efflux and cAMP concentration returned to their basal levels during the recovery periods. No significant change in ECF translocation was observed with PACAP27 (Fig. 5B). PACAP27 increased in a dose-dependent manner atrial stroke volume and pulse pressure, significantly at a dose of 100 nM (Fig. 5D and E). During recovery, the basal levels of atrial stroke volume and pulse pressure decreased slowly to below the initial basal level. The differences between basal and peak levels of cAMP efflux after the treatments were all significant (Student's *t*-test: 7.52 ± 1.00 vs. 4.27 ± 0.39, 14.87 ± 3.05 vs. 3.96 ± 0.45, 15.24 ± 3.21 vs. 4.53 ± 0.45 pmol/min/g for 1, 10 and 100 nM, respectively; all $P < 0.05$). As shown in Fig. 6, the incremental percent changes in cAMP efflux in response to PACAP27, 1, 10 and 100 nM, were 74.4 ± 11.8, 263.1 ± 51.6 and 225.8 ± 55.0% over the basal level. The differences between percent changes induced by 1 and 10 or 100 nM were significant ($P < 0.05$). The differences between basal and peak levels of atrial stroke volume were not significant (Student's *t*-test).

As a positive control, a series of experiments was done with isoproterenol. Fig. 7 shows dose-dependent responses to isoproterenol by cAMP efflux and atrial dynamics. Isoproterenol, 0.1 nM, tended to increase cAMP efflux, cAMP concentration and atrial dynamics (Fig. 7A, C and D, $n = 6$). Isoproterenol, 1 nM, increased cAMP efflux and atrial dynamics significantly. At a higher concentration, isoproterenol, 10 nM, further increased cAMP efflux, cAMP concentration and atrial dynamics. No significant change in ECF translocation was observed with isoproterenol (Fig. 7B). As shown in Fig. 8, the incremental percent changes in cAMP efflux and atrial stroke volume were well correlated. In time-matched controls ($n = 4$), cAMP efflux and atrial dynamics were relatively stable during the periods corresponding to the control and experimental observations (Fig. 9).

4. Discussion

Because the variation in atrial dynamics affects ECF translocation, and because the ECF translocation controls the appearance of atrial cellular products which were exported from cells into the interstitium (Cho et al., 1993, 1995), any agent which controls atrial dynamics could change the apparent amount of cellular products in the atrial lumen. Therefore, it is predicted that the appearance in the atrial lumen of atrial cellular products is a function of ECF translocation which is controlled by atrial dynamics. To more precisely quantify the amount of cAMP efflux into the interstitium in beating atria, cAMP efflux was analyzed in terms of ECF translocation, i.e., the concentration of cAMP in the interstitium. The latter may be the sum of changes in cAMP efflux from the cells and the metabolism of the cAMP in the interstitium. Therefore, it is likely that the cAMP exported into the atrial interstitium appears in the lumen through the endocardial endothelium as a result of the sum of at least two processes: paracellular translocation which is related with ECF translocation and metabolism in the interstitium or during the passage through the paracellular pathway. However, there is a limitation as some of the cAMP in the lumen may be derived directly from the endocardial endothelial cells. It is likely that cAMP efflux in terms of ECF translocation is more reliable than the simple cAMP efflux for quantification of the amount of cAMP exported from the atrial cells, and also for detection of an influence of atrial dynamics on the washout of cAMP in the interstitium.

As shown in Fig. 1, during the control periods, cAMP efflux from the atrium was relatively constant in response to changes in ECF translocation induced by variation of pacing frequency. The treatment with PACAP27 increased cAMP efflux and cAMP concentration in terms of ECF translocation, during which the cAMP efflux was a function of ECF translocation. Although the atrial efflux of cAMP is related to ECF translocation, the relationship between cAMP efflux and ECF translocation is affected by variations in cAMP production. That is, at higher rate of cAMP production, the slope of the relationship increased (Fig. 1). This may mean that, at higher rates of atrial cAMP production, the proportion of paracellular components of the pathways through which cAMP moves into the atrial lumen is increased.

In the present study, to analyze atrial production of cAMP in a real-time base, a relationship between cAMP efflux in the perfusate in terms of ECF translocation and atrial content of cAMP was established. The result suggests that cAMP efflux in terms of ECF translocation is a function of atrial content of cAMP. Therefore, it is suggested that an increase in cAMP efflux reflects the change in atrial production of cAMP in the beating atria. Since the transcellular export of cAMP may be affected by intracellular accumulation of cAMP and the activity of the cAMP transport system (Rindler et al., 1978), the cAMP efflux may not precisely represent the cellular production of cAMP. In this context, however, cAMP efflux has not yet been defined in the cardiac atrium. The linear relationship between cAMP efflux in the perfusate and cAMP content in the atrium shows that cAMP efflux reflects the changes in cAMP production of the atrium in the present protocol. This is consistent with previous findings for

neural tissue, showing a linear correlation between cAMP efflux and cAMP production (Florio et al., 1999).

It was shown for the first time that PACAP27 increased cAMP production in the beating atrium in a dose- and time-dependent manner. The response was rapid and reproducible. No tachyphylaxis was observed for the production of cAMP by PACAP27. However, the effect of PACAP27 on atrial stroke volume was different from that on cAMP production. An about three-fold increase in atrial cAMP production was not accompanied by the same change in atrial stroke volume (Fig. 4). Only a minor increase in atrial stroke volume was observed. During maintenance of the elevated cAMP production, the slightly increased atrial stroke volume even returned back to its basal level. Also, the basal levels of atrial stroke volume and pulse pressure decreased slowly during the recovery periods between repetitive administrations of PACAP27 (Fig. 5). A positive control such as isoproterenol, where cAMP is closely related to contraction, showed well the correlation between cAMP efflux and atrial stroke volume and pulse pressure (Figs. 7 and 8). The reasons for the discrepancy between changes in cAMP production and atrial stroke volume induced by PACAP27 are unknown at present. Recently, PACAP38 has been shown to activate the parasympathetic nervous system in isolated dog atria (Yonezawa et al., 1996). Should this have occurred in the present experiments, minor changes in atrial dynamics induced by PACAP27 may be related to counteraction of the system. Alternatively, extracellular cAMP may have effects different from those of intracellular cAMP, because paracrine/autocrine functions for extracellular cAMP have been proposed to exist in several tissues (Mi and Jackson, 1995). Since the concentration of cAMP exported into the atrial interstitium reaches micromolar concentrations, it is likely that the metabolic products of cAMP affect the force of contraction of the atrium (Johnson et al., 1988; Mi and Jackson, 1995).

The present finding of an increase in cAMP production induced by PACAP27 is consistent with the reports that PACAP receptor subtypes are coupled to adenylyl cyclase (Spengler et al., 1993; Ishihara et al., 1992). It was also shown that PACAP38 increases cAMP production in cultured neonatal cardiac myocytes from rats (Suzuki et al., 1993). Ross-Ascuitto et al. (1993) also observed an increase by IBMX of ventricular force of contraction in neonatal pig hearts. Based on this, the authors suggested that the positive inotropic effect of PACAP is caused by an increase in intracellular cAMP. Our present data are consistent with these results. Our finding of a positive inotropic effect of PACAP27 in the beating rabbit atria is consistent with previous reports in dogs (Yonezawa et al., 1996). However, the effect of PACAP27 on atrial dynamics was not as marked as that on cAMP production in beating atria. Therefore, it is hypothesized that the cAMP produced by PACAP27 may have functions other than the control of atrial dynamics.

In summary, PACAP27 increased cAMP production in a dose- and time-dependent manner with minor changes in atrial dynamics in the cardiac atrium.

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