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PACAP-38 activates parasympathetic nerves in isolated, blood-perfused dog atria

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Abstract

A pituitary adenylate cyclase-activating polypeptide (PACAP) activates PACAP and vasoactive intestinal peptide (VIP) receptors. We investigated the effects of PACAP-38 on the sinus rate and atrial contractile force in isolated, blood-perfused dog heart preparations and the stimulation by PACAP-38 of the parasympathetic nerve fibers. PACAP-38 (3–1000 pmol) caused positive and/or negative chronotropic responses and it dose dependently increased atrial and ventricular contractile force. The positive cardiac responses to PACAP-38 unlike those to VIP were much less than the positive responses to norepinephrine. Atropine inhibited the negative chronotropic responses to PACAP-38 and augmented the positive chronotropic and inotropic responses. Physostigmine potentiated the negative cardiac responses to PACAP-38 and acetylcholine. After physostigmine treatment, additionally, tetrodotoxin blocked the negative cardiac responses to PACAP-38 and intracardiac parasympathetic nerve stimulation. Propranolol did not inhibit the positive cardiac responses to PACAP-38 in atropine-treated atria. PACAP-(6–38) (1 and 3 nmol), an antagonist of PACAP-38, did not affect the cardiac responses to 100 pmol of PACAP-38. These results suggest that (1) PACAP-38 directly increases sinus rate and atrial contractile force and (2) PACAP-38 activates parasympathetic nerves and causes negative chronotropic and inotropic responses in the dog heart.

Keywords: PACAP-38 (pituitary adenylate cyclase-activating polypeptide-38); Parasympathetic nervous system; Acetylcholine; PACAP-(6–38) (pituitary adenylate cyclase-activating polypeptide-(6–38)); Chronotropism; Inotropism; Heart; (Dog)

1. Introduction

Pituitary adenylate cyclase-activating polypeptide (PACAP) is a newly discovered neuropeptide isolated from the ovine hypothalamus (Miyata et al., 1989). PACAP belongs to a family including secretin, vasoactive intestinal peptide (VIP), growth-hormone-releasing factor and glucagon (Miyata et al., 1989). PACAP-38 is widely distributed, not only in the hypothalamus, but also in the posterior pituitary, other brain areas and peripheral tissues such as testis, adrenal gland and gut (Arimura et al., 1991). PACAP is a potent stimulator of adenylate cyclase in rat anterior pituitary cell and the adenylate cyclase stimulating activity of PACAP is about 1000 times greater than that of VIP in pituitary cells (Miyata et al., 1989), PC12h cells (adrenal pheochromocytoma cell lines) (Watanabe et al., 1990) and glioma cells (Robberecht et al., 1994). PACAP is present in two molecular forms with 38 (PACAP-38) and 27 (PCAP-27) amino acid residues and its N-terminal (1–28) sequence is 68% homologous with VIP (Miyata et al., 1989, 1990).

Miyata et al. (1990) reported that PACAP had vasodepressor activity on the cardiovascular system in the rat when it was injected intravenously. PACAP evoked relaxation of de-endothelialized rabbit aortic rings in vitro with stimulation of cAMP production in aortic smooth muscles (Warren et al., 1991). It has also been demonstrated that PACAP-38 increases heart rate in intact conscious sheep (Sawangjaroen et al., 1992) and anesthetized dogs (Suzuki et al., 1993). Another report has shown that a low dose of PACAP-38 (10 pmol/kg) causes an increase in heart rate and a decrease in mean arterial blood pressure while a high dose of PACAP-38 (300 pmol/kg) induces bradycardia after transient tachycardia and produces hypertension after transient hypotension (Ishizuka et al., 1992). However, there is no report available on the direct effects of

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PACAP-38 on sinus rate and myocardial contractility, and on the mechanisms of the effects of PACAP-38 on the heart.

We investigated whether PACAP-38, like VIP, would increase sinus rate and myocardial contractile force in the isolated, blood-perfused dog atria or ventricles. PACAP-38 caused not only positive cardiac responses but also negative responses. Thus, we also analyzed the cardiac responses to PACAP-38 using pharmacological key substances.

2. Materials and methods

2.1. Preparations

An isolated right atrial or left ventricular preparation was perfused with heparinized arterial blood from an anesthetized support dog. The details of these preparations have been described in previous papers (Chiba et al., 1975; Chiba, 1976). Support dogs, weighing 9–28 kg, were anesthetized with pentobarbital sodium (30 mg/kg, i.v.) and ventilated artificially through a cuffed tracheal tube with room air by using a Harvard respirator (Harvard Apparatus, Millis, MA, USA, model 607). Sodium heparin (500 USP U/kg, i.v.) was administered to each dog at the beginning of the perfusion of the isolated atrial or ventricular preparation and 200 USP U/kg was given each hour thereafter.

Isolated right atrial or left ventricular preparations were obtained from other mongrel dogs weighing from 8 to 17 kg. Each dog was anesthetized with pentobarbital sodium (30 mg/kg, i.v.). After pentobarbital sodium, heparin (200 USP U/kg, i.v.) was administered, the right atrium or left ventricle was excised and immersed in cold Ringer's solution of the following composition (mM): NaCl, 154.0; KCl, 5.6; $CaCl_2$, 2.2 and $NaHCO_3$, 3.6. The sinus node artery of the isolated right atrium or the anterior descending branch of the left coronary artery of the isolated left ventricle was cannulated and each preparation was perfused with heparinized blood from the carotid artery of the anesthetized support dog by the aid of a peristaltic pump (Harvard Apparatus, model 1210). A pneumatic resistance was placed in parallel with the perfusion system so that the perfusion pressure could be maintained constant at 100 mm Hg. The rate of blood flow to the atrial or ventricular preparation was 3–10 ml/min. The venous effluent from the preparation was led to a collecting funnel and returned to the support dog through an external jugular vein.

The preparation was anchored to a stainless-steel bar and placed in a cup-shaped glass container kept at 37°C. The upper part of the cardiac preparation was connected to a force-displacement transducer (Nihon Kohden, Tokyo, Japan, AP-620G) by a silk thread. The cardiac tissue was usually stretched to a resting tension of 2 g. Isometric tension was recorded on a thermo-writing rectigraph (Nihon Kohden, RTA-1200). A pair of bipolar silver electrodes was brought into contact with the epicardial surface of the isolated preparation in order to record the atrial electrogram or to drive the left ventricle electrically. The left ventricular preparation was electrically paced at a frequency of 2 Hz with 1-s pulse duration and 4 V. The atrial rate was derived from the electrogram with a cardiotachometer (Nihon Kohden, AT-600G). The femoral arterial blood pressure and heart rate derived from lead II of the electrocardiograph of the support dog and the rate of blood flow to the preparation were monitored simultaneously.

2.2. Experimental protocols

First we examined the effects of PACAP-38 and norepinephrine on the SA nodal pacemaker activity and atrial myocardial contractility in the isolated, blood-perfused right atrium and on the left ventricular myocardial contractility in the isolated, blood-perfused left ventricle. PACAP-38 and norepinephrine at a dose of 3–1000 pmol were injected into the sinus node artery of the isolated atrium ($n = 13$) or the anterior descending branch of the left coronary artery of the isolated ventricle ($n = 3$). To investigate the effects of PACAP-38 on the autonomic nervous system in the isolated atrium, we studied the effects of atropine (10 nmol, $n = 6$) and propranolol (10 nmol, $n = 8$) after pretreatment with atropine (10 nmol) on the cardiac responses to PACAP-38 (300 pmol) in the isolated, blood-perfused atrium.

In the second series, to analyze the negative cardiac responses to PACAP-38, we studied the effects of physostigmine (30 nmol) on the chronotropic and inotropic responses to PACAP-38 (300 pmol, $n = 6$) and acetylcholine (0.3 or 1 nmol, $n = 6$). We also studied the effects of tetrodotoxin (10 nmol) after pretreatment with physostigmine (30 nmol) on the cardiac responses to PACAP-38 (300 pmol, $n = 6$) and intracardiac parasympathetic nerve stimulation. Intracardiac parasympathetic neuronal elements in the fatty tissue overlying the caval margin of the atrium were stimulated at a frequency of 10–20 Hz with 10 V and 10–20 ms pulse duration for 15 s in 6 isolated atria. The cardiac responses to PACAP-38 were observed before and 2 min after the application of physostigmine or tetrodotoxin. The changes in responses to PACAP-38 were determined 1.5, 3 and 4.5 min after the administration of PACAP-38. Each dose of PACAP-38 was injected at intervals of more than 30 min.

In the third series, to investigate the antagonistic effects of PACAP-(6–38), an antagonist of PACAP-38, we studied the effects of PACAP-(6–38) (1000 and 3000 pmol) on the positive and the negative cardiac responses to PACAP-38 (100 pmol) in the isolated, blood-perfused atrial preparation. The cardiac responses to PACAP-38 were observed before and 2 min after the application of PACAP-(6–38).

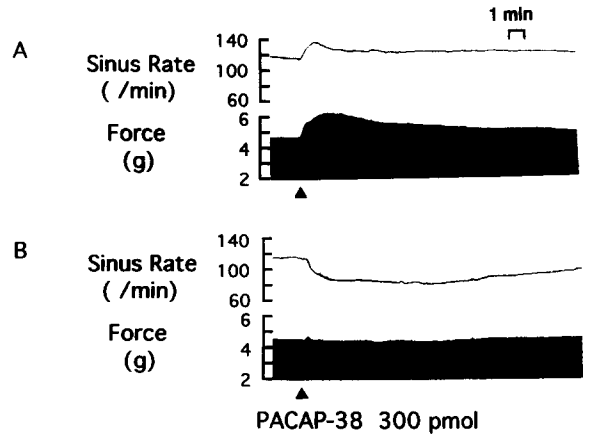

Fig. 1. Two types of chronotropic and inotropic effects of PACAP-38 at a dose of 300 pmol in isolated, blood-perfused right atria of dogs. Type A (upper panel), the positive responses predominated; type B (lower panel), the negative responses predominated.

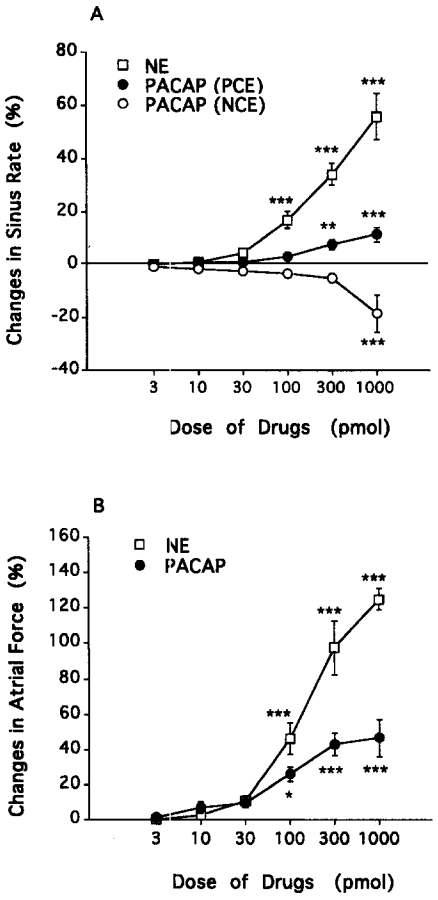

Fig. 2. Positive chronotropic (●, $n = 9$) and negative chronotropic (○, $n = 6$) responses to PACAP-38 and positive chronotropic responses to norepinephrine (□, $n = 13$) in 13 isolated, blood-perfused dog atria (A). PACAP-38 increased sinus rate in 9 out of 13 preparations, and decreased sinus rate with a transient positive effect in 6 preparations. Positive inotropic responses to PACAP-38 (●, $n = 13$) and norepinephrine (□, $n = 13$) at doses of 3 to 1000 pmol in the 13 atria (B). Vertical bars show S.E.M. The basal sinus rate and atrial contractile force in 13 isolated atria were 115 ± 2.9 (mean $\pm$ S.E.M.) beats/min and 2.3 ± 0.2 g, respectively.

To study the effects of PACAP-(6–38) on the negative cardiac responses to PACAP-38, physostigmine (30 nmol) was given 2.5 min before the injection of PACAP-38.

2.3. Drugs

The drugs used were pituitary adenylate cyclase-activating polypeptide 38 (human) (PACAP-38, Peptide Institute, Osaka, Japan), pituitary adenylate cyclase-activating polypeptide (human, 6–38) (PACAP-(6–38), Peptide Institute), acetylcholine chloride (Daiichi Seiyaku, Tokyo, Japan), atropine sulfate (Wako, Tokyo, Japan), norepinephrine hydrochloride (Sankyo, Tokyo, Japan), propranolol hydrochloride (Sigma, St. Louis, MO, USA), physostigmine (Sigma) and tetrodotoxin (Sankyo). PACAP 38 was dissolved in saline to make a stock solution of 10 μM and then diluted with saline to obtain lower concentrations. Other drugs were dissolved in saline before the start of the experiments. The amount of drug solution injected was 1–100 μl.

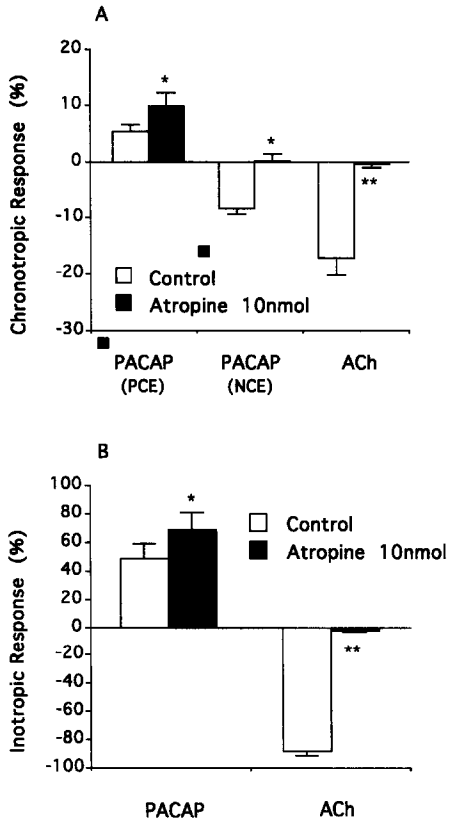

Fig. 3. Effects of 10 nmol of atropine on the positive ($n = 6$) and negative ($n = 2$) chronotropic (A) and positive inotropic (B, $n = 6$) responses to PACAP-38 (300 pmol) or to acetylcholine (ACh, 1 nmol, $n = 6$) in 6 isolated, blood-perfused dog atria. The basal sinus rate and atrial contractile force in 6 isolated atria were 115 ± 4.0 beats/min and 2.4 ± 0.2 g, respectively. Vertical bars show S.E.M. * $P < 0.05$; ** $P < 0.01$; PCE, positive chronotropic effect; NCE, negative chronotropic effect.

2.4. Statistical analysis

The data are shown as the maximal change in the response to each drug and are expressed as means ± S.E.M. The data were analyzed with an analysis of variance and Bonferroni's method for multiple comparisons of data. Student's *t*-test for paired or unpaired data was used for comparison between two groups. *P* values of less than 0.05 were considered statistically significant.

3. Results

3.1. Effects of PACAP-38 on the SA nodal pacemaker activity, atrial contractility and ventricular contractility

When PACAP-38 (3–1000 pmol) was injected into the sinus node artery of an isolated right atrium, PACAP-38 increased sinus rate and atrial contractile force (Fig. 1A) or decreased sinus rate with a small change in atrial force (Fig. 1B). PACAP-38 increased sinus rate in 5 out of 13 preparations, decreased sinus rate with a transient positive chronotropic effect in 4 preparations, only decreased sinus rate in 2 preparations and caused no chronotropic effect in 2 preparations. On the other hand, PACAP-38 increased the right atrial contractile force in 13 isolated atria, but in 2 of 13 atria, PACAP-38 caused a transient positive inotropic response followed by negative inotropic responses. Fig. 2 shows the summarized data from 13 experiments. While norepinephrine in doses of 3–1000 pmol increased sinus rate and atrial force dose dependently, PACAP-38 increased and/or decreased sinus rate and increased atrial contractile force. The positive cardiac responses to PACAP-38 were much less than the positive responses to norepinephrine.

PACAP-38 also increased left ventricular contractile force in the isolated, blood-perfused ventricle; 1000 pmol of PACAP-38 increased ventricular force by 25.4 ± 4.9% in 3 isolated ventricles.

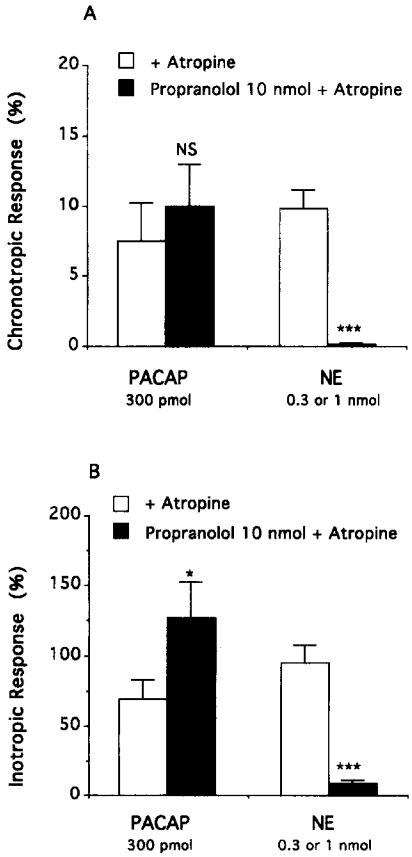

Fig. 4. Effects of 10 nmol of propranolol after pretreatment with 10 nmol of atropine on the chronotropic (A) and inotropic (B) responses to PACAP-38 (300 pmol) and the responses to norepinephrine (NE, 0.3 or 1 nmol) in 8 isolated, blood-perfused dog atria. The basal sinus rate and contractile force in 8 isolated atria were 113 ± 4.3 beats/min and 2.6 ± 0.2 g, respectively. Vertical bars show S.E.M. $^{***}P < 0.001$; NS, not significant vs. control.

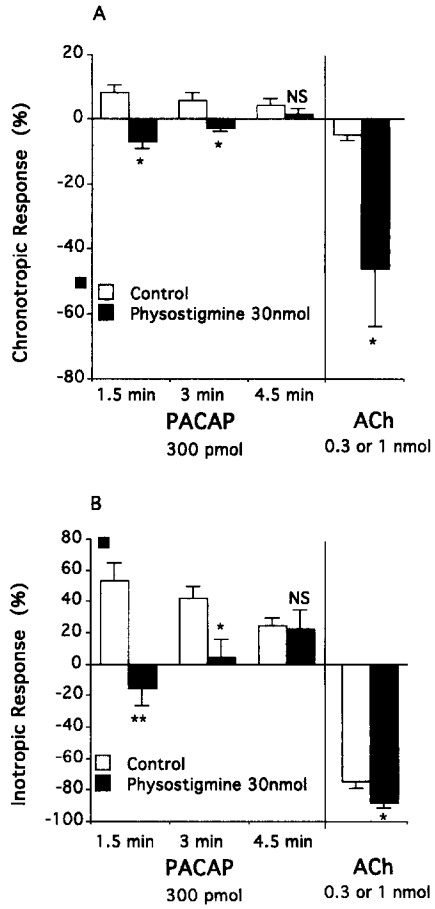

Fig. 5. Effects of physostigmine (30 nmol) on the negative chronotropic (A) and inotropic (B) responses to PACAP-38 (300 pmol) and acetylcholine (ACh, 0.3 or 1 nmol) in 6 isolated, blood-perfused dog atria. The responses to PACAP-38 at 1.5, 3 and 4.5 min after the administration are shown, while the responses to acetylcholine shown here are the minimal responses. Vertical bars show S.E.M. The basal sinus rate and atrial contractile force in 6 isolated atria were 117 ± 5.2 beats/min and 2.3 ± 0.3 g, respectively. $^{*}P < 0.05$; $^{**}P < 0.01$; NS, not significant vs. control.

3.2. Effects of atropine and propranolol

We tested whether the cardiac responses to PACAP-38 were mediated by activation of the autonomic nervous system. When atropine (10 nmol) blocked the negative chronotropic and inotropic responses to acetylcholine (1 nmol), it abolished the negative chronotropic responses to PACAP-38 (300 pmol) and significantly augmented the positive chronotropic and inotropic responses to PACAP-38 in 6 isolated atria (Fig. 3).

After the treatment with atropine (10 nmol), propranolol (10 nmol) did not change significantly the positive chronotropic responses to PACAP-38 (300 pmol) but, instead, significantly augmented the positive inotropic responses to PACAP-38, while propranolol suppressed the positive cardiac responses to norepinephrine (0.3 or 1 nmol) ($P < 0.001$) in 8 isolated atria (Fig. 4).

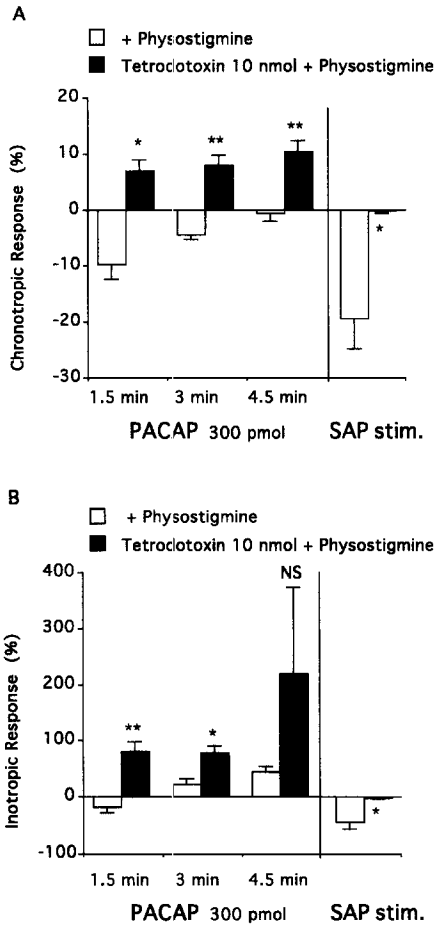

Fig. 6. Effects of tetrodotoxin (TTX, 10 nmol) after the pretreatment with 30 nmol of physostigmine on the negative chronotropic (A) and inotropic (B) responses to PACAP-38 (300 pmol; $n = 6$) and intracardiac parasympathetic nerve stimulation (SAP stimulation, 10 V, 10–20 μs and 10–20 Hz, $n = 4$) for 15 s in the isolated, blood-perfused dog atria. The responses to PACAP-38 at 1.5, 3 and 4.5 min after the administration are shown, while the responses to SAP stimulation shown here are the minimal responses. Vertical bars show S.E.M. The basal sinus rate and atrial contractile force in 6 isolated atria for the responses to PACAP-38 were 121±5.5 beats/min and 2.4±0.3 g, respectively. * $P < 0.05$; ** $P < 0.01$; NS, not significant vs. control.

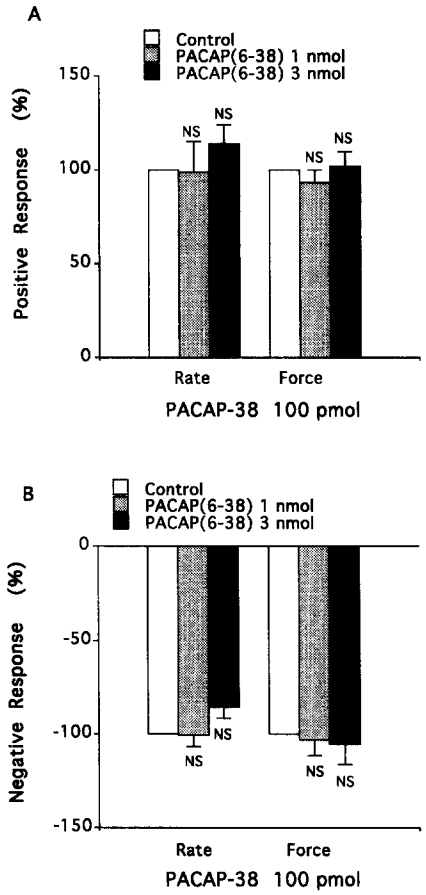

Fig. 7. Effects of PACAP (6–38) (1 and 3 nmol) on the negative (A) and the positive (B) cardiac responses to PACAP-38 (100 pmol) in 5 isolated, blood-perfused dog atria. The negative cardiac responses to PACAP-38 were determined 2.5 min after the treatment with physostigmine (30 nmol). Vertical bars show S.E.M. The basal sinus rate and atrial contractile force in 5 isolated atria were 114±5.1 beats/min and 2.4±0.2 g, respectively. NS, not significant vs. control.

3.3. Effects of physostigmine and tetrodotoxin

To investigate whether PACAP-38 stimulates parasympathetic nerve fibers and causes negative chronotropic and inotropic responses, we studied the effects of physostigmine and tetrodotoxin on the chronotropic and inotropic responses to PACAP-38 in the isolated, blood-perfused dog atrium.

Physostigmine (30 nmol) decreased the positive chronotropic and inotropic responses to PACAP-38 (300 pmol) significantly ($P < 0.05$) and turned them into negative responses 1.5 min after the injection of PACAP-38. Physostigmine, however, potentiated the negative chronotropic and inotropic responses to acetylcholine (0.3 or 1 nmol) (Fig. 5).

Fig. 6 shows the effects of tetrodotoxin (10 nmol) on the cardiac responses to PACAP-38 (300 pmol) after the treatment with physostigmine (30 nmol). In 6 isolated atria treated with physostigmine, tetrodotoxin inhibited the negative chronotropic and inotropic responses to PACAP-38 and reversed them to positive responses. Tetrodotoxin

treatment also suppressed the negative chronotropic and inotropic responses to intracardiac parasympathetic nerve stimulation (Fig. 6).

3.4. Effects of PACAP-(6–38) on the cardiac responses to PACAP-38

We tested the antagonistic effects of PACAP-(6–38) (1 and 3 nmol) on the positive chronotropic and inotropic responses to PACAP-38 (100 pmol) and on the negative chronotropic and inotropic responses to PACAP-38 (100 pmol) after pretreatment with physostigmine (30 nmol) in isolated dog atria (Fig. 7). PACAP-(6–38) at doses of 1 and 3 nmol did not cause any response in isolated dog atria. PACAP-(6–38) did not affect the positive or negative chronotropic and inotropic responses to PACAP-38.

4. Discussion

In the present study, we first demonstrated that PACAP-38 causes direct positive chronotropic and inotropic effects in the isolated, blood-perfused dog heart preparation and PACAP-38 decreases sinus rate and atrial contractility due to the activation of intracardiac parasympathetic neural elements.

PACAP-38 increased and/or decreased sinus rate, and increased atrial contractile force in the isolated, perfused dog atrium (Fig. 1 and Fig. 2). PACAP-38 also increased ventricular contractile force in the isolated perfused left ventricular preparation. The negative cardiac responses to PACAP-38 were blocked by atropine and augmented by physostigmine. Additionally, tetrodotoxin blocked the negative cardiac responses to PACAP-38. These results suggest that PACAP-38 causes negative chronotropic and inotropic responses due to the activation of the intracardiac parasympathetic neural elements in the dog heart. In anesthetized dogs, intravenous administration of PACAP-38 at a low dose increased heart rate and at a high dose caused positive, followed by negative, chronotropic responses (Ishizuka et al., 1992). However, the mechanism of the negative responses has not yet been studied in detail. Thus, our results could mean that the negative chronotropic response to PACAP-38 at a high dose was induced at least in part by the direct activation of the intracardiac parasympathetic neural elements in anesthetized dogs, although vasovagal reflex-induced chronotropic responses were not completely excluded. Parasympathetic activity is a very important factor to control heart rate in physiological and pathological conditions in humans as well as mammals (De Ferrari et al., 1993). It is, therefore, likely that PACAP-38 has a pathophysiological role through the direct positive cardiac effects and the activation of the parasympathetic nervous system in the heart in situ.

After treatment with atropine, addition of PACAP-38 caused only positive cardiac responses in the isolated atrium (Fig. 3) and the positive cardiac responses to PACAP-38 were not inhibited by propranolol (Fig. 4) or PACAP-(6–38) (Fig. 7). PACAP-(6–38) antagonized the increases in adenylate cyclase induced by PACAP-38 in human neuroblastoma NB-OK-1 cell membranes (Robberecht et al., 1992) and the relaxation of the guinea pig tenia coli muscle (Jin et al., 1994). PACAP-38 and PACAP-27 cause several effects mediated through three types of PACAP receptors (Harmar and Lutz, 1994). The PACAP type I receptor is highly specific for PACAP and the type I receptor is subdivided into two subtypes on the basis of binding experiments. $PACAP_{1A}$ receptors bind PACAP-27 with slightly higher affinity than PACAP-38, while $PACAP_{1B}$ receptors bind PACAP-38 with high affinity and PACAP-27 with low affinity. The PACAP type I receptor is coupled to adenylate cyclase and phospholipase C (Spengler et al., 1993). The PACAP type II receptor is identical with the vasoactive intestinal polypeptide (VIP) type I receptor (Ishihara et al., 1992; Sreedharan et al., 1995). The PACAP type II receptor binds PACAP-27, PACAP-38 and VIP with very similar affinities (Shivers et al., 1991) and is coupled to only adenylate cyclase (Ishihara et al., 1992; Spengler et al., 1993). The cDNA encoding third type of receptor is identical with the VIP type II receptor (Lutz et al., 1993) and PACAP-38, PACAP-27 and VIP activate the VIP type II receptor with the same rank order as for stimulation of cAMP production in the cAMP reporter LVIP cells (Usdin et al., 1994). However, in the isolated, blood-perfused dog heart preparations, VIP increases sinus rate and atrial contractile force, and the potency order for the positive cardiac effects of VIP and norepinephrine was VIP > norepinephrine (Karasawa et al., 1990). VIP never induced negative cardiac responses in the isolated perfused heart preparation of the dog. The order for the positive cardiac effects of PACAP-38 and norepinephrine was norepinephrine > PACAP-38 (Fig. 2). Even after atropine treatment, PACAP-38 (300 nmol) increased sinus rate and atrial contractile force less than did norepinephrine (Fig. 3). It is, therefore, unlikely that the positive cardiac responses to PACAP-38 are mediated through the VIP type I and II receptors in the isolated, perfused dog atrium. In the present study, 10–30-times higher doses of PACAP-(6–38) did not inhibit the PACAP-38-induced positive responses, although there remains the possibility that much higher doses of PACAP-(6–38) might inhibit the responses to PACAP-38 in the isolated dog atria. Jin et al. (1994) reported that 10 times higher concentration of PACAP(6–38) effectively inhibited the PACAP-38-induced relaxation of the guinea pig tenia coli muscle. Thus, the positive cardiac responses to PACAP-38 are probably produced through PACAP receptors which are not antagonized by PACAP-(6–38) or other unknown mechanism in the dog heart. It has been reported recently that PACAP-(6–38) did not inhibit melatonin formation induced by PACAP-38 in pineal glands of rats (Yuwiler et al., 1995).

The negative cardiac responses to PACAP-38 were blocked by atropine and tetrodotoxin, but not affected by PACAP-(6–38) in the isolated dog atrium (Fig. 3 and Fig. 6 and Fig. 7). Thus, our results suggest that PACAP-38 causes negative chronotropic and inotropic responses due to the direct activation of the intracardiac parasympathetic neural elements in the isolated, blood-perfused dog atrium. In the isolated, blood-perfused dog atrium, tetrodotoxin and atropine did not increase the sinus rate and atrial contractile force, indicating that there is no spontaneous tonic parasympathetic activity in the spontaneously beating, isolated right atrium of the dog (Furukawa et al., 1980). However, there is still uncertainty about the precise mechanism of the negative responses to PACAP-38 including that whereby PACAP receptors mediate the negative responses in the dog heart. In the rat dorsal hippocampus PACAP-38, PACAP-27 and VIP cause an increase of the spontaneous release of acetylcholine (Masuo et al., 1993). The order for the ability to enhance acetylcholine release was VIP > PACAP-38 > PACAP-27. On the other hand, neuropeptide Y attenuates the release of acetylcholine in the isolated dog atrium (Ren et al., 1991) and in anesthetized dogs (Potter, 1985; Warner and Levy, 1989). Although thus, neuropeptides modulate the parasympathetic nervous system at the presynaptic site, PACAP-38 seems to directly activate the parasympathetic neural elements in the isolated, perfused dog atrium. Therefore, we need further studies to define the physiological roles of PACAP and its precise working mechanisms in the heart.

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