Pituitary Adenylate Cyclase-Activating Polypeptide-27 Causes a Biphasic Chronotropic Effect and Atrial Fibrillation in Autonomically Decentralized, Anesthetized Dogs

MASAMICHI HIROSE, YASUYUKI FURUKAWA, YOSHTO NAGASHIMA, MANOJ LAKHE and SHIGETOSHI CHIBA

Department of Pharmacology, Shinshu University School of Medicine, Matsumoto 390, Japan

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ABSTRACT

We investigated the effects of a neuropeptide, pituitary adenylate cyclase-activating polypeptide-27 (PACAP-27), on the sinoatrial nodal pacemaker activity and the mechanisms for the cardiac effects of PACAP-27 in the autonomically decentralized heart of the anesthetized dog. PACAP-27 (0.01–0.3 nmol) injected into the sinus node artery increased followed by decreased sinus rate. PACAP-27 (0.1 and 0.3 nmol) caused atrial fibrillation spontaneously. After atropine, PACAP-27 never decreased but only increased sinus rate as did vasoactive intestinal peptide. However, propranolol did not affect the negative and positive chronotropic effects. Tetrodotoxin but not hexamethonium abolished the negative chronotropic response to PACAP-27 in atropine nontreated dogs, and tetrodotoxin also inhibited the positive chronotropic response by 34% in atropine-treated dogs. In atropine- and propranolol-treated dogs, positive chronotropic responses to PACAP-27 were inhibited by PACAP-(6–27), a PACAP receptor antagonist but not by vasoactive intestinal peptide (10–28), a vasoactive intestinal peptide receptor antagonist. These results indicate that PACAP-27 causes the negative chronotropic effect through the postganglionic parasympathetic nerve activation and it produces the positive chronotropic effect mediated by PACAP receptors with an activation of non-adrenergic, nonvasoactive intestinal peptide-ergic nerves at least in part in the dog heart. Atropine and tetrodotoxin abolished atrial fibrillation induced by PACAP-27 but other blockers did not. These results suggest that neurally released acetylcholine induced by PACAP-27 participates in the induction of atrial fibrillation.

PACAP is a newly discovered neuropeptide isolated originally from the ovine hypothalamus (Miyata et al., 1989). PACAP is widely distributed in the brain, testis, adrenal gland and gut (Arimura et al., 1991). PACAP acts as a neurotransmitter, a neuromodulator or a neurotrophic factor in the central nervous system (Arimura and Shinoda, 1995). However, the precise mechanism of PACAP on peripheral organs including the heart, has not been defined. PACAP is present in two molecular forms with 38 (PACAP-38) and 27 (PACAP-27) amino acid residues and its N-terminal (1–28) sequence is 68% homologous with VIP (Miyata et al., 1990). PACAP increases and IP₃ mediated by type I, II and III PACAP receptors and type II and III PACAP receptors are identical with type I and II VIP receptors, respectively (Shivers et al., 1991; Ishihara et al., 1992; Lutz et al., 1993; Spengler et al., 1993; Harmar and Lutz, 1994).

PACAP produced positive inotropic and lusitropic effects in the isolated neonatal pig heart (Ross-Ascuitto et al., 1993). An i.v. injection of PACAP increased heart rate in the anesthetized cat (Minkes et al., 1992) and the conscious rat (Gardiner et al., 1994), and increased followed by decreased heart rate in anesthetized dogs (Ishizuka et al., 1992). Recently, we observed that PACAP-38 increased followed by decreased heart rate in isolated and perfused dog atria (Yonezawa et al., 1996) and in anesthetized dogs (Hirose et al., 1997) when PACAP-38 was administered to the branch of the coronary artery to the SA nodal region, “sinus node artery.” We briefly reported that PACAP-38 decreased heart rate mediated by activation of parasympathetic nerves and sensitized the atrial fibrillation induced by ACh injection in anesthetized dog hearts (Hirose et al., 1997). However, there are little available reports in the heart that determine the mechanism of the cardiac responses to PACAP. Thus, we tried to investigate the chronotropic responses to PACAP-27 in the anesthetized dog heart when a substance was given into the sinus node artery. In the preliminary experiments, we observed that PACAP-27 was more potent than PACAP-38 and that at high doses PACAP-27 consistently caused atrial fibrillation.

It is well known that ACh induces atrial fibrillation when

ABBREVIATIONS: ACh, acetylcholine; IP₃, inositol 1,4,5-trisphosphate; PACAP, pituitary adenylate cyclase-activating polypeptide; SA, sinoatrial; TTX, tetrodotoxin; VIP, vasoactive intestinal peptide; cAMP, cyclic adenosine 3’,5’-monophosphate.
it is released by activation of vagus nerves or it is applied exogenously in mammalian hearts (Lewis et al., 1921; Goldenberg and Rothberger, 1934). However, there is no available report that an endogenous substance except ACh itself causes atrial fibrillation although aconitine (Scherf et al., 1948), hypertonic solution (Chiba et al., 1969) and mechanical interventions (Rothberger and Winterberg, 1910) can cause atrial fibrillation. Therefore, first, we verified that PACAP-27 causes atrial fibrillation in the anesthetized dog heart. Then, to elucidate the mechanism of the biphasic chronotropic effects and the induction of atrial fibrillation induced by PACAP-27, we analyzed PACAP-27-induced responses using pharmacological key drugs including PACAP receptor and VIP receptor antagonists in the autonomically decentralized heart of the open-chest anesthetized dog. To determine the direct chronotropic response, we injected substances directly into the sinus node artery in the anesthetized dog heart (Hashimoto et al., 1968).

Materials and Methods

The animal experiments were approved by the Shinshu University School of Medicine Animal Studies Committee.

Preparation

Forty-three mongrel dogs of either sex, weighing 10 to 28 kg, were anesthetized with sodium pentobarbital (35 mg/kg i.v.). A tracheal cannula was inserted and intermittent positive-pressure ventilation was started by a respirator (Harvard Apparatus, Millis, MA, model 607) with room air. The chest was opened transversely at the fifth intercostal space. Cervical vagus nerves were isolated bilaterally via a midline neck incision and crashed with tight ligature. Each stellate ganglion was isolated and crashed at its junction with the ansa subclavia. These maneuvers remove almost all tonic neural activity to the heart (Levy et al., 1966).

A bipolar electrode was placed on the base of the epicardial surface of the right atrium near the SA node to record electrical activity. Sinus rate was measured and displayed on an oscillograph (Nihon Kohden, Tokyo, Japan, model RTA-1200). The systemic arterial blood pressure was recorded from the left femoral artery by a pressure transducer. The left femoral vein was cannulated for drug infusion and for physiological saline infusion to adjust spontaneous fluid losses. To stimulate the intracardiac parasympathetic nerve fibers to the SA nodal region, a bipolar electrode, 1.5 mm interelectrode distance, was placed on the fat pad overlying the right atrial side of the junctions of the pulmonary veins and connected to a stimulator (Nihon Kohden, SEN 7103). This parasympathetic nerve stimulation was applied at 30 Hz with 0.05 msec or less pulse duration and a voltage of 10 V for 30 sec.

The direct perfusion of the sinus node artery was prepared by the method of Hashimoto et al. (Hashimoto et al., 1968). A polyethylene tubing (o.d. 2.2 mm) was tapered to fit a cannula, the tip of which had an outer diameter of 0.5 to 1 mm. A rubber tubing was connected to the shank of the cannula for the purpose of injecting drug solutions. The dorsal right atrial artery so called sinus node artery was carefully isolated from its origin and cannulated with the cannula. Then, it was perfused with heparinized blood from the right femoral artery. The perfusion pressure could be maintained constant at 90 mmHg by means of shunting the excess blood to the blood reservoir through a pneumatic resistance which was placed in parallel with the perfusion system. The perfusion blood flow rate was measured in the extracorporeal circuit of the perfusion system by an electromagnetic flowmeter (Nihon Kohden, MFV-2100). Sodium heparin (500 USP units/kg i.v.) was administered at the beginning of the perfusion and 200 USP units/kg were given subsequently at 1-hr intervals.

Experimental Protocol

SA nodal pacemaker activity. We carried out four series of experiments after 30 min stabilization from the surgical procedures. In the first series, to examine the effects of PACAP-27 and VIP on the SA nodal pacemaker activity, we studied the changes in sinus rate in response to PACAP-27 (0.01–0.3 nmol, n = 6) or VIP (0.003–0.03 nmol, n = 5) injected into the sinus node artery of the autonomically decentralized hearts in the open-chest, anesthetized dogs. Enough recovery time (usually 1 hr after injection of PACAP-27) has been allowed to avoid the effects of the former injection of PACAP-27 on the effects of the following injection of PACAP-27, that is, "tachyphylaxis." PACAP presents tachyphylaxis in neonatal pig hearts (Ross-Ascuitto et al., 1993).

In the second series, to determine whether the responses to PACAP-27 are mediated by autonomic nervous system, we examined the effects of propranolol (n = 6) and atropine (n = 5) on the chronotropic responses to PACAP-27. Propranolol at a dose of 3.4 µmol/kg i.v. was given at the beginning of the experiments and 1.7 µmol/kg were given subsequently at 1-hr intervals. Atropine at a dose of 0.7 µmol/kg i.v. was administered at the beginning of the experiments and 0.14 µmol/kg were given subsequently at 1-hr intervals.

In the third series, to examine whether neural elements participate in chronotropic responses to PACAP-27, we studied the effects of tetrodotoxin (TTX, 30 nmol, n = 5) and hexamethonium (1 µmol, n = 5) into the sinus node artery on the chronotropic responses to PACAP-27 (0.1 nmol) and intracardiac parasympathetic nerve stimulation. We studied the effects of a blocker on the chronotropic responses to PACAP-27 at 0.1 nmol 1 hr after the determination of the control responses to PACAP-27 at 0.1 nmol. The response to PACAP-27 was observed 2 min after a blocker. We also studied the effects of TTX (30 nmol) on the positive chronotropic response to PACAP-27 (0.1 nmol) and nicotine (10 nmol) in five anesthetized dogs in which atropine at 0.7 µmol/kg/i.v. was given. In four atropine-treated anesthetized dogs, we observed the chronotropic responses to PACAP-27 at 0.1 nmol repeatedly at 1-hr interval as a control study.

In the fourth series, to determine which receptors mediate the positive chronotropic responses to PACAP-27, we studied 1) the effects of PACAP-27 (0.1 nmol) on the chronotropic responses to PACAP-27 (0.05 nmol), ACh (3 nmol) and norepinephrine (0.3 nmol) in five dogs, 2) the effects of a PACAP receptor antagonist, PACAP-(6–27) (10 nmol) on the positive chronotropic responses to PACAP-27 at 0.1 nmol and VIP at 0.03 nmol in four dog hearts treated with atropine and propranolol and 3) the effects of a VIP receptor antagonist, VIP-(10–28) at 10 nmol on the positive chronotropic responses to PACAP-27 at 0.1 nmol and VIP at 0.03 nmol after pretreatment with atropine and propranolol in four anesthetized dogs. Responses to PACAP-27 and VIP were obtained before and 5 min after PACAP-(6–27) or VIP-(10–28) treatment.

Atrial Fibrillation. When PACAP-27 at 0.1 or 0.3 nmol was injected into sinus node artery, it usually caused spontaneous atrial fibrillation. Thus, we investigated the incidence of the atrial fibrillation induced by PACAP-27 in autonomically decentralized, open-chest anesthetized dogs. First, we examined the incidence of atrial fibrillation induced by PACAP-27 (0.01–0.3) in the absence of a blocker (n = 6) and in the presence of atropine (0.7 µmol/kg i.v., n = 5) or propranolol (3.4 µmol/kg i.v., n = 6). Second, we studied the effects of hexamethonium (1 µmol, n = 5), TTX (30 nmol, n = 5), phenolamine (355 nmol, n = 5) and PACAP-(6–27) (10 nmol, n = 5) injected into the sinus node artery on the incidence of the PACAP-27-induced atrial fibrillation. Additionally, to test whether intracardiac parasympathetic stimulation induces atrial fibrillation, we stimulated the intracardiac parasympathetic nerves to the SA nodal region at a frequency of 30 Hz for 30 sec in five autonomically decentralized hearts. The pulse duration of the stimulation was selected to evoke similar decreases in sinus rate induced by PACAP-27.
Drugs

Drugs were mixed fresh for each experiment. Pituitary adenylate cyclase-activating polypeptide-27 (human) (PACAP-27, Peptide Institute Inc., Osaka, Japan), vasoactive intestinal peptide (human, porcine) (VIP, Peptide Institute Inc.), vasoactive intestinal peptide 10–28 (human, porcine, rat) (VIP-(10–28), Peninsula Labo. Inc., Belmont, CA) and pituitary adenylate cyclase-activating polypeptide 6–27 (human, ovine, rat) (PACAP-(6–27), Peninsula Labo. Inc.) were dissolved in distilled water and kept frozen at -20°C as stock solutions, and diluted immediately before use. Acetylcholine chloride (ACh, Dainichi, Tokyo, Japan), atropine sulfate (Wako Pure Chemicals, Tokyo, Japan), hexamethonium bromide (Yamanouchi, Tokyo, Japan), TTX (Wako Pure Chemicals), norepinephrine hydrochloride (Sankyo, Tokyo, Japan), nicotine bitartrate (Tokyo Kasei Kogyo, Tokyo, Japan) and propranolol hydrochloride (Sigma Chemical Co., St Louis, MO) were dissolved and diluted in 0.9% NaCl. Drugs were injected into the sinus node artery through a rubber tube by a microsyringe (Terumo Co., Tokyo, Japan) or the left femoral vein. The amount of drug solution injected into the sinus node artery was 0.01 ml in a period of 4 sec, because 0.01 ml of 0.9% NaCl has no effect on the SA nodal pacemaker activity (Hashimoto et al., 1968).

Statistical Analysis

All data were shown as the maximum change in response to each drug and expressed as mean ± S.E.M. The biphasic chronotropic responses to PACAP-27 were determined at the same phase before and after the treatment with each blocker. To assess the potency of the PACAP-27 and VIP for the increases in sinus rate, the negative log doses causing an increase in sinus rate by 50 beats/min were determined. An analysis of variance with Bonferroni’s test was used for the statistical analysis of multiple comparisons of data. Student’s t-test for unpaired data was used for comparison between the two groups. Fisher’s exact test was used for comparison with the incidence of atrial fibrillation. P < 0.05 were considered statistically significant.

Results

Effects of PACAP-27 and VIP on SA nodal pacemaker activity. PACAP-27 at a dose of 0.03 nmol produced a biphasic effect on sinus rate characterized by an initial increase followed by decrease in sinus rate, when it was injected into the sinus node artery of an autonomically decentralized heart in the open-chest anesthetized dog (fig. 1A). The negative chronotropic response to PACAP-27 reached to the maximum level approximately 2 min after the injection. However, VIP at a dose of 0.03 nmol only increased sinus rate (fig. 1B). When we increased a dose of PACAP-27 to 0.3 nmol, PACAP-27 decreased atrial cycle length from 500 to 400 msec at 10 sec after the PACAP-27 injection (fig. 2A and B) and at 30 sec after the injection, it prolonged the cycle length to 840 msec followed by suddenly and spontaneously induced atrial fibrillation (fig. 2C).

Chronotropic responses to PACAP-27 and VIP injected into the sinus node artery are summarized in figure 3. PACAP-27 in doses of 0.01 to 0.3 nmol caused a transient positive chronotropic response followed by a dose-dependent negative chronotropic response (P < .01) in six dogs (fig. 3A). The negative response to PACAP-27 at 0.1 nmol lasted 5 min or more in four of six dogs in which atrial fibrillation did not occur. The positive chronotropic response to PACAP-27 varied in each experiment. In contrast, VIP in doses of 0.003 to 0.03 nmol produced only a dose-dependent positive chronotropic response (P < .001) in five anesthetized dogs (fig. 3B).

To avoid the tachyphylaxis caused by PACAP-27, we injected each dose of PACAP-27 after an interval of one hour or more.

Effects of propranolol and atropine on a biphasic response to PACAP-27. After propranolol at 3.4 μmol/kg i.v. was given, it abolished the increases (68 ± 9.2 beats/min) in sinus rate in response to norepinephrine at 0.3 or 1 nmol into the sinus node artery of six dogs (table II). However, the biphasic effects of PACAP-27 (0.01–0.3 nmol) in propranolol treated dogs were not significantly different from those in nontreated dogs (fig. 3A; table II).

When atropine at 0.7 μmol/kg i.v. was given, it abolished the decreases (71 ± 9.2 beats/min) in sinus rate in response to ACh at 3 or 10 nmol into the sinus node artery of five hearts (table III). After atropine treatment, PACAP-27 in doses of 0.01 to 0.3 nmol only increased sinus rate in a dose-dependent manner (P < .001) as did VIP (0.003–0.03 nmol) (figs. 1 and 3B; table III). The positive response to PACAP-27 at 0.3 nmol lasted 20 min or more. The mean negative log doses of the 50 beats/min increase in response to PACAP-27 and VIP were 9.7 and 10.6, respectively, in each five experiments. The dose for PACAP-27 was determined in atropine-treated dogs.

In four atropine-treated dogs, propranolol at 30 nmol into the sinus node artery did not affect the increases in sinus rate in response to PACAP-27 at 0.1 nmol, whereas it blocked the positive chronotropic response to norepinephrine (table II). Propranolol and atropine themselves did not change the basal sinus rate.

Effects of tetrodotoxin and hexamethonium. To determine whether the responses to PACAP-27 are mediated through neural activation, we examined the effects of TTX on the chronotropic responses to PACAP-27. TTX at 30 nmol injected into the sinus node artery abolished the negative chronotropic response to 0.1 nmol of PACAP-27 (P < .01), and it caused only a positive response in five hearts (fig. 4A; table III). TTX rather augmented the initial increase in sinus rate when the response was determined at the same phase before and after treatment with TTX. TTX also abolished the negative chronotropic response to stimulation of the parasympathetic nerves to the SA nodal region (fig. 4A; table III).

When a ganglionic nicotinic receptor blocker, hexamethonium at 1 μmol blocked the negative chronotropic response to parasympathetic nerve stimulation, it did not affect the negative chronotropic response to 0.1 nmol of PACAP-27 significantly (fig. 4B; table IV).

After i.v. treatment with atropine, we confirmed that PACAP-27 at 0.1 nmol repeatedly induced the positive chronotropic response with 1-hr interval in four dogs (fig. 5). TTX
Effects of each blocker on the chronotropic responses to PACAP-27 in autonically decentralized hearts of the anesthetized dogs

<table>
<thead>
<tr>
<th>Drug</th>
<th>Changes in Sinus Rate (Beats/Min)</th>
<th>Control</th>
<th>After drug treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>PACAP-27 (0.1 nmol)</td>
<td>8 ± 3</td>
<td>14 ± 8.7</td>
<td></td>
</tr>
<tr>
<td>PACAP-27 (0.3 nmol)</td>
<td>-60 ± 16</td>
<td>-51 ± 9.4</td>
<td></td>
</tr>
<tr>
<td>NE (0.3-1 nmol)</td>
<td>68 ± 9.4</td>
<td></td>
<td>0</td>
</tr>
<tr>
<td>PACAP-27 (0.1 nmol)</td>
<td>8 ± 3</td>
<td>35 ± 6.9</td>
<td></td>
</tr>
<tr>
<td>PACAP-27 (0.3 nmol)</td>
<td>-60 ± 16</td>
<td>35 ± 6.9</td>
<td></td>
</tr>
<tr>
<td>ACh (3-10 nmol)</td>
<td>-71 ± 9.2</td>
<td></td>
<td>0</td>
</tr>
<tr>
<td>PACAP-27 (0.1 nmol)</td>
<td>9 ± 4</td>
<td>28 ± 6.5</td>
<td></td>
</tr>
<tr>
<td>PACAP-27 (0.3 nmol)</td>
<td>-70 ± 19.8</td>
<td>34 ± 9.7</td>
<td></td>
</tr>
<tr>
<td>SAPS (30 Hz)</td>
<td>-57 ± 12.8</td>
<td></td>
<td>0</td>
</tr>
<tr>
<td>PACAP-27 (0.1 nmol)</td>
<td>11 ± 4.5</td>
<td>6 ± 3.8</td>
<td></td>
</tr>
<tr>
<td>PACAP-27 (0.3 nmol)</td>
<td>-66 ± 19.9</td>
<td>-69 ± 18.1</td>
<td></td>
</tr>
<tr>
<td>SAPS (30 Hz)</td>
<td>-57 ± 18.1</td>
<td>-2 ± 2.4</td>
<td></td>
</tr>
<tr>
<td>PACAP-27 (0.1 nmol)</td>
<td>23 ± 7.3</td>
<td>-12 ± 8.1</td>
<td></td>
</tr>
<tr>
<td>PACAP-27 (0.3 nmol)</td>
<td>4 ± 6.6</td>
<td>-21 ± 5.4</td>
<td></td>
</tr>
<tr>
<td>ACh (3 nmol)</td>
<td>-30 ± 6.3</td>
<td>36 ± 7.1</td>
<td></td>
</tr>
<tr>
<td>NE (1 nmol)</td>
<td>48 ± 5.4</td>
<td>39 ± 7.0</td>
<td></td>
</tr>
<tr>
<td>PACAP-(6-27) (10 nmol i.a., n = 5)</td>
<td>-55 ± 8.2</td>
<td>-51 ± 7.0</td>
<td></td>
</tr>
</tbody>
</table>

ACh, acetylcholine; NE, norepinephrine; SAPS, stimulation of intracardiac parasympathetic nerves to the SA nodal area; PACAP, pituitary adenylate cyclase-activating polypeptide; i.a., intraarterial injection.

\( ^{a} P < .05 \) vs. control.

\( ^{b} P < .01 \) vs. control.

Fig. 2. Changes in atrial cycle length in response to PACAP-27 at a dose of 0.3 nmol given to the sinus node artery in an autonically decentralized heart of an open-chest anesthetized dog. Ten sec after administration of PACAP-27, atrial cycle length shortened from 500 msec (A) to 400 msec (B) and then 30 sec later, the cycle length prolonged to 840 msec followed by suddenly occurring atrial fibrillation (af) in an autonically decentralized heart of the anesthetized dog. ECG, electrocardiogram from the body surface.

Table 1: Effects of each blocker on the chronotropic responses to PACAP-27 in autonically decentralized hearts of the anesthetized dogs

Effects of a high dose of PACAP-27. To investigate whether the chronotropic responses to PACAP-27 cause tachyphylaxis, we studied effects of PACAP-27 at a dose of 0.1 nmol on the chronotropic responses to PACAP-27 at a dose of 0.03 nmol, ACh at 3 nmol and norepinephrine at 0.3 nmol. PACAP-27 at 0.03 nmol and norepinephrine increased sinus rate, whereas ACh decreased sinus rate as shown figure 6A. One hour later, 0.1 nmol of PACAP-27 injected into the sinus node artery caused a biphasic effects. After returning to the pre-drug basal level, the second injection of PACAP-27 at 0.03 nmol only produced the negative chronotropic effect, whereas the chronotropic responses to norepinephrine and ACh were not changed (fig. 6A). Summarized data from five dogs are shown in figure 6B and table 1V. The positive chronotropic responses to 0.03 nmol of PACAP-27 were reversed to the negative one and the negative responses were potentiated (P < .05) by pretreatment with 0.1 nmol of PACAP-27, while the responses to ACh and norepinephrine were not affected significantly.

Effects of PACAP-(6-27) and VIP-(10-28). When we examined the effects of PACAP-(6-27) on the negative chronotropic response to PACAP-27, PACAP-(6-27) at 10 nmol in five dogs did not affect the decreases in sinus rate in response to 0.3 nmol of PACAP-27 (fig. 7B; Table 1VI). The negative response was determined just before the induction of atrial fibrillation.

A high dose of PACAP-27 presented tachyphylaxis on the positive response but not the negative one as presented in the previous session (fig. 6). Then, to determine which receptors mediate the positive chronotropic responses to PACAP-27, we studied the effects of a PACAP receptor antagonist, PACAP-(6-27) and a VIP receptor antagonist, VIP-(10-28) on the positive chronotropic response to PACAP-27 or VIP.

Fig. 3. A. Dose-response curves for the positive followed by negative chronotropic response to PACAP-27 in doses of 0.01 to 0.3 nmol injected into the sinus node artery before (○, n = 6) and after (●, n = 6) treatment with propranolol at 3.4 μmol/kg i.v. in the autonically decentralized heart of the anesthetized dog. B. Positive chronotropic responses to PACAP-27 (●) in doses of 0.01 to 0.3 nmol in five atropine-(0.7 μmol/kg i.v.) treated anesthetized dogs and to VIP (△) in doses of 0.003 to 0.03 nmol in five nonblockade dogs. Vertical bars show S.E.M. Control sinus rate before propranolol treatment in six dogs was 121 ± 8.3 (mean ± S.E.M.) beats/min and it was not significantly different from the sinus rates of other experimental groups.

at 30 nmol inhibited the positive chronotropic response to PACAP-27 by 34 ± 2.6% (P < .001) in five dogs, when TTX suppressed the increases in sinus rate induced by nicotine (fig. 5; table 2I). TTX and hexamethonium themselves did not change the basal sinus rate.
PACAP-(6–27) at 10 nmol attenuated the increase in sinus rate in response to 0.1 nmol of PACAP-27 in an atriope and propranolol treated dog (fig. 7A).

In four hearts after atropine and propranolol treatment, PACAP-(6–27) at 10 nmol significantly (P < .001) reduced the positive chronotropic response to 0.1 nmol of PACAP-27 but did not affect the positive chronotropic response to 0.03 nmol of VIP (fig. 8A; table 2-III).

However, VIP-(10–28) at 10 nmol did not change the positive chronotropic response to PACAP-27 (0.1 nmol), but it blocked the chronotropic response to VIP (0.03 nmol) significantly (P < .01) in four atropine and propranolol treated animals (fig. 8B; table 2IV). PACAP-(6–27) and VIP-(10–28) themselves did not change the basal sinus rate.

A pharmacological analysis of the PACAP-27-induced atrial fibrillation. PACAP-27 in doses of 0.1 and 0.3 nmol induced atrial fibrillation in a dose-dependent manner (P < .01) in six dogs (table 3). PACAP-27 at 0.3 nmol caused atrial fibrillation in all six dogs. The atrial fibrillation evoked by PACAP-27 continued 3 min or more and then spontaneously terminated. The longest duration of the atrial fibrillation was 54 min.

After propranolol treatment, 0.3 nmol of PACAP-27 induced atrial fibrillation in three of six dogs (table 3) and it was not significant. However, after atropine, PACAP-27 (0.01–0.3 nmol) did not evoke atrial fibrillation any more in five dogs (table 3). TTX also completely blocked the induction of atrial fibrillation evoked by PACAP-27 in five dogs (table 4). However, hexamethonium and phentolamine did not affect the induction of atrial fibrillation evoked by PACAP-27 in five and three dogs, respectively (table 4). Additionally, PACAP-(6–27) did not affect the induction of atrial fibrillation evoked by 0.3 nmol of PACAP-27 either, although PACAP-(6–27) attenuated the initial increase in sinus rate in response to PACAP-27 (fig. 7B; table 4). Even after 10 nmol of PACAP-(6–27) treatment, 0.3 nmol of PACAP-27 induced atrial fibrillation in all five examined dogs (table 4).

To study whether parasympathetic nerve stimulation induces atrial fibrillation, we stimulated the intracardiac parasympathetic nerves to the SA nodal region. The parasympathetic nerve stimulation at a frequency of 30 Hz for 30 sec decreased sinus rate by 102 ± 9.0 beats/min from the pre-stimulation rate, 132 ± 8.5 beats/min, in five dogs. However, atrial fibrillation did not occur in any examined cases.

Effects of PACAP-27 on flow rate of the sinus node artery. PACAP-27 in doses of 0.01 to 0.3 nmol increased the perfused blood flow in a dose-dependent manner (P < .005) in six hearts as shown in figure 9. Control flow rate for 6 dogs to the sinus node artery is 4 ± 1.3 ml/min.

Discussion

PACAP-27 on SA nodal pacemaker activity. PACAP-27 in doses of 0.01 to 0.3 nmol caused a biphasic dose-dependent chronotropic effect in the autonomically decentralized dog heart (figs. 1 and 3). PACAP-27 increased sinus rate to more than 180 beats/min in atropine-treated dog hearts and the order of the potency for the positive chronotropic response to PACAP-27, PACAP-38, VIP and norepinephrine is VIP > PACAP-27 > norepinephrine >
PACAP-38 in the dog heart (Yonezawa et al., 1996; Hirose et al., 1997). PACAP-38 at 1 nmol increased sinus rate by 20% followed by decreased sinus rate by 20%, respectively, in the anesthetized dog heart (Hirose et al., 1997). Thus, the negative chronotropic response to PACAP-27 was much greater than that to PACAP-38 and PACAP-27 at a high dose (0.3 nmol) sometimes caused sinus arrest transiently. ACh at 3 nmol or more into the sinus node artery induced sinus arrest in the autonomically decentralized dog hearts. Therefore, it is suggested that PACAP-27 is a very potent endogenous substance on the heart rhythmicity.

The negative chronotropic response to PACAP-27 was blocked by atropine and tetrodotoxin but not by hexametho-
nium (figs. 1, 3 and 4; table 1), indicating that PACAP-27 decreases sinus rate due to an activation of the postganglionic parasympathetic nerves in the dog heart but the negative response is not mediated by ganglionic nicotinic receptors. PACAP contracts the guinea pig ileum via the release of ACh from postganglionic cholinergic neuron (Katsuoulis et al., 1993). A high dose of PACAP-27 did not inhibit the negative chronotropic response to a low dose of PACAP-27, indicating that the negative response to PACAP-27 did not present tachyphylaxis (fig. 6). Additionally, a PACAP receptor antagonist, PACAP-(6–27) did not inhibit the negative chronotropic response to PACAP-27, although the blocker attenuated the increases in sinus rate (figs. 7 and 8; table 1). Thus, we suggest that the release of ACh evoked by PACAP-27 is mediated by the PACAP receptors which are different from the PACAP-(6–27)-sensitive PACAP receptors although other unknown mechanisms may exist including direct effects independent of PACAP receptors, e.g., direct ACh release or direct muscarinic receptor activation by PACAP-27. In the distal colon of the rat, a PACAP antagonist, PACAP-(6–38) inhibited the muscle relaxation induced by electrical field stimulation (Kishi et al., 1996). However, PACAP-(6–38) did not affect the positive as well as negative chronotropic responses to PACAP-38 in the isolated, perfused dog atrium (Yonezawa et al., 1996).

Neither propranolol injected into the sinus node artery did affect the increase in sinus rate in response to PACAP-27 in atropine-treated dogs nor intravenous treatment with propranolol affected the biphasic effects of PACAP-27 in nonatropine treated dogs (fig. 3; table 2), indicating that the positive chronotropic response to PACAP-27 is not mediated through beta adrenoceptors in the dog heart. However, a high dose of PACAP-27 attenuated the positive chronotropic response to a low dose of PACAP-27 (fig. 6; table 1) and PACAP-(6–27) inhibited the positive chronotropic response to PACAP-27 but not to VIP (figs. 7 and 8; table 2). However, VIP-(10–28) inhibited the positive chronotropic response to VIP but not to PACAP-27 (fig. 8; table 2). From these results, therefore, we suggest that PACAP-27 increases sinus rate mediated by PACAP receptors that are different from VIP receptors in the dog heart.

**Fig. 7.** A, A blocking effect of a PACAP receptor antagonist, PACAP-(6–27) at 10 nmol on the increase in sinus rate in response to PACAP-27 at 0.1 nmol in an atropine- and propranolol-treated anesthetized dog heart. Atropine at 0.7 μmol/kg i.v. and propranolol at 3.4 μmol/kg i.v. were given before starting the experiment and PACAP-27 was administered into the sinus node artery with 1-hr intervals. B, Effects of PACAP-(6–27) at 10 nmol on the PACAP-27 (0.3 nmol) induced atrial fibrillation in an autonomically decentralized heart of the anesthetized dog. PACAP-(6–27) attenuated the initial positive chronotropic response to PACAP-27 but did not inhibited the induction of atrial fibrillation. PACAP-27 was administered into the sinus node artery with 1-hr intervals.

**Fig. 8.** A, Effects of PACAP-(6–27) at a dose of 10 nmol on the positive chronotropic response to PACAP-27 at 0.1 nmol and VIP at 0.03 nmol in 4 atropine- (0.7 μmol/kg i.v.) and propranolol- (3.4 μmol/kg i.v.) treated dogs. Control sinus rate for four dogs was 123 ± 1.3 (mean ± S.E.M.) beats/min. PACAP-27 and VIP increased sinus rate by 59 ± 12.3 and 32 ± 2.9 beats/min before PACAP-(6–27) treatment, respectively. B, Effects of VIP-(10–28) at a dose of 10 nmol on the positive chronotropic response to PACAP-27 at 0.1 nmol and VIP at 0.03 nmol in four atropine- (0.7 μmol/kg i.v.) and propranolol- (3.4 μmol/kg i.v.) treated dogs. PACAP-27 and VIP increased sinus rate by 62 ± 6.3 and 47 ± 1.7 beats/min before VIP-(10–28) treatment, respectively. Open and closed columns present a response to intervention before and after treatment with a blocker, respectively. Control sinus rates before PACAP-(6–27) and VIP-(10–28) treatment were 123 ± 1.3 and 123 ± 4.0 (mean ± S.E.M.) beats/min, respectively. Vertical bars show S.E.M. **P < .01; ***P < .01; NS, not significant vs. values in control.
through three types of PACAP receptors (Harmar and Lutz, 1994). The type I PACAP receptor is subdivided into two subtypes on the basis of binding studies. PACAP1A receptors bind PACAP-27 with slightly higher affinity than PACAP-38, although PACAP1B receptors bind PACAP-38 with high affinity and PACAP-27 with low affinity. The type I PACAP receptor is coupled to adenylate cyclase and phospholipase C (Spengler et al., 1993). The type II PACAP receptor is identical with the type I VIP receptor (Ishihara et al., 1992; Sreedharan et al., 1995). The type II PACAP receptor binds PACAP-27, PACAP-38 and VIP with similar affinities (Shiv-ers et al., 1991) and is coupled to only adenylate cyclase (Ishihara et al., 1992; Spengler et al., 1993). The third type of receptor is identical to the type II VIP receptor (Lutz et al., 1993) and three neuropeptides activate the type II VIP receptor with the same rank order as for stimulation of cAMP production in the cAMP reporter LVIP cells (Usdin et al., 1994). Therefore, we suggest that the positive chronotropic response to PACAP-27 is mediated by type I PACAP receptors, probably PACAP1A or PACAP1A-like receptors in the dog heart. However, we need further studies including the receptor binding study and measurements of cAMP and IP<sub>3</sub> in the dog heart, because the type I PACAP receptor mRNA was expressed abundantly in the brain, but there was little expression in peripheral tissues (Spengler et al., 1993; Hashimoto et al., 1993).

In our study, tetrodotoxin attenuated the positive chronotropic responses to PACAP-27 by 34% in atropine-treated dogs (fig. 5; table 2). These results in addition to other our results suggest that PACAP-27 neurally increases sinus rate in part and the neural activation is nonadrenergic and non-VIP-ergic in the dog heart. From our results, however, we could not define which receptors mediated the tetrodotoxin-sensitive positive response to PACAP-27.

**Atrial fibrillation induced by PACAP-27.** In our study, we first demonstrated that PACAP-27 injected into the sinus node artery caused atrial fibrillation in the autonomically decentralized heart of the open-chest anesthetized dog (fig. 2; table 3). The atrial fibrillation induced by PACAP-27 was abolished by atropine and TTX but not hexamethonium (tables 3 and 4), indicating that the PACAP-27-induced ACh release from intracardiac parasympathetic nerves participates in an induction of atrial fibrillation in the dog heart. It is well known that vagus stimulation and other cholinomimetic drugs evoke atrial fibrillation in anesthetized dog hearts (Lewis et al., 1921). However, in the autonomically decentralized dog heart, vagus stimulation caused sinus arrest but not induce atrial fibrillation (Furukawa et al., 1996). ACh in doses of 3 to 30 nmol injected into sinus node artery also hardly caused atrial fibrillation (Hirose et al., 1997). In addition, when we stimulated the intracardiac parasympathetic nerves to the SA nodal region for 30 sec, the stimulation decreased sinus rate more than did PACAP-27, but did not cause atrial fibrillation in our present study. Therefore, the PACAP-27-induced ACh release mainly affects the induction of atrial fibrillation but we cannot neglect other mechanisms may be involved in that of atrial fibrillation in the dog heart.

When ACh causes atrial fibrillation, it shortens the atrial refractory period heterogenously and induces multiple pacemaker activation (Schuessler et al., 1991). Sympathetic stimulation also shortens atrial refractory period in the anesthetized dog (Zipes et al., 1974; Takei et al., 1991). The shortening atrial refractory period is related to the augmentation of the relaxation after the increase in tissue cAMP mediated by beta adrenoceptors. Additionally, simultaneous stimulation of the sympathetic and parasympathetic nerves additively shortens the atrial refractory period in anesthetized dog hearts (Takei et al., 1991). Under sympathetic tone, thus, atrial fibrillation induced by ACh occurs easier (Hashimoto et al., 1968). In our study, propranolol tended to decrease the incidence of the atrial fibrillation induced by PACAP-27 (table 3). However, propranolol affected neither
the positive nor negative chronotropic responses to PACAP-27 (fig. 3; tables 1 and 2). Thus, it is unlikely that beta adrenergic mechanism participates in the PACAP-27-induced atrial fibrillation. PACAP-38 induced a biphasic inotropic and chronotropic responses in isolated perfused dog atria, and those cardiac responses were not inhibited by propranolol (Yonezawa et al., 1996). In addition, i.v. injection of propranolol did not change the basal sinus rate in our study, indicating that circulatory catecholamines hardly affected the positive chronotropic and inotropic effects of propranolol, so called membrane stabilizing action (Davis and Temte, 1968), related to decrease the induction of atrial fibrillation induced by PACAP-27, although beta adrenoceptor blocking effects might be involved.

However, phenolamine administered into the SA node artery did not decrease the incidence of the PACAP-27-induced atrial fibrillation (table 4). The positive cardiac responses to alpha adrenoceptor agonists were not inhibited by alpha adrenoceptor antagonists in isolated dog atria (Chiba, 1977). Furthermore, alpha adrenoceptors exist in the dog heart but alpha adrenoceptor-mediated responses are not determined (Endoh et al., 1991). Therefore, alpha adrenoceptors may not relate to the induction of atrial fibrillation induced by PACAP-27 in the dog heart.

A PACAP receptor antagonist, PACAP-(6-27) did not attenuate the incidence of PACAP-27-induced atrial fibrillation (table 4), although it partly inhibited the positive chronotropic response to PACAP-27 (fig. 8; table 2). These results suggest that PACAP-(6-27)-sensitive PACAP receptors do not mainly act on the induction of atrial fibrillation in the dog heart, but they may support in part the induction of atrial fibrillation.

Thus, our results suggest that the PACAP-27-induced ACh release from intracardiac parasympathetic nerves but not adrenergic mechanism mainly participates in the induction of atrial fibrillation in the autonomically decentralized dog heart, although we cannot neglect other mechanisms, which induce positive chronotropic responses to PACAP-27, participate in the induction of atrial fibrillation. PACAP-27 increases cAMP in several tissues (Miyata et al., 1989; Spengler et al., 1993; Tong et al., 1993). IBMX, a nonspecific phosphodiesterase inhibitor, augmented the increase in left ventricular dp/dtmax in response to PACAP in neonatal pig hearts (Ross-Ascuitto et al., 1993). Thus, PACAP-27 might increase tissue cAMP and shorten the atrial refractory period in the dog atrial muscles. We need further studies to define the precise mechanisms of the induction of atrial fibrillation induced by PACAP-27, especially the relationship between atrial fibrillation and mechanisms causing positive chronotropic responses to PACAP-27, in the heart.

It has been suggested that the positive inotropic and lusitropic effects of PACAP are useful as a cardiotonic agent (Ascuitto et al., 1996). However, PACAP has several actions in addition to the hormonal actions (Arimura et al., 1995). Furthermore, in our study, we demonstrated that PACAP acts on the peripheral nervous system as well as on the heart and it induces atrial fibrillation. Although many reports suggest a role for PACAP in humans (Kimura et al., 1990; Shen et al., 1992; Guijarro et al., 1995; Suda et al., 1991), further studies with PACAP are still needed before clinical application.

References


Send reprint requests to: Dr. Y. Furukawa, Department of Pharmacology, Shinshu University School of Medicine, Matsumoto 390, Japan.