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PACAP-27 causes negative and positive dromotropic effects in anesthetized dogs

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Abstract

While pituitary adenylate cyclase-activating polypeptide (PACAP) has been identified radioimmunologically in the rat heart, the physiological role of PACAP has not been elucidated in the regulation of the atrioventricular conduction in the heart. We, therefore, determined the dromotropic effects of PACAP-27 injected into the cannulated atrioventricular node artery in the autonomically decentralized heart of the open-chest, anesthetized dog. PACAP-27 caused transient positive followed by negative dromotropic responses in a dose-dependent manner, whereas vasoactive intestinal peptide (VIP) caused only a positive dromotropic response. Atropine and tetrodotoxin blocked the negative dromotropic response to PACAP-27 and after blockade PACAP-27 caused only a positive dromotropic response. Tetrodotoxin and propranolol did not affect the positive dromotropic response to PACAP-27 in atropine-treated dogs. PACAP-27 altered the atrio–His bundle interval but did not alter the His–ventricle interval. These results demonstrate that PACAP-27 prolongs the atrio–His bundle interval due to the liberation of acetylcholine from parasympathetic nerves and decreases it by a non-adrenergic mechanism in the dog heart in situ. © 1997 Elsevier Science B.V.

Keywords: PACAP (pituitary adenylate cyclase-activating polypeptide); Atrioventricular conduction; Parasympathetic nerve; VIP (vasoactive intestinal peptide)

1. Introduction

Pituitary adenylate cyclase-activating polypeptide (PACAP) is a newly discovered neuropeptide isolated originally from the ovine hypothalamus (Miyata et al., 1989). 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 vasoactive intestinal peptide (VIP) (Miyata et al., 1990). PACAP is widely distributed in the brain and peripheral tissues, including the heart, and its receptors have been detected in the heart (Arimura et al., 1991; Inagaki et al., 1994; Usdin et al., 1994; Sreedharan et al., 1995; Wei and Mojsov, 1996). Plasma levels of PACAP have also been reported for the rat and humans (Wang et al., 1992). Additionally, in an immunohistochemical study, double-immunostaining for VIP and PACAP revealed coexistence of the two peptides in nerve cell bodies and fibers in the gut of humans and chickens and in fibers in the gastric mucosa of mice and rats, although PACAP has not yet been identified as a cotransmitter with VIP in cardiac neurons (Sundler et al., 1992). Therefore, PACAP, like VIP, may have important physiological and pathophysiological roles in the regulation of the heart in situ.

An intravenous injection of PACAP-38 induced bradycardia after producing transient tachycardia and produced hypertension after producing transient hypotension in anesthetized dogs (Ishizuka et al., 1992). Another PACAP, PACAP-27, is approximately 3 or 4 times more potent than PACAP-38 in eliciting the positive inotropic response in neonatal pig hearts (Ascuitto et al., 1996). It has been also demonstrated that PACAP-38 first increases and then decreases the sinus rate and myocardial contractile force in isolated perfused dog atria (Yonezawa et al., 1996). Recently, we demonstrated that PACAP-38 directly induces a biphasic chronotropic response and reduced acetylcholine-induced atrial fibrillation threshold in the anesthetized dog (Hirose et al., 1997). It is, thus, conceivable that PACAP induces cardiac arrhythmias. Atrioventricular conduction is one of the key steps governing cardiac rhythm. However,

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there are no reports of the dromotropic effect of PACAP in the heart. We, therefore, investigated the effects of PACAP-27 on atrioventricular conduction in the anesthetized dog heart. To study the direct effect of PACAP-27 on atrioventricular conduction, we determined changes in the atrioventricular interval in response to PACAP-27 and VIP injected into the atrioventricular node artery in the autonomically decentralized heart of the anesthetized dog. In the present study, we verified the negative and positive dromotropic responses to PACAP-27 and then analyzed the dromotropic responses pharmacologically.

2. Materials and methods

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

2.1. Preparation

Fifteen mongrel dogs of either sex, weighing 10 to 20 kg, were anesthetized with sodium pentobarbital (35 mg/kg i.v.). A tracheal cannula was inserted and intermittent positive-pressure ventilation was started with a respirator (Model 607, Harvard Apparatus, Millis, MA, USA) and room air. The chest was opened transversely at the fifth intercostal space. Cervical vagus nerves were isolated bilaterally via a midline neck incision and crushed with a tight ligature. Each stellate ganglion was also isolated and ligated tightly at its junction with the ansa subclavia. These maneuvers remove almost all tonic neural activity to the heart (Levy et al., 1966).

Two bipolar electrodes, each used to record the atrial electrogram and to pace the atrium, were placed on the epicardial surface of the right atrial appendage and a bipolar electrode was placed on the base of the epicardial surface of the right ventricle to record the ventricular electrogram. A His catheter was placed in the noncoronary cusp of the aorta via the left femoral artery to record the His-bundle electrogram. Atrial pacing at a fixed atrial interval (400 ms) was performed with an electrical stimulator (Nihon Kohden, SEN 7103, Tokyo, Japan) which delivered a 1 ms rectangular pulse at twice the diastolic voltage threshold. The atrioventricular conduction time (atrioventricular interval) was measured with an atrioventricular interval counter (Nihon Kohden ET-601G) that detected the upstroke of the atrial and ventricular electrograms. The atrioventricular nodal conduction time was also estimated from the a–h interval measured from the atrial deflection (a) in the His-bundle recording to the His-bundle potential (h). The His bundle–ventricle conduction (h–v interval) was derived from the His-bundle potential to the ventricular deflection (v) in the His-bundle recording. Systemic arterial blood pressure was recorded from the left femoral artery with a pressure transducer. All electrograms, atrioventricular interval and systemic arterial blood pressure were recorded and displayed on an oscillograph (Nihon Kohden, model RTA-1200). The left femoral vein was cannulated for drug injection and for physiological saline infusion to adjust for spontaneous fluid loss.

Direct perfusion of the atrioventricular node artery was achieved by using the method developed by Chiba and Hashimoto (1970). A polyethylene tubing (o.d. 2.2 mm) was tapered to fit a cannula, the tip of which had an outer

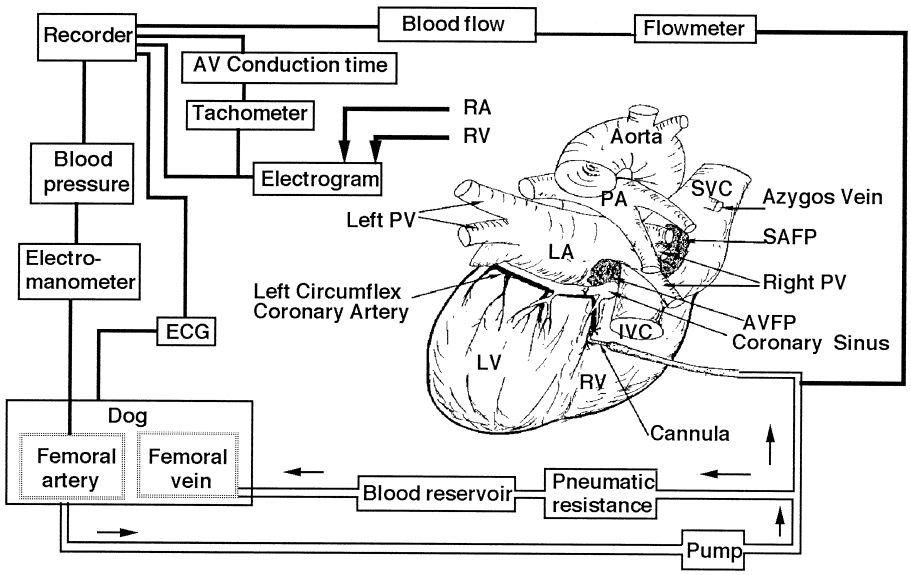

Fig. 1. Schematic diagram of the atrioventricular node artery perfusion system. Distal branch of the left circumflex coronary artery (so-called atrioventricular node artery) was cannulated and perfused at a constant pressure of 90 mmHg with heparinized blood from a femoral artery. RA, right atrium; SVC, superior vena cava; IVC, inferior vena cava; PA, pulmonary artery; PV, pulmonary vein; RV, right ventricle; LA, left atrium; LV, left ventricle; SAFP, fat pad overlying the right atrial side of the atrial junctions of the pulmonary veins; AVFP, fat pad at the junction of the inferior vena cava and left atrium.

diameter of 0.5 to 1 mm. A piece of rubber tubing was connected to the shank of the cannula for injection of drug solution. The distal branch of the left circumflex coronary artery was carefully isolated from the origin of the atrioventricular node artery and cannulated with the cannula. Then, the atrioventricular node artery was perfused with heparinized blood from the right femoral artery. The perfusion pressure was 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 using an electromagnetic flowmeter (Nihon Kohden, MFV-2100). A schematic diagram of the atrioventricular node artery perfusion system is shown in Fig. 1. Sodium heparin (500 USP u/kg i.v.) was administered at the beginning of perfusion and 200 USP u/kg were given subsequently at 1-h intervals.

2.2. Experimental protocols

We carried out two 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 atrioventricular conduction, we studied the changes in atrioventricular interval in response to PACAP-27 (0.01–0.3 nmol, $n = 5$) or VIP (0.03–0.3 nmol, $n = 5$) injected into the atrioventricular node artery of the autonomically decentralized hearts in the open-chest, anesthetized dogs. Enough recovery time (usually 1 h) after injection of PACAP-27 was allowed to prevent the former injection of PACAP-27 from affecting the following injection of PACAP-27, that is, 'tachyphylaxis'. PACAP caused tachyphylaxis in neonatal pig hearts (Ascuitto et al., 1996). To determine the regions of the atrioventricular conduction system that were affected by PACAP-27, we studied changes in a–h and h–v intervals in response to PACAP-27 (0.3 nmol) in the His-bundle recording from 3 dogs.

In the second series, to determine whether the dromotropic responses to PACAP-27 are mediated by the autonomic nervous system, we examined the effects of atropine ($n = 5$) and propranolol ($n = 4$) on the dromotropic response to PACAP-27. Atropine at a dose of 0.7 μmol/kg i.v. was administered at the beginning of the experiments and 0.14 μmol/kg i.v. was given subsequently at 1-h intervals. After atropine treatment, PACAP-27 (0.01–0.3 nmol) was injected into the atrioventricular node artery and the dromotropic effects of PACAP-27 were determined. PACAP-27 (0.1 nmol) and norepinephrine (0.3 nmol) were also injected into the atrioventricular node artery before and after propranolol (30 nmol) in atropine-treated dogs.

Additionally, to examine whether neural elements participate in dromotropic responses to PACAP-27, we studied the effects of tetrodotoxin (30 nmol, $n = 4$) injected into the atrioventricular node artery on the dromotropic responses to PACAP-27 (0.1 nmol) and to nicotine (a ganglionic nicotinic receptor agonist, 10 nmol). We also studied the effects of tetrodotoxin (30 nmol) on the positive dromotropic response to PACAP-27 (0.1 nmol, $n = 4$)

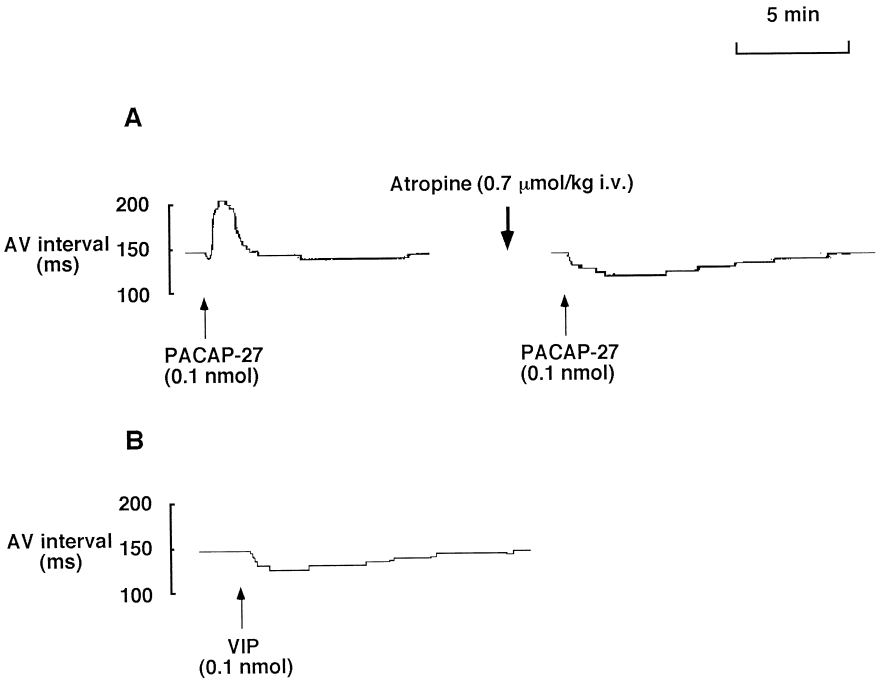

Fig. 2. Changes in atrioventricular interval in response to PACAP-27 at a dose of 0.1 nmol injected into the atrioventricular node artery before and after treatment with atropine at 0.7 μmol/kg i.v. (A) and in response to VIP at a dose of 0.1 nmol (B) in an autonomically decentralized heart of an open-chest, anesthetized dog. AV, atrioventricular.

in anesthetized dogs in which atropine at 30 nmol was injected into the atrioventricular node artery. We studied the effects of each blocker on the dromotropic responses to PACAP-27 (0.1 nmol) 1 h after the determination of the control responses to PACAP-27 (0.1 nmol). The responses to PACAP-27 were observed 2 min after each blocker treatment.

The control blood flow rate to the atrioventricular node artery was 4.6 ± 0.3 (mean $\pm$ S.E., $n = 12$) ml/min in the present study.

2.3. Drugs

Drugs were mixed fresh for each experiment. Pituitary adenylate cyclase-activating polypeptide 27 (Human) (PACAP-27, Peptide Institute, Osaka, Japan) and vasoactive intestinal peptide (Human, Porcine) (VIP, Peptide Institute) were dissolved in distilled water and kept frozen at -20°C as stock solutions, and diluted immediately before use. Acetylcholine chloride (ACh, Daiichi, Tokyo, Japan), atropine sulfate and tetrodotoxin (Wako, Tokyo, Japan), nicotine bitartrate (Tokyo Kasei Kogyo, Tokyo, Japan), norepinephrine hydrochloride (Sankyo, Tokyo, Japan) and propranolol hydrochloride (Sigma, St. Louis, MO, USA) were dissolved and diluted in 0.9% NaCl. Drugs were injected into the atrioventricular node artery or left femoral vein through a rubber tube with a microsyringe (Ito, Shizuoka, Japan). The amount of drug solution injected into the atrioventricular node artery was 0.01 ml over a period of 4 s.

2.4. Statistical analysis

All data are shown as the maximum change in response to each drug and expressed as means $\pm$ S.E. 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. *P* values of less than 0.05 were considered statistically significant.

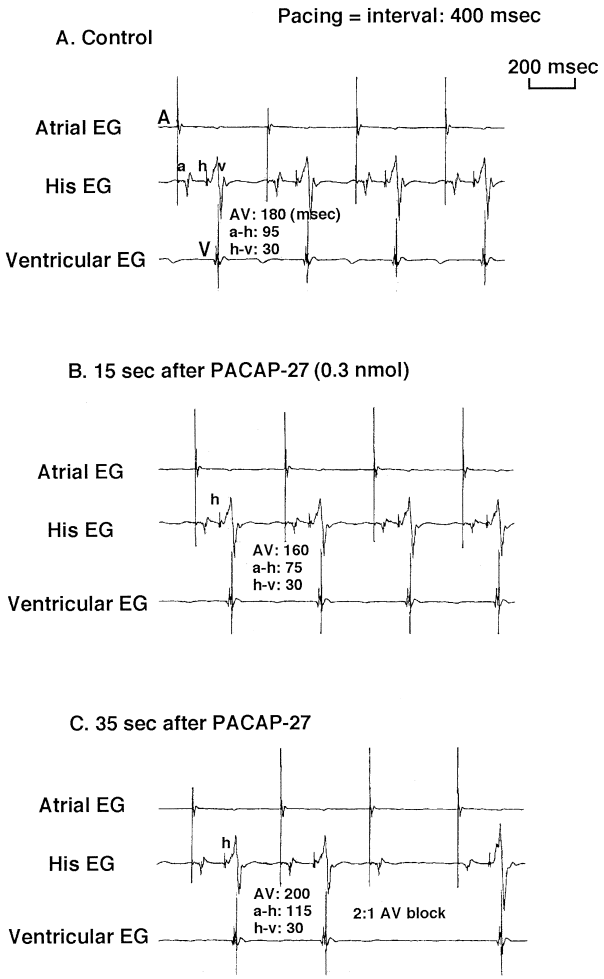

Fig. 3. Recordings of electrical changes in the atrium, His-bundle and ventricle induced by PACAP-27 in an autonomically decentralized, open-chest, anesthetized dog when the atrium was paced at 400 ms intervals. Data were obtained before (Control), at 15 s after PACAP-27 (0.3 nmol) injection and at 35 s after the injection. A, electrogram from the base of the right atrial appendage; V, electrogram from the right ventricular surface; a, electrogram from the atrium by the His electrode; h, electrogram from the His-bundle by the His electrode; v, electrogram from the ventricle by the His electrode, AV, atrioventricular interval; a–h, atrial–His bundle interval; h–v, His–ventricle interval; Atrial EG, atrial electrogram; His EG, His electrogram; Ventricular EG, ventricular electrogram.

3. Results

3.1. Effects of PACAP-27 and VIP on atrioventricular conduction

PACAP-27 had a biphasic effect on the atrioventricular interval which was characterized by an initial decrease followed by increase in the atrioventricular interval, when it was injected into the atrioventricular node artery of an autonomically decentralized heart in the open-chest, anesthetized dog (Fig. 2A, left panel and Fig. 3). The negative dromotropic response to PACAP-27 reached a maximum within 2 min after the injection. VIP (0.1 nmol) only decreased the atrioventricular interval (Fig. 2B). Atrial, His-bundle and ventricular electrograms from a representative anesthetized dog are portrayed in Fig. 3. These electrograms were recorded during atrial pacing at a fixed interval (400 ms). The control atrioventricular, a–h and h–v intervals were 180, 95 and 30 ms, respectively. At 15 s after the PACAP-27 injection (0.3 nmol), the atrioventricular and a–h intervals decreased by 20 ms to 160 and 75 ms, respectively, but the h–v interval did not change. Then, PACAP-27 prolonged the atrioventricular and a–h intervals by 20 ms to 200 and 115 ms, respectively, followed by a second-degree atrioventricular conduction block (Fig. 3C).

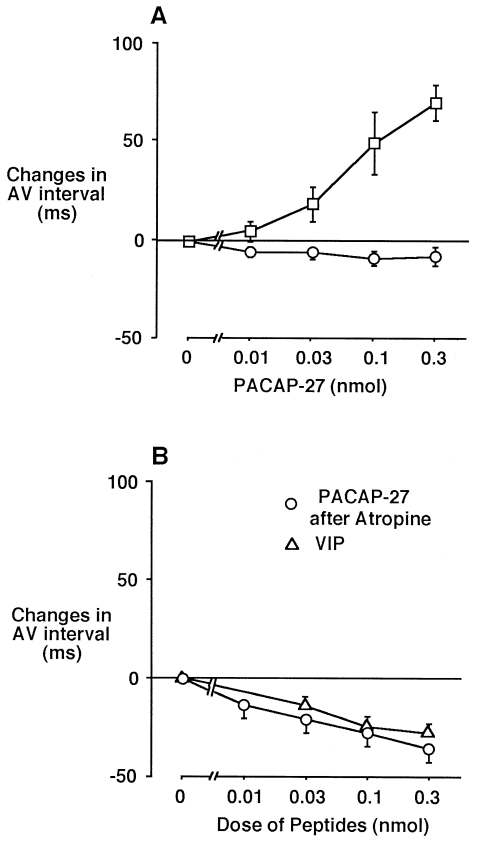

Fig. 4. (A) Dose–response curves for the positive (○) followed by negative (□) dromotropic responses to PACAP-27 in doses of 0.01 to 0.3 nmol injected into the atrioventricular node artery in the autonomically decentralized hearts of 5 anesthetized dogs. (B) Positive dromotropic responses to PACAP-27 (○) in doses of 0.01 to 0.3 nmol in 5 atropine (0.7 μmol/kg i.v.)-treated anesthetized dogs and to VIP (△) in doses of 0.03 to 0.3 nmol in 5 non-treated dogs. Vertical bars show SE. Control atrioventricular interval for 5 dogs was 158 ± 12 (mean ± S.E.) ms and it was not significantly different from the atrioventricular intervals of other experimental groups. AV, atrioventricular.

Dromotropic responses to PACAP-27 and VIP are summarized in Fig. 4. PACAP-27 (0.01–0.3 nmol) caused a transient positive dromotropic response followed by a dose-dependent negative dromotropic response ($P < 0.001$) in the autonomically decentralized hearts of 5 anesthetized dogs (Fig. 4A). The positive dromotropic response to PACAP-27 varied in each experiment. PACAP-27 (0.3 nmol) induced second-degree atrioventricular block in all 5 anesthetized dogs. The second-degree atrioventricular block evoked by PACAP-27 continued for 3 min or longer. In contrast, VIP (0.03–0.3 nmol) produced only a dose-dependent positive dromotropic response ($P < 0.005$) in 5 anesthetized dogs (Fig. 4B). To avoid the tachyphylaxis caused by PACAP-27, we injected each dose of PACAP-27 at intervals of 1 h or longer, since our preliminary experiments revealed that repeated injections of PACAP-27 at 1-h intervals induced almost equal changes in the atrioventricular interval. Femoral artery blood pressure did not change significantly through the experiment.

3.2. Effects of atropine and propranolol

When atropine (0.7 μmol/kg i.v.) was given, it abolished the increases (72 ± 4 ms) in the atrioventricular interval in response to ACh (3 nmol) injected into the atrioventricular node artery in 5 anesthetized dog hearts. After atropine treatment, PACAP-27 (0.1 nmol) did not increase the atrioventricular interval but decreased it (Fig. 2A, right panel). The positive dromotropic response to PACAP-27 reached a maximum 2 min after PACAP-27 injection and continued for 15 min (Fig. 2A, right panel).

The positive dromotropic responses to PACAP-27 in 5 atropine-treated dog hearts are summarized in Fig. 4B. PACAP-27 (0.01–0.3 nmol) decreased the atrioventricular interval in a dose-dependent manner ($P < 0.01$), as did VIP (0.03–0.3 nmol). The doses causing a 25 ms decrease in atrioventricular interval in response to PACAP-27 and VIP were 0.07 and 0.1 nmol, respectively. PACAP-27 significantly ($P < 0.001$) decreased the a–h interval from 90 ± 0.1 to 58 ± 4.4 ms in 3 atropine-treated dogs but it caused no consistent changes in the h–v interval at 2 min after PACAP-27 injection.

In 4 atropine-treated dogs, propranolol (30 nmol) injected into the atrioventricular node artery did not affect the decreases in atrioventricular interval in response to PACAP-27 (0.1 nmol), whereas it inhibited those elicited by norepinephrine (0.3 nmol) (Fig. 5).

3.3. Effects of tetrodotoxin

To determine whether the responses to PACAP-27 are mediated through neural activation, we examined the effects of tetrodotoxin on the dromotropic responses to PACAP-27. After tetrodotoxin (30 nmol) was injected into

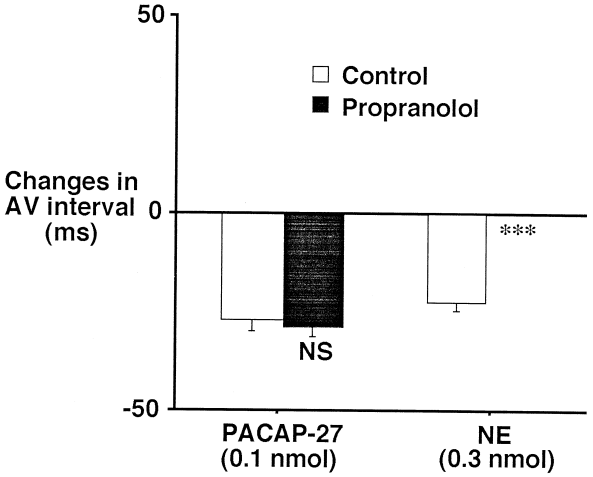

Fig. 5. Effects of propranolol at 30 nmol given into the atrioventricular node artery on the positive dromotropic responses to PACAP-27 at 0.1 nmol and norepinephrine at 0.3 nmol in 4 atropine (0.7 μmol/kg i.v.)-treated anesthetized dogs. Open and closed columns present responses to each intervention before and after treatment with propranolol, respectively. $^{***}P < 0.001$; NS (not significant) vs. control. Vertical bars show SE. Control atrioventricular interval before propranolol for 4 dogs was 167 ± 12 ms. AV, atrioventricular; NE, norepinephrine.

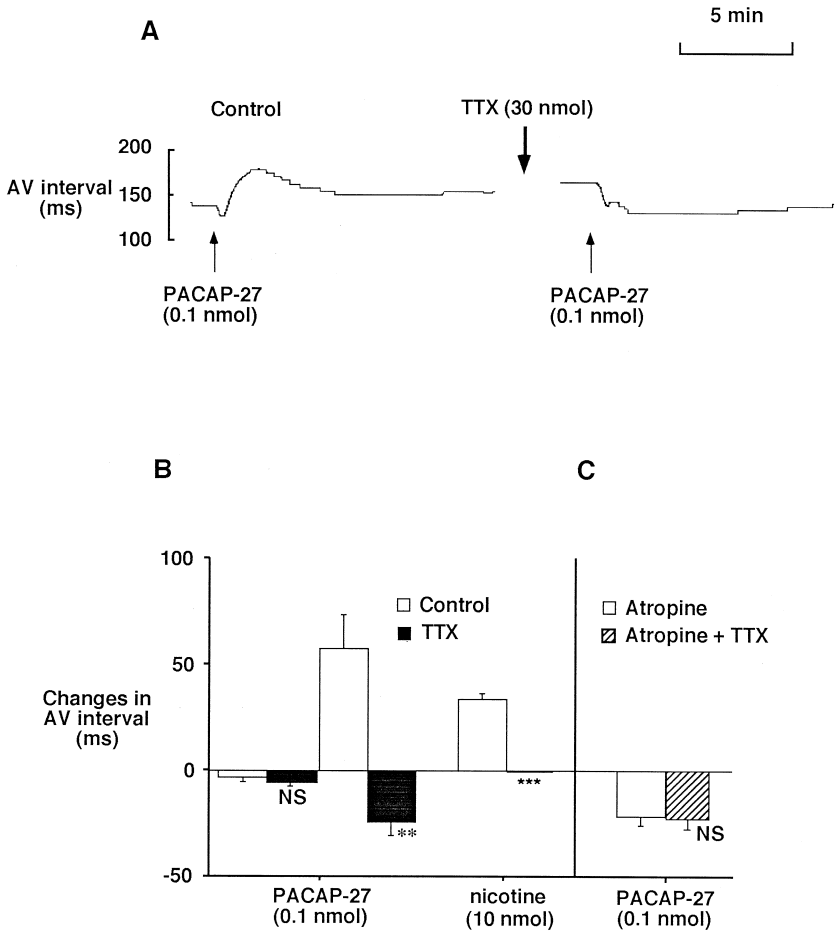

Fig. 6. (A) Changes in atrioventricular interval in response to PACAP-27 at a dose of 0.1 nmol injected into the atrioventricular node artery before and after treatment with tetrodotoxin at 30 nmol in an open-chest anesthetized dog. (B) Effects of tetrodotoxin at 30 nmol injected into the atrioventricular node artery on the positive followed by negative dromotropic responses to PACAP-27 at 0.1 nmol and the negative dromotropic response to nicotine at 10 nmol in 4 anesthetized dogs. Open and closed columns present responses to each intervention before and after treatment with tetrodotoxin, respectively. (C) Effects of tetrodotoxin at 30 nmol on the positive dromotropic response to PACAP-27 at 0.1 nmol in 4 atropine (30 nmol)-treated dog hearts. Open and hatched columns present a response to PACAP-27 before and after treatment with tetrodotoxin, respectively. $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$; NS (not significant) vs. control. Vertical bars show S.E. Control atrioventricular interval before tetrodotoxin for 4 dogs was 169 ± 14 ms. AV, atrioventricular; TTX, tetrodotoxin.

the atrioventricular node artery, PACAP-27 (0.1 nmol) only decreased the atrioventricular interval (Fig. 6A). Tetrodotoxin abolished the negative dromotropic response to PACAP-27 (0.1 nmol) significantly ($P < 0.01$) in 4 anesthetized dogs but did not alter the initial increase in the atrioventricular interval in response to PACAP-27 when the response was determined at the same phase before and after treatment with tetrodotoxin (Fig. 6B). Tetrodotoxin also abolished the negative dromotropic response to nicotine (10 nmol).

After atropine (30 nmol) was injected into the atrioventricular node artery, tetrodotoxin (30 nmol) did not inhibit the positive dromotropic response to PACAP-27 (0.1 nmol) (Fig. 6C).

4. Discussion

In the present study, we demonstrated that PACAP-27 caused negative and positive dromotropic effects in the autonomically decentralized hearts of open-chest anesthetized dogs. The negative dromotropic response to PACAP-27 was blocked by atropine and tetrodotoxin, suggesting that PACAP-27 releases acetylcholine from the parasympathetic nerves around the atrioventricular node of the dog heart (Fig. 2A, Fig. 4B and Fig. 6). Our previous study, however, demonstrated that hexamethonium, a ganglionic nicotinic receptor antagonist, did not block the negative chronotropic response to PACAP-38 (Hirose et al., 1997). Therefore, it is likely that negative dromotropic effects of PACAP-27 are due to activation of intracardiac parasympathetic nerves and not to a direct interaction between PACAP-27 and nicotinic receptors.

PACAP and VIP contracted the guinea pig ileum by releasing ACh from the postganglionic cholinergic neuron (Katsuoulis et al., 1993). In the rat dorsal hippocampus, PACAP and VIP also caused an increase in the spontaneous release of acetylcholine (Masuo et al., 1993). However, in the present study, VIP produced only a positive

dromotropic response, indicating that VIP did not activate the parasympathetic nerves in the dog heart. PACAP causes several effects mediated through type I, II and III PACAP receptors (Harmar and Lutz, 1994). The type I PACAP receptor binds PACAP with higher affinity than it binds VIP (Harmar and Lutz, 1994). It was reported that the type I PACAP receptor is present in the human heart (Wei and Mojsov, 1996). Thus, it is likely that the release of ACh from cardiac parasympathetic nerves evoked by PACAP-27 is mediated by the type I PACAP receptor in the dog heart, although other unknown mechanisms may exist, including direct effects independent of PACAP receptors.

In atropine-treated dogs, PACAP-27 caused a long-lasting positive dromotropic response which was not blocked by tetrodotoxin (Fig. 6). The positive dromotropic response was not inhibited by propranolol in doses which completely inhibited the norepinephrine-induced positive dromotropic response in the atropine-treated dog heart (Fig. 5). Therefore, we suggest that PACAP-27, like VIP, elicits a direct positive dromotropic response that is not due to activation of the adrenergic neurotransmission in the anesthetized dog heart. The potency of the positive dromotropic effects of PACAP-27 was almost equal to that of the effects elicited by VIP (Fig. 2 and Fig. 4B). In addition, PACAP-27 shortened the a–h interval only. Rigel and Lathrop (1990) demonstrated that the intravenous injection of VIP shortened the a–h interval but did not alter intra-atrial, intra-ventricular, or His–Purkinje conduction, and propranolol did not affect the positive dromotropic effect of VIP. Type II and III PACAP receptors are identical with type I and II VIP receptors, respectively, and bind PACAP and VIP with similar affinity (Shivers et al., 1991; Ishihara et al., 1992; Lutz et al., 1993; Inagaki et al., 1994). Thus, the positive dromotropic responses to PACAP-27 and VIP may be mediated by the same receptors. However, our unpublished results suggest that PACAP-27 and VIP may act on distinct receptors for sinoatrial pacemaker rhythmicity in the dog hearts. In addition, the distribution and density of the three types of PACAP receptors have not been determined in the different sites of the heart such as sinus node, atrioventricular node, atrial and ventricular muscles. Therefore, further studies, including receptor binding studies, are needed to determine which type of PACAP receptors acts on atrioventricular conduction in the dog heart. It was recently reported that PACAP and VIP act on distinct receptors in cat systemic vascular beds, whereas these peptides bound to the same receptor in rat blood vessels (Nandha et al., 1991; Minkes et al., 1992).

In the present study, PACAP-27 affected the atrioventricular interval in the anesthetized dog heart (Figs. 2 and 4). Numerous previous studies suggested that VIP has physiological and pathophysiological roles in the regulation of the heart (Weihe et al., 1984; Rachardt et al., 1986; Rigel, 1988; Rigel and Lathrop, 1990; Karasawa et al., 1990). The plasma concentrations of PACAP-38 and VIP in the rat are 8.4 ± 0.9 and 8.3 ± 1.0 pM, respectively (Wang et al., 1992; Ye and Duggan, 1996). The concentration of PACAP is approximately 1.13 ng/g wet tissue (Arimura et al., 1991) and that of VIP is 183.6 ± 32.6 fmol/g tissue (approximately 0.61 ng/g tissue) in the rat heart (Ye and Duggan, 1996). Therefore, the present observations combined with those of previous studies suggest that endogenous PACAP-27, like VIP, may modulate atrioventricular conduction of the heart in physiological or pathophysiological states.

Ascuitto et al. (1996) suggested that because of its positive inotropic and lusitropic effects, PACAP would be useful as a cardiotonic agent. However, PACAP has several actions including hormonal actions (Arimura and Shinoda, 1995). Furthermore, in the present study, we demonstrated that PACAP acted on the peripheral nervous system as well as on the heart and it induced atrioventricular conduction block. Therefore, further studies are needed to determine the drug's clinical applications.

In conclusion, we demonstrated in the present study that PACAP-27 caused negative and positive dromotropic effects in the anesthetized dog heart, and suggest that the negative dromotropic effect is due to the liberation of acetylcholine from parasympathetic nerves and that the positive dromotropic effect is induced by a non-adrenergic mechanism.

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