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Pituitary adenylate cyclase-activating polypeptide: Localization and differential influence on isolated hearts from rats and guinea pigs

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Abstract

This study was done to determine if pituitary adenylate cyclase-activating peptide (PACAP)-immunoreactive nerve fibers occur in cardiac muscle as well as intracardiac ganglia of rats and guinea pigs and to clarify the chronotropic actions of PACAP27 in the same species using isolated heart preparations. PACAP nerve fibers were not detected in atrial or ventricular muscle of rat or guinea pig but a few stained nerve fibers occurred in the atrioventricular bundle of the guinea pig. Stained nerve fibers were prominent in intracardiac ganglia of both species. PACAP27 caused a dose-dependent tachycardia in isolated rat hearts ($+39 \pm 3$ beats/min with 1 nmol, $n=6$). Positive and/or negative chronotropic responses were evoked by PACAP27 in guinea pig heart, depending on dose and prior exposure to the peptide. PACAP27 also caused arrhythmias in several guinea pig hearts. Treatment with atropine eliminated or prevented PACAP-evoked bradycardia and arrhythmias, implicating cholinergic neurons in these responses. Positive chronotropic responses to PACAP were unaffected by beta-adrenergic receptor blockade in either species, suggesting that tachycardia resulted from a direct action on the heart. These observations support the conclusion that endogenous PACAP could have a role in regulating parasympathetic input to the heart but through different mechanisms in rats versus guinea pigs. A direct positive chronotropic influence of endogenous PACAP is unlikely since atrial muscle lacks PACAP-immunoreactive nerve fibers.

Keywords: Arrhythmias; Cholinergic neurons; Heart rate; Immunohistochemistry; Intracardiac ganglia; PACAP

1. Introduction

Pituitary adenylate cyclase-activating polypeptide (PACAP) was first isolated from ovine hypothalamus and subsequently identified in neurons and nerve fibers at various sites within the central and peripheral nervous system [1]. Full-length PACAP (PACAP38) and a shorter form (PACAP27) are derived from a single precursor and have similar biological actions and potencies. Vasoactive intestinal polypeptide (VIP) belongs to the same peptide family as PACAP and has about 68% sequence homology with PACAP27. Some overlap in the pharmacological profiles of these peptides exists due to the fact that VIP and both forms of PACAP have high and comparable affinities for VPAC1 and VPAC2 receptors [1,2]. PACAP27 and PACAP38 can also have unique effects through high affinity binding to PAC1 receptors, which have only low affinity interactions with VIP [1]. Both anatomical and functional studies have provided evidence that VIP could have a role in local neuroregulation of the heart [3,4]. Recent investigations have shown that PACAP likewise has potent pharmacological effects on the heart and exhibits some distinct effects compared to VIP [1,5,6].

The influence of PACAP on cardiac function has been studied most thoroughly in canine models where the peptide causes biphasic changes in heart rate, conduction velocity and atrial contractility [6–10]. Cholinergic neurons mediate the inhibitory responses to PACAP27 and PACAP38 in isolated canine heart preparations whereas the excitatory actions of PACAP occur as direct effects on cardiac tissue.

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Studies with isolated atrial ganglia from guinea pigs have shown that PACAP stimulates intracardiac cholinergic neurons in this species as well through a direct effect on neuronal cell bodies [11]. Application of PACAP to these cells increased their excitability and frequently evoked action potentials. The same investigators demonstrated that PACAP is localized to preganglionic cholinergic nerve fibers that innervate the intracardiac ganglia suggesting that PACAP could have a physiological role in regulation of parasympathetic input to the guinea pig heart [12]. In accord with these observations, functional studies with spontaneously beating atria from guinea pigs have demonstrated that PACAP but not VIP causes bradycardia mediated by cholinergic neurons [5]. However, a significant number of the atria in that study did not respond to PACAP and neither peptide evoked a positive chronotropic response. Cholinergic nerves that innervate rat intracardiac ganglia also contain PACAP [13] but application of the peptide to these cells has not been reported to evoke action potentials [14,15]. Furthermore, experiments with spontaneously beating rat atria have not demonstrated a negative chronotropic response to PACAP [5,16], although a small magnitude of tachycardia was noted in one report [5]. Positive chronotropic responses to VIP have been well documented in experiments with isolated perfused hearts from guinea pigs and rats [17,18].

The present study was initiated to determine if PACAP-immunoreactive nerve fibers occur in cardiac muscle as well as intracardiac ganglia of rats and guinea pigs and to clarify the chronotropic responses evoked by PACAP27 in isolated perfused hearts from the same species. PACAP immunoreactivity was identified in tissue sections by immunohistochemistry using the avidin biotinylated enzyme complex method. Functional studies were done using non-working hearts perfused at a constant rate with physiological buffer.

2. Materials and methods

2.1. Tissue preparation for immunohistochemistry

Sprague–Dawley rats (200–250 g) of either sex or male Hartley guinea pigs (300–400 g) were pretreated with heparin (500 units/kg, i.p.) 15–20 min before they were anesthetized using sodium pentobarbital (70 mg/kg, i.p.). Deeply anesthetized animals were perfused transcardially with 0.1 M phosphate buffered saline (PBS, pH 7.4) followed by 4% paraformaldehyde in PBS. Hearts were removed and postfixed overnight at 4 °C. For some experiments, the initial fixation was accomplished by in vitro perfusion of the hearts with PBS and paraformaldehyde/PBS (10 ml each). Tissues were subsequently transferred to 20% sucrose in PBS and stored 2 days at 4 °C for cryoprotection. Frozen 30 μm short axis sections were cut through the base of the heart and ventricular myocardium, using an IEC cryostat microtome, and mounted on slides that were double-coated with chrom-alum gelatin.

2.2. Immunohistochemistry

Slide-mounted sections were permeabilized with 0.4% Triton X-100, treated with 3% H_2O_2 for 10 min to inhibit endogenous peroxidase activity and blocked for 2 h in PBS that contained 10% normal goat serum, 1% bovine serum albumen (BSA) and 0.4% Triton X-100. They were then incubated overnight (4 °C) with rabbit anti-PACAP27 (1:500; Phoenix Pharmaceuticals, Belmont, CA). After washing with PBS, tissues were incubated with biotinylated goat anti-rabbit IgG (1:200; Vector Laboratories, Burlingame, CA) for 2 h at room temperature. Tissues were washed with PBS again and incubated for 1–1.5 h with avidin:biotinylated horseradish peroxidase complex (Vector). Stain was developed by incubating tissues in a solution of 3,3'-diaminobenzidine and H_2O_2. After drying the sections, coverglasses were applied using Cytoseal 60 mounting medium (Stephens Scientific, Kalamazoo, MI). Sections were viewed with an Olympus BX41 microscope and photographed using a MagnaFire SP digital camera.

2.3. Isolated heart preparations

Sprague–Dawley rats (200–250 g) of either sex and male Hartley guinea pigs (400–600 g) were pretreated with heparin before they were decapitated under pentobarbital anesthesia (75 mg/kg, i.p.). The heart was removed rapidly, flushed with perfusion buffer and transferred to an isolated heart apparatus for perfusion by a modification of the Langendorff technique [19]. The perfusion solution was a modified Krebs–Ringer bicarbonate buffer (pH 7.35–7.4) that contained 0.1% BSA and the following components (mM): NaCl 127, KCl 4.7, $CaCl_2$ 2.5, KH_2PO_4 1.2, $NaHCO_3$ 24.9, $MgSO_4$ 1.2, sodium pyruvate 2.0 and dextrose 5.5. A Masterflex peristaltic pump (Cole Parmer, St. Louis, MO) was used to perfuse the hearts at a constant rate of 8 ml/min. The buffer was continuously gassed with 95% O_2–5% CO_2 and maintained at 37 °C. Cardiac contractions were measured by attaching one end of a silk suture to the apex of the heart and the other end to an isometric force transducer. Diastolic tension on the heart was adjusted to ~1 g. Output from the force transducer was sent to a Gould universal amplifier and a Gould Biotach amplifier to monitor ventricular contractions and heart rate, respectively. Perfusion pressure was measured using a pressure transducer that was connected to the side arm of a three-way stopcock located at the proximal end of the aortic cannula. Experiments were started after a 30–40 min stabilization period.

2.4. Preparation, storage and administration of drugs

PACAP27 (100 μM) and calcitonin gene-related peptide (CGRP)8–37 (1 mM) were dissolved in sterile saline and 50