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PAC₁ receptors mediate positive chronotropic responses to PACAP-27 and VIP in isolated mouse atria

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Abstract

PACAP and VIP have prominent effects on cardiac function in several species, but little is known about their influence on the murine heart. Accordingly, we evaluated the expression of PACAP/VIP receptors in mouse heart and the response of isolated atria to peptide agonists. Quantitative PCR demonstrated that PAC₁, VPAC₁, and VPAC₂ receptor mRNAs are present throughout the mouse heart. Expression of all three receptor transcripts was low, PAC₁ being the lowest. No regional differences in expression were detected for individual receptor mRNAs after normalization to L32. Pharmacological effects of PACAP-27, VIP, and the selective PAC₁ agonist maxadilan were evaluated in isolated, spontaneously beating atria from C57BL/6 mice of either sex. Incremental additions of PACAP-27 at 1 min intervals caused a concentration-dependent tachycardia with a log EC₅₀ = -9.08 ± 0.15 M (n=7) and a maximum of $96.3 \pm 5.9\%$ above baseline heart rate. VIP and maxadilan also caused tachycardia but their potencies were about two orders of magnitude less. Increasing the dosing interval to 5 min caused a leftward shift of the concentration-response curve to maxadilan but no changes in the curves for PACAP-27 or VIP. Under this condition, neither the potency nor the efficacy of maxadilan differed from those of PACAP-27. Neither PACAP-27 nor maxadilan caused tachyphylaxis, and maximal responses to maxadilan were maintained for at least 2h. We conclude that all three VIP/PACAP family receptors are expressed by mouse cardiac tissue, but only PAC₁ receptors mediate positive chronotropic responses to PACAP-27 and VIP.

Keywords

heart rate; isolated atria; PACAP; neuropeptides; relative potency; VIP

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1. Introduction

Pituitary adenylate cyclase-activating polypeptide (PACAP) and vasoactive intestinal peptide (VIP) are members of a large superfamily of biologically active peptides (Vaudry et al., 2009; Harmar et al., 2012). While separate genes code PACAP and VIP, the mature peptides have significant sequence homology and significant overlap of their pharmacological and biological actions. Both peptides are localized to neurons in the central and peripheral nervous systems, where they often function as co-transmitters. PACAP also has significant non-neuronal presence, being localized to the gonads, placenta, and cells of the immune system. The widespread distribution of these peptides has resulted in numerous studies, which suggest important physiological functions for PACAP and VIP and potential therapeutic use of PACAP/VIP analogues for anti-inflammatory, neuroprotective, and cardioprotective effects (Vaudry et al., 2009).

PACAP occurs as a full-length 38-amino acid peptide (PACAP-38) and as a truncated form that contains amino acids 1 through 27 (PACAP-27) (Vaudry et al., 2009). VIP is a 28-amino acid peptide that has 68% sequence homology with PACAP-27. These peptides can evoke biological responses by activating any of three G protein-coupled receptors named PAC₁, VPAC₁ and VPAC₂, but their relative potency is receptor dependent (Vaudry et al., 2009; Harmar et al., 2012). Specifically, PACAP 27 and PACAP-38 have a comparable high affinity at PAC₁ receptors, while the affinity of VIP at these sites is two-to-three orders of magnitude lower. In contrast, all three peptides have a high affinity at VPAC₁ and VPAC₂ receptors. Each of these receptors has been identified at various sites throughout the body including the nervous and cardiovascular systems (Vaudry et al., 2009; Harmar et al., 2012).

Previous studies have evaluated the cardiovascular pharmacology of PACAP and VIP in several species, and this work established that these peptides are potent vasodilators in most vascular beds (Vaudry et al., 2009) and have both direct and indirect effects on heart rate. Studies of isolated canine and guinea-pig hearts established that PACAP, but not VIP, can evoke bradycardia by stimulating cholinergic neurons in cardiac parasympathetic ganglia (Hirose et al., 1997a; Hirose et al., 1997b; Braas et al., 1998; Chang et al., 2005; Tompkins et al., 2007; Hoover et al., 2009). This effect is mediated by PAC₁ receptors in the guinea pig (Braas et al., 1998; Tompkins et al., 2007; Hoover et al., 2009). Tachycardia can occur *in vivo* as a reflex response to the primary vasodilator effect of these peptides, but PACAP can also evoke tachycardia by stimulating PAC₁ receptors at sympathetic ganglia (Vaudry et al., 2009). Work with isolated hearts from dogs, guinea pigs, and rats further demonstrated that PACAP and VIP increase heart rate by a direct effect on the atrial myocardium (Hoover, 1989; Henning and Sawmiller, 2001; Chang et al., 2005). All three PACAP/VIP receptors are expressed in atrial and ventricular myocardium of the rat and human (Baron et al., 2001; Saetrum Opgaard et al., 2001), but definitive evidence implicating a specific receptor (s) in the positive chronotropic response to PACAP and VIP is lacking. However, the selective PAC₁ agonist maxadilan does not cause tachycardia in isolated guinea-pig hearts (Hoover et al., 2009), suggesting that VPAC receptors mediate PACAP/VIP-evoked tachycardia in this species.

Mouse models are used commonly in cardiovascular research, yet very little is known about the cardiac pharmacology of PACAP and VIP in this species. Accordingly, our goals for this study were to 1) evaluate the expression of mRNAs for PAC₁, VPAC₁, and VPAC₂ receptors in mouse atrial and ventricular tissue and 2) quantify responses of isolated mouse atria to PACAP-27, VIP, and maxadilan.

2. Materials and methods

2.1 Animals

Adult C57BL/6 mice of either sex (Harlan, Indianapolis, IN) were used for this study. The East Tennessee State University Committee on Animal Care and the University of Vermont Institutional Animal Care and Use Committee approved animal protocols, which also conformed to guidelines of the National Institutes of Health as published in the *Guide for the Care and Use of Laboratory Animals* (Eight Edition, National Academy of Sciences, 2011).

2.2. Quantification of receptor mRNA

Mice were anesthetized with isoflurane, and thoracotomy was performed to collect the heart. Total RNA was extracted from heart samples using the STAT-60 total RNA/mRNA isolation reagent (Tel-Test 'B', Friendswood, TX, USA). One μg of RNA per sample was used to synthesize complementary DNA using a mix of random hexamers and oligo dT primers with M-MLV reverse transcriptase (Promega Corp.). Primers were identical to those used in previous work (Tompkins et al., 2010). Complementary DNA templates, diluted 10-fold, were assayed using HotStart-IT SYBR Green qPCR Master Mix (USB, Cleveland, OH, USA). Real-time quantitative PCR was performed (Applied Biosystems 7500 Fast real-time PCR system, Foster City, CA, USA) using the following standard conditions: (i) serial heating at 94°C for 2 min; (ii) amplification over 45 cycles at 94°C for 15 s and 60–65°C depending on primer sets for 30 s. The amplified product from these amplification parameters was subjected to SYBR Green I melting analysis by ramping the temperature of the reaction samples from 60 to 95°C. A single DNA melting profile was observed under these dissociation assay conditions demonstrating amplification of a single unique product free of primer dimers or other anomalous products. For data analyses, a standard curve was constructed by amplification of serially diluted plasmids containing the target sequence. Data were analyzed at the termination of each assay using sequence detection software (Sequence Detection Software, version 1.3.1; Applied Biosystems, Norwalk, CT, USA). In standard assays, default baseline settings were selected. The increase in SYBR Green I fluorescence intensity (ΔRn) was plotted as a function of cycle number and the threshold cycle was determined by the software as the amplification cycle at which the ΔRn first intersects the established baseline. All data were expressed first as the relative quantity of the gene of interest normalized to the relative quantity of the housekeeping gene L32. To standardize the presentation of these results, expression of each gene in the LA was set at 100, and values for other regions were calculated relative to the LA.

2.3. Concentration-response studies with isolated atrial preparations

Mice were deeply anesthetized with 5% isoflurane and euthanized by decapitation. Hearts were removed and placed in cold, oxygenated (95% O₂, 5% CO₂) Krebs-Ringer bicarbonate buffer (pH 7.35 to 7.4) of the following composition (in mM): 120 NaCl, 4.7 KCl, 1.2 KH₂PO₄, 25 NaHCO₃, 2.5 CaCl₂, 1.2 MgCl₂, and 11.1 D-glucose. The entire atrium was dissected from the ventricles, and each appendage was impaled with a small metal hook (#28 trout hooks) with 5-0 suture attached. The left atrium was anchored to the bottom of a vertical support rod in a 15 mL tissue bath, and the right atrium was attached to a 25-g force transducer (World Precision Instruments, Sarasota, FL). Krebs-Ringer buffer in the tissue bath was oxygenated continuously and maintained at 37°C. Spontaneous atrial contractions were recorded at a resting tension of 0.3 to 0.5 g using a ML224 Bridge Amplifier (ADInstruments, Colorado Springs, CO), a PowerLab/8SP, and a computer running Chart software version 5.2. Aliquots of peptide stock solutions were stored at –80°C and diluted with a solution of 0.1% bovine serum albumin in saline. Concentration-response (CR) data for peptides were collected in a cumulative fashion allowing 1 or 5 min between each concentration. Collections of CR data for two peptides in the same preparation were

separated by at least 30 min. Responses to L-isoproterenol were evaluated in separate atria as a positive control. Non-cumulative CR data were collected for this agonist to preclude desensitization. Incremental doses of isoproterenol were separated by at least 20 min. Data were analyzed using Prism software version 4.01 (GraphPad Software, La Jolla, CA).

2.4. Agonists

PACAP-27 and porcine VIP were purchased from Bachem Bioscience (King of Prussia, PA). Recombinant maxadilan was provided by Dr. Ethan Lerner (Lerner and Shoemaker, 1992). L-isoproterenol hydrochloride was purchased from Sigma-Aldrich (St. Louis, MO).

2.5. Statistical Analysis

Statistical comparisons and graphing of data were accomplished using GraphPad Prism version 4.01 (GraphPad Software, San Diego, CA). Group data are presented as the arithmetic mean $\pm$ S.E.M. (n). Statistical comparisons were made using an unpaired t-test or one-way ANOVA. The Newman-Keuls procedure was used for post hoc comparisons after ANOVA. A probability level of 0.05 or smaller was used to indicate statistical significance.

3. Results

3.1. Regional expression of PAC₁, VPAC₁, and VPAC₂ receptor genes in the mouse heart

All three PACAP/VIP receptor genes were expressed bilaterally in the mouse heart, in atrial and ventricular samples, but each mRNA occurred in low abundance. VPAC₁ and VPAC₂ mRNAs were detected after 32 cycles but PAC₁ mRNA was not detected until around 36 cycles. Relative expression levels after normalization to L32 housekeeping gene are shown in Fig. 1 for left atrium (LA), right atrium (RA), left ventricular free wall (LV), and right ventricular wall (RV). Separate one-way ANOVAs performed for each receptor showed no significant regional differences (P 0.05, $n=6$).

3.2. Effects of PACAP-27, VIP, and maxadilan on spontaneously beating mouse atria

Isolated atria exhibited spontaneous beating at rates that were usually below the intrinsic heart rate determined *in situ* (Mabe and Hoover, 2011). Resting heart rates were stable in each atrium but varied somewhat between preparations. Baseline heart rates for preparations used to evaluate peptides and maximum heart rates evoked by each peptide are shown in Table 1.

For the first series of experiments, we collected cumulative CR data for each peptide allowing 1 min for equilibration after each addition of peptide to the bath. In some cases, it was possible to collect data for VIP and PACAP-27 from the same atrium. VIP was evaluated first in these experiments, since heart rate returned to baseline more rapidly for VIP than PACAP-27 after washing the preparation. Identical results were obtained when PACAP-27 was tested in separate atria; so all data for this peptide were pooled for analysis. Maxadilan was evaluated separately in all cases since the maximal response to this peptide was maintained for at least two hours despite repeated washing of the preparation. Each of the peptides caused a concentration-dependent increase in heart rate when studied in this manner but PACAP-27 had a much higher potency than VIP or maxadilan (Fig. 2A). Nonlinear regression analysis of the PACAP-27 data yielded a sigmoidal curve with a log EC₅₀ of -9.08 ± 0.15 M and a maximum increase of $96.3 \pm 5.9\%$ baseline ($n=7$). The rank order of potency was PACAP-27 $\gg$ VIP $\approx$ maxadilan.

The low potency of maxadilan relative to that of PACAP-27 in the preceding experiments was surprising since maxadilan is a selective PAC₁ agonist (Moro and Lerner, 1997; Vaudry et al., 2009). Since the molecular mass of maxadilan is significantly greater than that of

PACAP-27 and VIP, we reasoned that maxadilan might require a longer exposure to reach equilibrium in the tissue bath. Therefore, a second series of cumulative CR experiments were performed allowing 5 min for equilibration after each addition of peptide to the bath. For this series, only one peptide was evaluated per atrial preparation. This change of equilibration time resulted in a leftward shift of the CR curve for maxadilan (Fig. 2B), and the rank order of potency was now PACAP-27 $\approx$ maxadilan $\gg$ VIP. PACAP-27 and maxadilan data could be fit accurately to sigmoidal curves, which did not differ with respect to log EC₅₀ or maximum % increase (log EC₅₀ of -9.17 ± 0.52 M for PACAP-27, $n=5$, and -8.97 ± 0.38 M for maxadilan, $n=3$; maximum % increase of $75.2 \pm 12.2\%$ for PACAP-27 and $73.3 \pm 10.5\%$ for maxadilan). Responses to each concentration of the peptides were maintained at their respective plateaus indicating the absence of tachyphylaxis. Return of heart rate to baseline after the highest dose of peptide was not studied systematically, but it was noted that the maximal tachycardic response to maxadilan was maintained until the end of the experiments despite several changes of buffer in the chambers. For maxadilan, no recovery of heart rate occurred over 92 ± 36 min ($n=3$; range of 47–164 min). Positive chronotropic responses to PACAP-27 were also prolonged, but heart rate did return to baseline if the experiment was continued long enough. Time for 50% recovery of heart rate after the maximal response was 57 ± 22 min ($n=5$, range of 6–120 min).

Negative chronotropic responses to each peptide were associated with concentration-dependent decreases in force of atrial contraction. This effect is shown in Figure 3 for experiments with 5 min equilibration. The rank order of potency for this decrease in force was PACAP-27 $\approx$ maxadilan $\gg$ VIP.

3.3. Effects of isoproterenol on spontaneously beating mouse atria

Isoproterenol caused concentration-dependent increases in rate and force of atrial contraction (Fig. 4). The log EC₅₀ was -9.00 ± 0.28 M for the positive chronotropic response and -7.43 ± 0.35 M for the positive inotropic response ($n=3$). However, positive inotropic responses were usually not maintained at the peak value and were often preceded by a decrease in force that coincided with the rapid increase in heart rate. The lower concentrations of isoproterenol often evoked small decreases in atrial force that coincided with increases in rate.

4. Discussion

Results of this study establish that transcripts for all three PACAP/VIP receptors (*i.e.*, PAC₁, VPAC₁, and VPAC₂) are expressed in mouse cardiac tissue. Furthermore, expression of each receptor was uniform at the message level across chambers and sides of the heart. Our functional studies demonstrate for the first time that PACAP and VIP have a direct positive chronotropic action on the mouse atrium, but neither peptide evokes a positive inotropic response. PACAP-27 had a much higher potency than VIP for increasing atrial rate, which supports the conclusion that positive chronotropic responses to these peptides are mediated by PAC₁ receptors. Further evidence for this conclusion comes from experiments with the PAC₁ selective agonist maxadilan. This peptide was equipotent with PACAP-27 for causing tachycardia when evaluated under equilibrium conditions. Maxadilan was notable also for evoking prolonged tachycardia that was resistant to washout.

Our finding that multiple PACAP/VIP receptors are expressed in mouse heart concurs with previous results obtained for atrial and ventricular tissue from rats and humans (Baron et al., 2001; Saetrum Opgaard et al., 2001). However, neither of the former studies evaluated tissues obtained from both sides of the heart, and levels of expression were not quantified. Our findings demonstrate that each receptor mRNA is expressed at a uniform level throughout the hearts. Thus, differences in chamber pressure do not appear to affect

expression of PACAP/VIP receptors in myocardial tissue. While the cellular localization of these receptors was not evaluated in the present study, previous work with atrial myocyte cultures from neonatal rats demonstrated the same receptor profile as occurred in adult rat atrial and ventricular tissue (Baron et al., 2001). Nonetheless, whole tissue expression levels undoubtedly include some contribution from vascular cells since VIP and PACAP cause vasodilatation of coronary blood vessels (Vaudry et al., 2009). Cardiac ganglia are another potential source of PACAP/VIP receptor mRNA in atrial samples (Braas et al., 1998; DeHaven and Cuevas, 2002).

Previous studies with isolated heart preparations from various species have established that PACAP can have direct and indirect effects on heart rate. PACAP-evoked tachycardia occurs consistently across species and results from direct stimulation of atrial receptors (Yonezawa et al., 1996; Hirose et al., 1997a; Hirose et al., 1997b; Chang et al., 2005). PACAP-evoked bradycardia has been reported in studies with guinea pig and canine heart preparations (Seebeck et al., 1996; Hirose et al., 1997a; Hirose et al., 1997b; Hirose et al., 2001; Chang et al., 2005), and this effect often occurs in concert with tachycardia, resulting in biphasic heart rate changes. Several lines of evidence have established that PACAP-evoked bradycardia is caused by PAC₁ receptor-mediated activation of cholinergic neurons, which are located in the intrinsic cardiac ganglia, and release of ACh from cholinergic nerve fibers (Seebeck et al., 1996; Yonezawa et al., 1996; Hirose et al., 1997a; Hirose et al., 1997b; Braas et al., 1998; Chang et al., 2005; Tompkins et al., 2007; Hoover et al., 2009). PACAP does not evoke bradycardia in isolated rat hearts, despite evidence that PAC₁ receptors are present in some intracardiac neurons (DeHaven and Cuevas, 2002; Chang et al., 2005). Negative chronotropic responses to PACAP-27 did not occur in isolated mouse atria even though cholinergic neurons and nerve fibers are intact in these preparations. Therefore, mice are similar to rats in showing only positive chronotropic responses to PACAP.

Two lines of evidence from our study implicate PAC₁ receptors in positive chronotropic responses to PACAP-27 and VIP in the mouse. First, the potency of PACAP-27 was about two orders of magnitude higher than that of VIP. This rank order is characteristic of PAC₁ receptors (Vaudry et al., 2009; Harmar et al., 2012). Second, PACAP-27 and the PAC₁-selective agonist maxadilan had comparable potencies for increasing heart rate when evaluated under equilibrium conditions. Previous CR studies of cAMP production by isolated neonatal rat atrial cells also implicated PAC₁ receptors based on relative potencies of PACAP-38 and VIP, but some contribution from VPAC receptors was still proposed for that response (Baron et al., 2001). PAC₁ receptors do not appear to mediate positive chronotropic responses to PACAP-27 in isolated guinea pig hearts, since tachycardia was not evoked by maxadilan in this preparation, even when infused, and desensitization with VIP eliminated PACAP-27-evoked tachycardia without affecting PAC₁-mediated bradycardia (Hoover et al., 2009). In contrast to these results obtained with small animals, there is evidence that PACAP-27 and VIP cause tachycardia in the dog through stimulation of PAC₁ and VPAC receptors, respectively (Hirose et al., 1997a). Our study is the first to demonstrate a positive chronotropic response to the PAC₁-selective agonist maxadilan.

The response characteristics of maxadilan differed from those of PACAP-27 in two ways in the present study. First, maxadilan took longer to reach equilibrium. This requirement might be due to different rates of tissue penetration since the molecular weight of maxadilan is 2.6 times greater than that of PACAP-27. Alternatively, maxadilan might associate with PAC₁ receptors at a slower rate than PACAP-27. Second, maxadilan caused a more prolonged tachycardia, which was maintained for at least two hours despite repeated replacement of buffer in the tissue chamber. In previous studies with isolated perfused guinea-pig hearts, maxadilan was found to cause prolonged effects to dilate coronary resistance vessels and

increase excitability of intracardiac neurons (Hoover et al., 2009). The mechanism responsible for such prolonged effects is unknown, but slow dissociation of maxadilan from the PAC₁ receptor is one possibility.

Previous studies with isolated, blood perfused canine atria and ventricles have demonstrated that PACAP has a positive inotropic action in this species (Yonezawa et al., 1996), but neither PACAP nor maxadilan increased contractile force in isolated mouse atria. Instead, both peptides caused concentration-dependent decreases in contractile force, which coincided with positive chronotropic responses. In marked contrast, isoproterenol evoked positive chronotropic and inotropic responses, demonstrating that contractility of isolated mouse atria can be increased when treated with a known positive inotropic agent. While the peptide-evoked decreases in contractile force could be due to a true negative inotropic action, it is more likely that they occurred secondary to tachycardia. This conclusion is supported by the observations that small decreases in atrial contractility often occurred along with tachycardia at lower concentrations of isoproterenol and preceded positive inotropic responses at higher concentrations of isoproterenol.

PACAP is localized to preganglionic vagal nerve fibers that innervate the intracardiac ganglia of guinea pigs and rats (Calupca et al., 2000; Richardson et al., 2003), but PACAP-immunoreactive nerve fibers are rarely detected in the myocardium of these species (Chang et al., 2005). In contrast, we have not detected PACAP-27- or PACAP-38-immunoreactive nerves in mouse intracardiac ganglia or cardiac tissue (unpublished observation). Consequently, it is unlikely that PAC₁ receptors in the atria contribute to direct neural regulation of cardiac function. Nevertheless, it is possible atrial PAC₁ receptors could be activated by PACAP that has been released into the circulation from nerve fibers located in other tissues such as the adrenal gland (Lamouche and Yamaguchi, 2003). Evidence from several recent studies suggests that PAC₁ receptors in the heart could be activated by PACAP that is derived from an extrinsic or non-neuronal source. PACAP-immunoreactivity has been detected in human plasma and atrial appendage samples by radioimmunoassay (Borzsei et al., 2009; Szanto et al., 2012), and levels are higher in atrial samples from patients with ischemic versus valvular heart disease (Szanto et al., 2012). PACAP immunoreactivity also is expressed by macrophages that infiltrate infarcted mouse myocardium (Alston et al., 2011). Our findings suggest the possibility that elevated PACAP levels in patients with cardiac disease might affect heart rate by stimulating atrial PAC₁ receptors.

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References

- Alston EN, Parrish DC, Hasan W, Tharp K, Pahlmeyer L, Habecker BA. Cardiac ischemia-reperfusion regulates sympathetic neuropeptide expression through gp130-dependent and independent mechanisms. *Neuropeptides*. 2011; 45:33–42. [PubMed: 21035185]
- Baron A, Monnier D, Roatti A, Baertschi AJ. Pituitary adenylate cyclase-activating polypeptide activates K(ATP) current in rat atrial myocytes. *Am J Physiol Heart Circ Physiol*. 2001; 280:H1058–65. [PubMed: 11179047]
- Borzsei R, Mark L, Tamas A, Bagoly T, Bay C, Csanaky K, Banki E, Kiss P, Vaczy A, Horvath G, Nemeth J, Szauer E, Helyes Z, Reglodi D. Presence of pituitary adenylate cyclase activating polypeptide-38 in human plasma and milk. *Eur J Endocrinol*. 2009; 160:561–565. [PubMed: 19174531]

- Braas KM, May V, Harakall SA, Hardwick JC, Parsons RL. Pituitary adenylyl cyclase-activating polypeptide expression and modulation of neuronal excitability in guinea pig cardiac ganglia. *J Neurosci*. 1998; 18:9766–9779. [PubMed: 9822736]
- Calupca MA, Vizzard MA, Parsons RL. Origin of pituitary adenylyl cyclase-activating polypeptide (PACAP)-immunoreactive fibers innervating guinea pig parasympathetic cardiac ganglia. *J Comp Neurol*. 2000; 423:26–39. [PubMed: 10861534]
- Chang Y, Lawson LJ, Hancock JC, Hoover DB. Pituitary adenylyl cyclase-activating polypeptide: localization and differential influence on isolated hearts from rats and guinea pigs. *Regul Pept*. 2005; 129:139–146. [PubMed: 15927709]
- DeHaven WI, Cuevas J. Heterogeneity of pituitary adenylyl cyclase-activating polypeptide and vasoactive intestinal polypeptide receptors in rat intrinsic cardiac neurons. *Neurosci Lett*. 2002; 328:45–49. [PubMed: 12123856]
- Harmar AJ, Fahrenkrug J, Gozes I, Laburthe M, May V, Pisegna JR, Vaudry D, Vaudry H, Waschek JA, Said SI. Pharmacology and functions of receptors for vasoactive intestinal peptide and pituitary adenylyl cyclase-activating polypeptide: IUPHAR review 1. *Br J Pharmacol*. 2012; 166:4–17. [PubMed: 22289055]
- Henning RJ, Sawmiller DR. Vasoactive intestinal peptide: cardiovascular effects. *Cardiovasc Res*. 2001; 49:27–37. [PubMed: 11121793]
- Hirose M, Furukawa Y, Nagashima Y, Lakhe M, Chiba S. Pituitary adenylyl cyclase-activating polypeptide-27 causes a biphasic chronotropic effect and atrial fibrillation in autonomically decentralized, anesthetized dogs. *J Pharmacol Exp Ther*. 1997a; 283:478–487. [PubMed: 9353360]
- Hirose M, Furukawa Y, Nagashima Y, Yamazaki K, Hoyano Y, Chiba S. Effects of PACAP-38 on the SA nodal pacemaker activity in autonomically decentralized hearts of anesthetized dogs. *J Cardiovasc Pharmacol*. 1997b; 29:216–221. [PubMed: 9057071]
- Hirose M, Leatmanorath Z, Laurita KR, Carlson MD. Effects of pituitary adenylyl cyclase-activating polypeptide on canine atrial electrophysiology. *Am J Physiol Heart Circ Physiol*. 2001; 281:H1667–74. [PubMed: 11557557]
- Hoover DB. Effects of guinea pig vasoactive intestinal peptide on the isolated perfused guinea pig heart. *Peptides*. 1989; 10:343–347. [PubMed: 2755874]
- Hoover DB, Tompkins JD, Parsons RL. Differential activation of guinea pig intrinsic cardiac neurons by the PAC1 agonists maxadilan and pituitary adenylyl cyclase-activating polypeptide 27 (PACAP27). *J Pharmacol Exp Ther*. 2009; 331:197–203. [PubMed: 19602551]
- Lamouche S, Yamaguchi N. PACAP release from the canine adrenal gland in vivo: its functional role in severe hypotension. *Am J Physiol Regul Integr Comp Physiol*. 2003; 284:R588–597. [PubMed: 12414436]
- Lerner EA, Shoemaker CB. Maxadilan. Cloning and functional expression of the gene encoding this potent vasodilator peptide. *J Biol Chem*. 1992; 267:1062–1066. [PubMed: 1730635]
- Mabe AM, Hoover DB. Remodeling of cardiac cholinergic innervation and control of heart rate in mice with streptozotocin-induced diabetes. *Auton Neurosci*. 2011; 162:24–31. [PubMed: 21334985]
- Moro O, Lerner EA. Maxadilan, the vasodilator from sand flies, is a specific pituitary adenylyl cyclase activating peptide type I receptor agonist. *J Biol Chem*. 1997; 272:966–970. [PubMed: 8995389]
- Richardson RJ, Grkovic I, Anderson CR. Immunohistochemical analysis of intracardiac ganglia of the rat heart. *Cell Tissue Res*. 2003; 314:337–350. [PubMed: 14523644]
- Saetrum Opgaard O, Knutsson M, de Vries R, Tom B, Saxena PR, Edvinsson L. Vasoactive intestinal peptide has a direct positive inotropic effect on isolated human myocardial trabeculae. *Clin Sci (Lond)*. 2001; 101:637–643. [PubMed: 11724651]
- Seebeck J, Schmidt WE, Kilbinger H, Neumann J, Zimmermann N, Herzig S. PACAP induces bradycardia in guinea-pig heart by stimulation of atrial cholinergic neurones. *Naunyn-Schmiedeberg's Arch Pharmacol*. 1996; 354:424–430. [PubMed: 8897444]
- Szanto Z, Sarszegi Z, Reglodi D, Nemeth J, Szabadfi K, Kiss P, Varga A, Banki E, Csanaky K, Gaszner B, Pinter O, Szalai Z, Tamas A. PACAP Immunoreactivity in Human Malignant Tumor Samples and Cardiac Diseases. *J Mol Neurosci*. 2012; 48:667–673. [PubMed: 22648511]

- Tompkins JD, Ardell JL, Hoover DB, Parsons RL. Neurally released pituitary adenylate cyclase-activating polypeptide enhances guinea pig intrinsic cardiac neurone excitability. *J Physiol.* 2007; 582:87–93. [PubMed: 17495034]
- Tompkins JD, Girard BM, Vizzard MA, Parsons RL. VIP and PACAP effects on mouse pelvic ganglia neurons. *J Mol Neurosci.* 2010; 42:390–396. [PubMed: 20428965]
- Vaudry D, Falluel-Morel A, Bourgault S, Basille M, Burel D, Wurtz O, Fournier A, Chow BK, Hashimoto H, Galas L, Vaudry H. Pituitary adenylate cyclase-activating polypeptide and its receptors: 20 years after the discovery. *Pharmacol Rev.* 2009; 61:283–357. [PubMed: 19805477]
- Yonezawa T, Furukawa Y, Lakhe M, Nagashima Y, Hirose M, Chiba S. PACAP-38 activates parasympathetic nerves in isolated, blood-perfused dog atria. *Eur J Pharmacol.* 1996; 315:289–296. [PubMed: 8982667]

Fig. 1.

Regional expression of PAC₁, VPAC₁, and VPAC₂ receptor genes in the mouse heart determined by quantitative PCR. RNA was extracted from left atrium (LA), right atrium (RA), left ventricular wall (LV) and right ventricular wall (RV) of six mice (n=6). Receptor expression was normalized to that of the housekeeping gene L32. Expression of each gene in the LA was set at 100, and values for other regions are presented relative to the LA. Expression of each receptor was compared across regions by one-way ANOVA, and no significant differences were detected. PAC1/L32: $F_{3,20}=1.178$, $P=0.3432$; VPAC1/L32: $F_{3,20}=2.608$, $P=0.0800$; and VPAC2/L32: $F_{3,20}=2.970$, $P=0.0565$.

Fig. 2.

Cumulative concentration-response curves for the positive chronotropic action of PACAP-27, VIP, and maxadilan in spontaneously beating mouse atria. Separate panels show curves obtained with equilibration times of 1 min (A) and 5 min (B) for each peptide. Values are means $\pm$ SEM. Values for n are shown in Table 1.

Fig. 3. Cumulative concentration-response curves for the decrease in contractile force evoked by PACAP-27, VIP, and maxadilan in spontaneously beating mouse atria. Baseline force was 223 ± 21 mg for PACAP-27 ($n=5$), 235 ± 58 mg for maxadilan ($n=3$) and 296 ± 46 mg for VIP ($n=3$).

Fig. 4.

Non-cumulative concentration-response curves for positive chronotropic (A) and positive inotropic (B) responses to isoproterenol in isolated spontaneously beating mouse atria. Baseline heart rate and contractile force at the start of experiments were 319 ± 1 beats per min and 204 ± 10 mg, respectively ($n=3$).

Table 1

Baseline heart rates and maximum heart rates (beats per min) evoked by peptide agonists in concentration-response experiments with 1 and 5 min equilibration time for incremental additions to the tissue bath.

Peptide	1 min equilibration		5 min equilibration	
	Baseline	Maximum	Baseline	Maximum
PACAP-27	318±16 (7)	614±10	363±32 (5)	620±17
VIP	313±8 (4)	464±60 ^b	333±37 (3)	458±33 ^c
Maxadilan	401±26 (3) ^a	532±3	354±34 (3)	605±8

Values are mean ± SEM (n).

^a $P < 0.05$ vs VIP and PACAP-27.

^b $P < 0.05$ vs PACAP-27.

^c $P < 0.01$ vs PACAP-27 and Maxadilan.