VPAC Receptor Modulation of Neuroexcitability in Intracardiac Neurons

DEPENDENCE ON INTRACELLULAR CALCIUM MOBILIZATION AND SYNERGISTIC ENHANCEMENT BY PAC1 RECEPTOR ACTIVATION*

Wayne I. DeHaven and Javier Cuevas‡

From the Department of Pharmacology and Therapeutics, University of South Florida College of Medicine, Tampa, Florida 33612

Pituitary adenylate cyclase-activating polypeptide (PACAP) and vasoactive intestinal polypeptide (VIP) have been found within mammalian intracardiac ganglia, but the cellular effects of these neuropeptides remain poorly understood. Fluorometric calcium imaging and whole cell patch clamp recordings were used to examine the effects of PACAP and VIP on [Ca^{2+}]i, and neuroexcitability, respectively, in intracardiac neurons of neonatal rats. PACAP and VIP evoked rapid increases in [Ca^{2+}]i, that exhibited both transient and sustained components. Pharmacological experiments using PAC1 and VPAC receptor-selective antagonists demonstrated that the elevations in [Ca^{2+}]i result from the activation of VPAC receptors. The transient increases in [Ca^{2+}]i were shown to be the product of Ca^{2+} mobilization from caffeine/ryanodine-sensitive intracellular stores and were not due to inositol 1,4,5-trisphosphate-mediated calcium release. In contrast, the sustained [Ca^{2+}]i elevations were dependent on extracellular Ca^{2+} and were blocked by the transient receptor channel antagonist, 2-aminoethoxydiphenyl borate, which suggests that they are due to Ca^{2+} entry via store-operated channels. In addition to elevating [Ca^{2+}]i, both PACAP and VIP depolarized intracardiac neurons, and PACAP was further shown to augment action potential firing in these cells. Depolarization of intracardiac neurons by the neuropeptides was dependent on activation of VPAC receptors and the concomitant increases in [Ca^{2+}]i. Although activation of PAC1 receptors alone had no direct effects on neuroexcitability, PAC1 receptor stimulation potentiated the VPAC receptor-induced depolarizations. Furthermore, enhanced action potential firing was only observed upon concurrent stimulation of PAC1 and VPAC receptors, which indicates that these receptors act synergistically to enhance neuroexcitability in intracardiac neurons.

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‡ To whom correspondence should be addressed: Dept. of Pharmacology and Therapeutics, University of South Florida College of Medicine, 12901 Bruce B. Downs Blvd., MDC 9, Tampa, FL 33612-4799. Tel.: 813-974-4678; Fax: 813-974-2565; E-mail: jcuevas@hsf.usf.edu.

1 The abbreviations used are: PACAP, pituitary adenylate cyclase-activating polypeptide; VIP, vasoactive intestinal polypeptide; fura-2/AM, fura-2/acetoxymethylester; FSS, physiological saline solution; GRF, growth hormone-releasing factor; RMP, resting membrane potential; TTX, tetrodotoxin; ∆[Ca^{2+}]i, change in intracellular calcium concentration; YF-GRF, [N-acetyl-Tyr1'-p-Phε]-GRF (1-29); nAChR, nicotinic acetylcholine receptor; ACh, acetylcholine; IP3, inositol 1,4,5-trisphosphate; 2-ABP, 2-aminoethoxydiphenyl borate; L-8-K, VIP receptor binding inhibitor.
in rat cardiac ganglia by potentiating nicotinic acetylcholine receptors (22, 23), whereas studies on guinea pig intracardiac neurons indicate that PACAP, but not VIP, regulates the excitability of intrinsic cardiac neurons, possibly by influencing the K+ conductance, $I_K$ (19).

PACAP and VIP evoke their effects via the activation of specific G-protein-coupled receptors (24, 25). PAC 1 or PAC 2-selective receptors, bind both active forms of PACAP, PACAP-27 and PACAP-38, with equally high affinities but bind VIP with much lower affinity (26), whereas nonselective VPAC (VPAC 1 and VPAC 2) receptors recognize PACAP-27, PACAP-38, and VIP with similar high affinities (27–33). At least seven isoforms of the PAC 1 receptor exist due to alternative splicing of the mRNA, whereas no splice variants of PAC 2 or VPAC 2 are presently known to exist. These different PAC isoforms and VPAC receptor types couple to distinct second messenger pathways (34–36). Rat intrinsic cardiac neurons express several isoforms of the PAC 1 (PAC 1–short, PAC 1–HOP 1, PAC 1–HOP 2) as well as both VPAC receptors (37).

The functional consequences of the PAC 1/VPAC receptor heterogeneity, which is also observed in other neuron types, such as mammalian cortical neurons, remain to be fully elucidated. The present study examined the ability of PACAP and VIP to regulate the electrical properties of intrinsic cardiac neurons from neonatal rats and to modulate [Ca 2+]i in these cells. Both PACAP and VIP depolarize intracardiac neurons and increase [Ca 2+]i, by evoking Ca 2+ release from ryanodine-sensitive intracellular stores and promoting Ca 2+ entry through the plasma membrane. PACAP, but not VIP, also increased the number of action potentials fired by neurons in response to depolarizing current pulses. The changes in neuroexcitability evoked by PACAP and VIP were blocked by inhibiting the [Ca 2+]i elevations produced by the neuropeptides.

**EXPERIMENTAL PROCEDURES**

Membrane currents and [Ca 2+]i were investigated in isolated intracardiac ganglion neurons of neonatal rats (4–7 days old). The preparation and culture of isolated intrinsic cardiac neurons have been previously described (38). Dissociated neurons were plated onto poly-l-lysine-coated glass coverslips and incubated at 37°C for 36–72 h under a 95% air, 5% CO2 environment prior to the experiments.

**Microfluorometric Measurements**—The calcium-sensitive dye fura-2/AM was used for measuring intracellular free calcium concentrations in intracardiac neurons, as previously described (39). Cells plated on coverslips were incubated for 1 h at room temperature in physiological saline solution (PSS) consisting of 140 mM NaCl, 3 mM KCl, 2.5 mM CaCl2, 1.2 mM MgCl2, 7.7 mM glucose, and 10 mM HEPES (pH 7.2 with NaOH), which also contained 1 mM fura-2/AM and 0.1% Me 2SO. The coverslips were then washed in PSS (fura-2/AM-free) prior to the experiments being carried out. All solutions were applied via a rapid application system identical to that previously described (40). A DG-4 high speed wavelength switcher (Sutter Instruments Co., Novato, CA) was used to apply alternating excitation with 340- and 380-nm UV light. Fluorescent emission at 510 nm was captured using the Sensicam digital CCD camera (Cooke Corp., Auburn Hills, MI) and values were determined to be 189 μg/ml amphotericin B, and 0.4% Me 2SO.

Membrane potential responses to hyperpolarizing and depolarizing current pulses (−100 to +200 pA) were determined in the absence and presence of VIP/PACAP receptor ligands. The mean resting membrane potential, latency for action potential firing, action potential duration, amplitude of afterhyperpolarization, and the number of action potentials elicited by depolarizing current pulses were determined in the absence and presence of PACAP and VPAC receptor ligands. Membrane input resistance was also determined as described previously (42).

**Reagents and Statistical Analysis**—All chemicals used in this investigation were of analytic grade. The following drugs were used: amphotericin B, Me 2SO, mecamylamine, caffeine, and 2-aminoethoxydiphenyl borate (2-APB) (Sigma); PACAP-27, PACAP-38, 1-8-R (VIP receptor binding inhibitor), [N-Ac-Tyr3-11]PACAP(1–27), and VIP (American Peptide Co., Sunnyvale, CA); tetrodotoxin (TTX), ryanodine, thapsigargin, and forskolin (Alomone Labs, Jerusalem, Israel); barium chloride (Fisher); and fur-2/AM (Molecular Probes, Inc., Eugene, OR). Recombinant maxadilan and M65 were generous gifts from E. A. Lerner (Massachusetts General Hospital, Harvard Medical School). All data and error bars were subjected to the S.E. of the mean. All values are derived from statistical analysis. Statistical analysis was conducted using SigmaPlot 8 (SPSS, Chicago, IL), and paired and unpaired t-tests were used for within and between group comparisons, respectively.

**RESULTS**

PACAP and VIP Directly Increase Free [Ca 2+]i—The most abundant PAC 1 receptor isoform expressed in neonatal rat intrinsic cardiac neurons, PAC 1–HOP, has been shown to elicit elevations in intracellular calcium in cortical neurons (44). However, the relationship between PACAP and intracellular calcium has not been investigated in the autonomic neurons. The effect of PACAP on Ca 2+ handling in intracardiac neurons was studied using fur-2 Ca 2+–imaging techniques. Fig. 1A shows a representative trace of change in [Ca 2+]i, ($\Delta$[Ca 2+]), as a function of time recorded from a neuron before, during and following a 2-min application of 100 nM PACAP-27. Intracellular Ca 2+ concentrations increased by >400 nM when PACAP-27 was applied and returned to near control levels upon washout of drug. In similar experiments, PACAP-27 significantly increased intracellular calcium by ~400%, from a baseline of 65.3 ± 5.5 nM to a peak value of 335.1 ± 65.4 nM (n = 23) (Fig. 1B). In all cells responding to PACAP, the elevations in [Ca 2+]i, were reversed when PACAP was removed from the bath, and subsequent applications of PACAP to the same cell produced [Ca 2+]i increases of similar magnitude.

In a series of experiments, the PAC 1 receptor-selective agonist, maxadilan, and VPAC receptor-selective agonist, VIP, were used to determine the PACAP/VIP receptor subtype(s) mediating the PACAP-induced elevations in [Ca 2+]i. Fig. 1A shows representative traces of change in [Ca 2+]i, recorded from two neurons as a function of time before, during and following a 2-min application of maxadilan and VIP, respectively. Maxadilan, at a concentration (10 nM) specific for PAC 1 (45), failed to elicit a change in [Ca 2+]i. In contrast, VIP, at a concentration (100 nM) that preferentially
antagonists were shown to block the effects of PACAP on [Ca$^{2+}$]. A bar graph of results obtained in similar experiments is shown in Fig. 2D and reveals that application of either l-8-K (n = 9) or YF-GRF (n = 5) significantly attenuated PACAP-evoked elevations in [Ca$^{2+}$], in isolated intracardiac neurons, whereas M65 (n = 5) had no effects on the neuropeptide-induced changes in [Ca$^{2+}$]. These data further support the conclusion that the PACAP and VIP evoked increases in [Ca$^{2+}$], are mediated by VPAC receptors and not PAC1 receptors in these cells.

PACAP and VIP are known to modulate nicotinic acetylcholine receptors (nAChRs) in intrinsic cardiac neurons (29). This raises the possibility that the observed PACAP- and VIP-induced elevations in [Ca$^{2+}$], may be due to regulation of presynaptic or postsynaptic nAChRs by the neuropeptides. Thus, the Na$^+$ channel blocker, TTX (400 nM), and ganglionic nAChR blocker, mecamylamine (25 μM), were used to block nicotinic neurotransmission pre- and postsynaptically, respectively, to verify that the effects of exogenously applied PACAP and VIP on [Ca$^{2+}$], are not indirectly caused by nAChR modulation. Neither the application of TTX (n = 4) nor mecamylamine (n = 3) prevented the PACAP-induced rise in [Ca$^{2+}$], in rat intracardiac neurons. The application of PACAP-27 (100 nM) in the presence of TTX significantly (p < 0.01) elevated [Ca$^{2+}$], from 107.1 ± 13.8 to 286.8 ± 25.1 nM, values comparable with the calcium elevations seen in the absence of TTX. Similarly, PACAP-27 increase in [Ca$^{2+}$], was unaffected by the application of mecamylamine (65.4 ± 9.5 to 236.6 ± 31.2 nM), a 265% increase in intracellular calcium (data not shown). These results suggest that the observed PACAP-induced rise in [Ca$^{2+}$], is not dependent on neurotransmission.

**PACAP and VIP Mobilize Ca$^{2+}$ from Intracellular Stores and Evoke Ca$^{2+}$ Entry through the Plasma Membrane—PAC** and VPAC receptors have been linked to both Ca$^{2+}$ entry through the plasma membrane via the activation of L-type calcium channels (36, 46) and Ca$^{2+}$ release from intracellular stores (47). Experiments were conducted to determine the source of Ca$^{2+}$ responsible for the VPAC-mediated increase in [Ca$^{2+}$]. Application of PACAP-27 produced an increase in [Ca$^{2+}$], that exhibited both a transient and a sustained component (Fig. 3A). However, when PACAP was applied in Ca$^{2+}$-free PSS, the initial transient increase in [Ca$^{2+}$], was observed, but the sustained component was abolished (Fig. 3A). Similar results were observed when VIP was used as the agonist (data not shown). Fig. 3B shows a bar graph of mean peak and sustained [Ca$^{2+}$], recorded under normal and Ca$^{2+}$-free conditions from 12 isolated intracardiac neurons. Although the magnitude of the transient peak [Ca$^{2+}$], remained unchanged (normal PSS: 179.2 ± 17.6 nM; Ca$^{2+}$-free PSS: 173.4 ± 17.8 nM), the sustained component significantly decreased from 103.6 ± 11.5 to 45.7 ± 6.7 nM following the removal of extracellular Ca$^{2+}$. This latter [Ca$^{2+}$], value was equivalent to control baseline [Ca$^{2+}$]. Thus, the sustained component is dependent on extracellular Ca$^{2+}$ and is probably due to Ca$^{2+}$ influx through the plasmalemma.

The presence of a PACAP-induced transient elevation in [Ca$^{2+}$], in the absence of extracellular calcium suggested that activation of VPAC receptors mobilized Ca$^{2+}$ from intracellular stores in these neurons. In order to test this hypothesis, we used the endoplasmic reticulum Ca$^{2+}$-ATPase inhibitor, thapsigargin, which specifically blocks Ca$^{2+}$ reuptake, leading to depletion of the endoplasmic reticulum Ca$^{2+}$ stores (48). Prior to the experiments, dissociated intracardiac neurons were incubated for 1 h in 20 μM thapsigargin (in PSS, 37°C). Experiments were then conducted to determine whether PACAP increased [Ca$^{2+}$], under these conditions. Untreated neurons collected from either the same neonatal rat or animals from the same litter were used as positive controls. Fig. 3C shows traces

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**FIG. 1. PACAP and VIP, but not maxadilan, reversibly increase [Ca$^{2+}$], in neonatal rat intracardiac neurons.** A, graph of change in [Ca$^{2+}$], (Δ[Ca$^{2+}$]), defined as peak [Ca$^{2+}$], minus baseline [Ca$^{2+}$], as a function of time for single intracardiac neurons recorded before, during, and following a 2-min bath application of 100 nM PACAP-27 (black trace), 100 nM VIP (gray trace), and the PAC1-selective agonist maxadilan (10 nM) (dotted trace). The solid line above the traces indicates peptide application times. Individual images were captured at 0.5 Hz. B, bar graph of mean peak [Ca$^{2+}$], ± S.E. recorded before (Baseline), during (Peptide), and following (Wash) PACAP-27 (n = 23), VIP (n = 14), and maxadilan (n = 7) application in different preparations. The asterisks denote significant difference (p < 0.01) from respective baseline [Ca$^{2+}$], values. No significant difference exists between the PACAP-27- and VIP-induced responses.
of change in \([Ca^{2+}]_i\), recorded from two neurons preincubated in 20 \(\mu M\) thapsigargin and exposed to either 100 \(nM\) PACAP-27 or 500 \(\mu M\) acetylcholine (ACh). PACAP failed to elicit a change in \([Ca^{2+}]_i\) in these neurons, but ACh, which promotes \(Ca^{2+}\) entry via plasma membrane nicotinic acetylcholine receptors in intracardiac neurons (49), produced a pronounced transient elevation in \([Ca^{2+}]_i\). In similar experiments, thapsigargin pretreatment significantly decreased the peak PACAP response from 370.3 \(\pm\) 40.9 to 105.0 \(\pm\) 8.3 \(nM\) (\(n=6\); Fig. 3D), with the latter value being indistinguishable from the baseline \([Ca^{2+}]_i\), measured in this group of cells (104.4 \(\pm\) 16.5 \(nM\)). In contrast, thapsigargin preincubation had no effect on the peak \([Ca^{2+}]_i\), elevations produced by rapid focal application of 500 \(\mu M\) ACh (Fig. 3D). Thapsigargin also eliminated the rise in \([Ca^{2+}]_i\), elicited by bath application of 5 \(\mu M\) caffeine (Fig. 3D), which has been shown to evoke release of \(Ca^{2+}\) from intracellular stores in these cells (49). Therefore, depleting intracellular \(Ca^{2+}\) stores via thapsigargin pretreatment abolished both the transient and sustained PACAP-induced changes in \([Ca^{2+}]_i\).

**PACAP Induces Mobilization of \([Ca^{2+}]_i\), from Caffeine- and Ryanodine-sensitive Stores**—Previous studies have demonstrated that rat intrinsic cardiac neurons have two distinct endoplasmic reticulum \(Ca^{2+}\) stores, one that is sensitive to inositol 1,4,5-trisphosphate (IP\(_3\)) and a second that is sensitive to ryanodine and caffeine (39, 49). The PACAP/VIP receptors have been shown to mobilize \(Ca^{2+}\) from both IP\(_3\)- and ryanodine/caffeine-sensitive stores in other cell types (44, 46). To determine whether PACAP evokes release of \(Ca^{2+}\) from ryanodine/caffeine-sensitive stores we used two approaches: 1) depleting the ryanodine/caffeine-sensitive stores of \(Ca^{2+}\) by bath-applying 5 \(mM\) caffeine and 2) blocking the release of \(Ca^{2+}\) from these stores by bath-applying 10 \(\mu M\) ryanodine. Fig. 4 shows representative traces of change in \([Ca^{2+}]_i\), in response to 5 \(mM\) caffeine (Fig. 4A) or 10 \(\mu M\) ryanodine (Fig. 4C) recorded from two neurons and to PACAP-27 (100 \(nM\)) in the absence and presence of these drugs (Fig. 4, A and C, respectively) in the same cells. Both the transient and sustained PACAP-evoked elevations in \([Ca^{2+}]_i\), were suppressed by pretreatment with caffeine or ryanodine. In six identical experiments, caffeine significantly decreased peak \([Ca^{2+}]_i\), increase from 139.3 \(\pm\) 32.5 to 21.3 \(\pm\) 4.6 \(nM\) (Fig. 4B). Similarly, ryanodine depressed the peak elevation in \([Ca^{2+}]_i\), from 162.2 \(\pm\) 27.0 to 32.7 \(\pm\) 10.9 \(nM\) (Fig. 4D). The initial application of caffeine produced an increase in \([Ca^{2+}]_i\), (236.8 \(\pm\) 34.1 \(nM\)) similar to that elicited by PACAP but, unlike PACAP, was often associated with \(Ca^{2+}\) oscillations (see Fig. 4A). Ryanodine, however, did not promote calcium release in any of the cells tested, which is consistent with the drug acting as an inhibitor of the ryanodine receptor at this concentration (50).

Results from our experiments with ryanodine, however, cannot rule out the possibility that neuropeptide-stimulated IP\(_3\) production evokes small, local changes in \([Ca^{2+}]_i\), that initiate the release of \(Ca^{2+}\) from the caffeine/ryanodine stores. The IP\(_3\) receptor antagonist 2-APB (51) was used to determine whether IP\(_3\)-mediated \(Ca^{2+}\)-induced \(Ca^{2+}\) release is responsible for the transient elevation in \([Ca^{2+}]_i\), observed following VPAC recep-

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**Fig. 2. PACAP-induced mobilization of \([Ca^{2+}]_i\), in rat intracardiac neurons is via VPAC receptor activation.** Shown are representative traces of \(\Delta[Ca^{2+}]_i\), as a function of time recorded from three neurons (A–C, respectively) in response to 100 \(nM\) PACAP-27 application in the absence (black traces) and presence (gray traces) of the PAC\(_1\) receptor antagonist, M65 (A; 100 \(nM\)), or the VPAC receptor antagonist l-8-K (B; 5 \(\mu M\)) or YF-GRF (C; 100 \(nM\)). The solid line above the traces indicates PACAP-27 bath application times. All antagonists were preapplied for 5 min prior to and during the administration of PACAP. D, bar graph of mean peak \([Ca^{2+}]_i\), ± S.E. before (Baseline), during (Drug), and following washout (Wash) of PACAP or PACAP and the indicated PAC\(_1\)/VPAC antagonists. The asterisk denotes significant difference (\(p < 0.01\)) from the baseline \([Ca^{2+}]_i\), recorded for each experimental condition: PACAP-27 (\(n = 19\)), M65 + PACAP-27 (\(n = 5\)), l-8-K + PACAP-27 (\(n = 9\)), and YF-GRF + PACAP-27 (\(n = 5\)). #, significant difference from PACAP control (\(p < 0.01\)).
The transient, but not the sustained, component of the PACAP-induced increase in $[Ca^{2+}]_i$, is dependent on extracellular $Ca^{2+}$. A, typical traces of $\Delta [Ca^{2+}]$, recorded from a single neuron in response to a 2-min bath application of PACAP-27 (100 nM) in normal physiological extracellular $Ca^{2+}$ (Control, black trace) and $Ca^{2+}$-free conditions (0 Ca$^{2+}$, gray trace). Cells were exposed to the Ca$^{2+}$-free PSS only during the PACAP application to prevent depletion of Ca$^{2+}$ from intracellular stores. B, bar graph of mean $[Ca^{2+}]_i$, ± S.E. before application of PACAP (Baseline), at the peak of the PACAP response (Transient PACAP), and at the end of the PACAP application (Sustained PACAP) recorded in normal and calcium-free conditions ($n = 12$). The asterisks denote significant difference ($p < 0.01$) from baseline [Ca$^{2+}]$, #, significant difference from control PACAP response. C, representative $\Delta [Ca^{2+}]$, traces recorded in response to 1.5-s application (arrow) of 500 μM acetylcholine (ACh) (gray trace) and 1-min application 100 nM PACAP (black trace) from two neurons preincubated in thapsigargin (20 μM, 1 h, 37°C). D, bar graph of mean baseline and mean peak $[Ca^{2+}]_i$, ± S.E. following the addition of acetylcholine (500 μM), PACAP-27 (100 nM), and caffeine (5 mM) in control neurons and neurons pretreated for 1 h in thapsigargin. The asterisks denote significant difference ($p < 0.01$) from baseline [Ca$^{2+}]$, #, significant difference ($p < 0.01$) from control PACAP and caffeine responses.

VPAC and PAC$_1$ Control of Excitability

Fig. 3. The transient, but not the sustained, component of the PACAP-induced increase in $[Ca^{2+}]_i$, is dependent on extracellular $Ca^{2+}$. A, typical traces of $\Delta [Ca^{2+}]$, recorded from a single neuron in response to a 2-min bath application of PACAP-27 (100 nM) in normal physiological extracellular $Ca^{2+}$ (Control, black trace) and $Ca^{2+}$-free conditions (0 Ca$^{2+}$, gray trace). Cells were exposed to the Ca$^{2+}$-free PSS only during the PACAP application to prevent depletion of Ca$^{2+}$ from intracellular stores. B, bar graph of mean $[Ca^{2+}]_i$, ± S.E. before application of PACAP (Baseline), at the peak of the PACAP response (Transient PACAP), and at the end of the PACAP application (Sustained PACAP) recorded in normal and calcium-free conditions ($n = 12$). The asterisks denote significant difference ($p < 0.01$) from baseline [Ca$^{2+}]$, #, significant difference from control PACAP response. C, representative $\Delta [Ca^{2+}]$, traces recorded in response to 1.5-s application (arrow) of 500 μM acetylcholine (ACh) (gray trace) and 1-min application 100 nM PACAP (black trace) from two neurons preincubated in thapsigargin (20 μM, 1 h, 37°C). D, bar graph of mean baseline and mean peak $[Ca^{2+}]_i$, ± S.E. following the addition of acetylcholine (500 μM), PACAP-27 (100 nM), and caffeine (5 mM) in control neurons and neurons pretreated for 1 h in thapsigargin. The asterisks denote significant difference ($p < 0.01$) from baseline [Ca$^{2+}]$, #, significant difference ($p < 0.01$) from control PACAP and caffeine responses.
PACAP significantly decreased the action potential latency (1.6). VIP affected a population of neurons similar to PACAP 
but was statistically significant (p = 0.01) from PACAP control peak Δ[Ca\textsuperscript{2+}]/nM. The asterisk denotes significant difference (p < 0.01) from PACAP control peak Δ[Ca\textsuperscript{2+}].

In most neurons tested, the depolarizing current pulses evoked short, adapting trains of action potentials (46 of 73 neurons), whereas 15 neurons fired single action potentials and 12 neurons exhibited tonic firing when challenged with a similar current pulses. In a population of neurons (42 of 73), the number of action potentials elicited was increased by 115\%, from 3.2 ± 0.3 to 6.9 ± 0.5, by 100 nM PACAP-27 (Fig. 6C). Following a 15-min washout of the neuropeptide, the number of action potentials induced by the current pulses returned to near control levels (3.9 ± 0.5). The increase in action potentials observed in the presence of PACAP was statistically significant when compared with either control or washout. The population of neurons exhibiting augmented action potential firing included phasic, adapting, and tonic neurons. Thus, the effect of PACAP was not associated with a cell type with distinct active membrane properties. Examining the action potential characteristics of neurons that showed increased firing revealed that PACAP significantly decreased the action potential latency period from 6.0 ± 0.7 to 4.7 ± 0.5 ms (n = 7; p < 0.01) and increased the afterhyperpolarization from −13.3 ± 6.1 to −21.4 ± 6 mV (n = 7; p < 0.05). However, other parameters including action potential duration and overshoot were not altered (data not shown). It is important to note that the membrane depolarization and increase in action potential firing were not correlated, and only 30 of 73 cells showed both a depolarization and increased action potential firing. This observation suggests that two distinct mechanisms are mediating these membrane responses.

The effects of PACAP were in part mimicked by the related neuropeptide, VIP (Fig. 7). Bath application of VIP depolarized a population of neurons (8 of 13) from −47.3 ± 1.7 to −44.5 ± 1.5 mV (Fig. 7B). This depolarization was of lesser magnitude than the PACAP-induced depolarization but was statistically significant and reversed upon washout of the peptide (−45.9 ± 1.6). VIP affected a population of neurons similar to PACAP (−60\%). However, unlike PACAP, VIP had no effects on action potential firing in these cells (Fig. 7C). The number of action potentials fired in response to 150-pA depolarizing current pulses before, during, and after washout of 100 nM VIP were 2.8 ± 0.5, 3.2 ± 0.6, and 3.4 ± 0.6, respectively.

Membrane input resistance was investigated to determine whether the neuropeptide-induced changes in excitability were associated with opening and/or closing of ion channel(s). Current pulses (−100 pA) were applied to elicit hyperpolarizations before, during, and after PACAP or VIP (100 nM) application. The amplitude of the sustained hyperpolarization was used to determine input resistances. Neither PACAP nor VIP significantly altered the input resistance. This observation suggests that the neuropeptides are probably exerting their effects by simultaneously opening and closing multiple membrane chan-

![Figure 4](http://www.jbc.org/)

**Fig. 4.** PACAP evokes a rapid transient elevation of Δ[Ca\textsuperscript{2+}], in intracardiac neurons by releasing Ca\textsuperscript{2+} from caffeine/ryanodine-sensitive internal stores. A, typical time courses of Δ[Ca\textsuperscript{2+}], recorded in response to 100 nM PACAP-27 (black trace) and caffeine (gray trace) and to PACAP (100 nM) after caffeine (5 mM) preincubation (dotted trace). Caffeine was applied until Δ[Ca\textsuperscript{2+}], baseline stabilized (−10 min) and was present during the PACAP application (PACAP-27 + Caffeine). B, bar graph of mean Δ[Ca\textsuperscript{2+}], (± S.E.) evoked by 100 nM PACAP, 5 mM caffeine, and 100 nM PACAP following 5 mM caffeine preincubation (n = 6). The asterisk denotes significant difference (p < 0.01) from the PACAP control. C, representative Δ[Ca\textsuperscript{2+}], trace recorded in response to application of PACAP-27 (100 nM), ryanodine (10 μM), or PACAP following ryanodine preincubation. PACAP was bath applied for 2 min, whereas ryanodine was applied for −10 min. D, bar graph of mean Δ[Ca\textsuperscript{2+}], ± S.E. recorded under the same conditions as C; n = 9 for each condition. The asterisk denotes significant difference (p < 0.01) from PACAP control peak Δ[Ca\textsuperscript{2+}].
nels, resulting in no net change in input resistance. Likewise, there was no correlation between changes in input resistance and PACAP-induced modulation of neuroexcitability in guinea pig intrinsic cardiac neurons (19).

**Increases in Neuroexcitability Evoked by PACAP and VIP Are in Part Mediated by a VPAC Receptor**—In order to substantiate that VPAC receptors contribute to the PACAP- and VIP-induced changes in intrinsic cardiac neuron excitability, the VPAC receptor antagonist [N-Ac-Tyr\(^1\),\-D-Phe\(^2\)]GRF-(1–29) was used. Fig. 8A shows representative action potentials evoked from a single neuron in response to a depolarizing current pulse (300 ms, 150 pA) in the absence and presence of 100 nM PACAP-27 and/or 100 nM [N-Ac-Tyr\(^1\),\-D-Phe\(^2\)]GRF-(1–29). Whereas PACAP depolarized the cell by 3.6 mV and increased action potential firing in the cell when administered alone, PACAP failed to change the resting membrane potential when co-applied with [N-Ac-Tyr\(^1\),\-D-Phe\(^2\)]GRF-(1–29) (PACAP-27 + YF-GRF). Bath application of YF-GRF alone had no direct effects on the active or passive membrane properties of the cell. In similar experiments, co-application of [N-Ac-Tyr\(^1\),\-D-Phe\(^2\)]GRF-(1–29) blocked PACAP-induced depolarizations in cells that were shown to significantly respond to the neuropeptide (Fig. 8B). Fig. 8C shows a bar graph of the number of action potentials elicited by identical depolarizing current pulses in cells exposed to PACAP prior to or following incubation in [N-Ac-Tyr\(^1\),\-D-Phe\(^2\)]GRF-(1–29). Whereas PACAP increased firing from 2.7 ± 0.3 to 6.3 ± 1.5 action potentials under control conditions, the neuropeptide had no effect on action potential firing following application of [N-Ac-Tyr\(^1\),\-D-Phe\(^2\)]GRF-(1–29).

Although VIP in part mimicked the depolarizing effects of PACAP, the magnitude of the VIP-induced depolarization was only ~60% of that elicited by PACAP. Also, VIP had no effects on action potential firing in these cells. This observation suggests that PAC\(_1\) receptors may also contribute to the PACAP-induced changes in the electrical properties of intracardiac neurons. To examine this hypothesis, the PAC\(_1\)-selective antagonist, M65, was used in a series of experiments to determine whether inhibition of PAC\(_1\) receptors alters the PACAP-evoked increase in excitability. Co-application of M65 (10 nM) depressed both the PACAP-induced depolarization and increase in action potential firing in neurons shown to respond to 100 nM PACAP-27 (Table I). Surprisingly, bath application of maxadilan (10–100 nM) failed to produce depolarizations in these neurons (Table I). Taken together, these data suggest that stimulation of VPAC receptors increases neuroexcitability in intrinsic cardiac neurons and that this increase in neuroexcitability is enhanced by concurrent activation of PAC\(_1\) receptors.

**Effects of Ca\(^{2+}\)-free Extracellular Conditions on PACAP-induced Intracardiac Neuroexcitability**—Our observation that stimulation of VPAC receptors evokes elevations in [Ca\(^{2+}\)], raises the possibility that this change in [Ca\(^{2+}\)], may contribute to the observed enhancement in neuroexcitability evoked by PACAP. This hypothesis is further supported by the noted
association of increases in the amplitude of the [Ca$^{2+}$]-dependent action potential after hyperpolarization with augmented action potential firing in the cells. To determine the role of PACAP-induced Ca$^{2+}$ mobilization in intracardiac neuroexcitability, whole cell perforated patch experiments were conducted in current clamp mode under Ca$^{2+}$-free extracellular conditions. In rat intracardiac neurons, removal of extracellular Ca$^{2+}$ over a short period of time (minutes) not only prevents Ca$^{2+}$ conductance through the plasma membrane but also results in depletion of Ca$^{2+}$ from internal stores. Thus, PACAP-induced [Ca$^{2+}$]$_i$ mobilization is blocked by washing the intracardiac neurons with Ca$^{2+}$-free PSS. Fig. 9A shows representative action potentials evoked from a neuron by depolarizing current pulses (300 ms, 150 pA) in the absence and presence of PACAP-27 (100 nM) under control conditions (2.5 mM Ca$^{2+}$) and in Ca$^{2+}$-free conditions (0 Ca$^{2+}$, 1 mM EGTA). In the presence of external Ca$^{2+}$, PACAP depolarized the neuron from −51.5 to −47.8 mV and increased the number of action potentials fired in response to the current pulse. However, PACAP failed to produce either of these changes when extracellular Ca$^{2+}$ was removed. In similar experiments, Ca$^{2+}$-free conditions inhibited the ability of PACAP to alter the resting membrane potential (Fig. 9B) and the action potential firing properties (Fig. 9C) of neurons shown to respond to the neuropeptide under control conditions. In normal extracellular Ca$^{2+}$ (2.5 mM), PACAP-27 (100 nM) depolarized the cells from −51.1 ± 1.6 to −46.8 ± 1.9 mV, whereas in Ca$^{2+}$-free PSS the resting membrane potential was −50.9 ± 1.0 and −51.9 ± 1.8 mV in the absence and presence of PACAP, respectively (n = 6). Similarly, in five neurons that exhibited increased action potential firing when PACAP was bath-applied in PSS containing normal Ca$^{2+}$ (2.5 mM), removal of extracellular Ca$^{2+}$ abolished the neuropeptide-mediated effects (Fig. 9C). Thus, PACAP-evoked changes in excitability are dependent on the neuropeptide-elicited elevation in [Ca$^{2+}$]$_i$.

In order to confirm that in the absence of extracellular Ca$^{2+}$ intracardiac neurons were capable of firing multiple action potentials, 1 mM Ba$^{2+}$ was bath-applied in Ca$^{2+}$-free PSS. Barium (1 mM) has previously been shown to block membrane K$^+$ channels and to increase neuroexcitability in intracardiac neurons (42). Under these calcium-free conditions, barium significantly depolarized (p < 0.01) the intracardiac neurons from −51.1 ± 1.9 to −43.8 ± 1.5 mV and increased action potential firing (p < 0.01) from a mean of 1.8 ± 0.3 to 5.8 ± 0.5 action potentials (n = 5). These effects were reversible upon washout of Ba$^{2+}$. Thus, removal of extracellular Ca$^{2+}$ does not prevent intracardiac neurons from exhibiting increased neuroexcitability.

Effects of Ryanodine on PACAP-induced Intracardiac Neuroexcitability—Electrophysiological experiments conducted under Ca$^{2+}$-free conditions suggested that PACAP-induced changes in excitability were at least in part due to the effect of the neuropeptide on [Ca$^{2+}$]$_i$. Further experiments were conducted to determine if PACAP-induced mobilization of Ca$^{2+}$ from ryanodine-sensitive intracellular stores contributed to the increased neuroexcitability observed in the presence of the neuropeptide. Fig. 10A shows representative action potentials evoked in response to a depolarizing current pulse (300 ms, 150 pA) during bath application of 100 nM PACAP-27 before and after pretreatment with ryanodine (10 μM). Whereas PACAP increased action potential firing in this cell under control conditions, preincubation in ryanodine eliminated the PACAP-induced changes in firing characteristics. Moreover, preincubation in ryanodine also prevented PACAP from depolarizing the

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*W. I. DeHaven and J. Cuevas, unpublished observation.*
cell, since PACAP depolarized the cell by 3.7 mV under control conditions but failed to alter the resting membrane potential in the presence of ryanodine. In similar experiments, preincubation in ryanodine blocked PACAP-induced depolarizations in cells that were shown to depolarize in the presence of the neuropeptide (Fig. 10B). Fig. 10C shows a bar graph of the number of action potentials elicited by identical depolarizing current pulses in cells exposed to PACAP prior to and following incubation in ryanodine. Although PACAP increased firing from 1.7 ± 0.3 to 5.3 ± 0.9 action potentials under control conditions, PACAP had no effects on action potential firing following application of ryanodine. This observation further establishes elevations in [Ca\(^{2+}\)], as an obligatory component in the PACAP/VIP enhancement of excitability in these cells.

**DISCUSSION**

The major finding reported here is that activation of VPAC receptors increases neuroexcitability in intrinsic cardiac neurons and that concurrent activation of VPAC and PAC1 receptors results in a synergistic augmentation of neuroexcitability. Furthermore, the PACAP- and VIP-mediated increase in excitability is dependent on VPAC receptor-induced Ca\(^{2+}\) release from caffeine- and ryanodine-sensitive intracellular stores.

Our studies show that bath application of VIP or PACAP evokes an elevation in intracellular free Ca\(^{2+}\) that is completely blocked by the VPAC receptor antagonists L-8-K and [N-Ac-Tyr\(^{6}\),d-Phe\(^{2}\)]GRF-1(1–29). Furthermore, the effects of PACAP and VIP were not mimicked by the PAC1-selective agonist maximadilan. Taken together, these data suggest that the effects of PACAP and VIP on [Ca\(^{2+}\)], are mediated by activation of VPAC receptors in these cells. Previous studies have shown that rat intracardiac neurons express three PAC1 receptor isoforms (PAC1-short, PAC1-HOP1, and PAC1-HOP2) as well as VPAC1 and VPAC2 receptors (37). The predominant PAC1 receptor, PAC1-HOP, has been shown to evoke release of calcium from intracellular stores via both cAMP and IP\(_3\) pathways in various cell types including neuroepithelial cells, chromaffin cells, astrocytes, and cortical neurons (44, 53, 54). However, our data indicate that stimulation of PAC1 has no direct effect on [Ca\(^{2+}\)], in neonatal rat intracardiac neurons. Earlier studies using single-cell reverse transcription-PCR detected VPAC1 and VPAC2 receptor transcripts (37). The predominant PAC1 receptor, PAC1-HOP, has been shown to evoke release of calcium from intracellular stores via both cAMP and IP\(_3\) pathways in various cell types including neuroepithelial cells, chromaffin cells, astrocytes, and cortical neurons (44, 53, 54). However, our data indicate that stimulation of PAC1 has no direct effect on [Ca\(^{2+}\)], in neonatal rat intracardiac neurons. Earlier studies using single-cell reverse transcription-PCR detected VPAC1 and VPAC2 receptor transcripts (37). In light of the fact that PACAP increased [Ca\(^{2+}\)], in over >95% of the cells tested, VPAC2 receptors are likely to be mediating these increases.

To date, there have been no reports of VPAC receptors evoking elevations of [Ca\(^{2+}\)], in neurons, and there is limited information concerning VPAC receptor-mediated regulation of [Ca\(^{2+}\)], in other cell types. Stimulation of VPAC receptors with low concentrations of VIP (1 nM) has been shown to evoke calcium oscillations in pancreatic acinar cells (55). In contrast to our observations, however, higher concentrations of VIP (100 nM) failed to elicit elevations in [Ca\(^{2+}\)], and had an inhibitory effect on ACh-evoked [Ca\(^{2+}\)], responses in acinar cells (55). VPAC-mediated increases in [Ca\(^{2+}\)], have also been reported in the lymphoblastic MOLT4 and SUPT1 cells (56, 57). Exogenously expressed VPAC1 and VPAC2 receptors have also been shown to stimulate [Ca\(^{2+}\)], in COS7 cells and Chinese hamster ovary cells (47, 58).

The VPAC-evoked increases in [Ca\(^{2+}\)], exhibited both a tran-

![Fig. 7. VIP-induced changes in neuroexcitability in neonatal rat intracardiac neurons.](http://www.jbc.org/Downloaded from)

*Fig. 7. VIP-induced changes in neuroexcitability in neonatal rat intracardiac neurons. A, action potentials elicited form a single current-clamped neuron in response to 150-pA depolarizing current pulses in the absence (Control) and presence of 100 nM VIP (VIP) and following washout of drug (Wash). B, scatter plot of mean resting membrane potential ± S.E. recorded from eight neurons under identical conditions as in A. C, bar graph of the mean number of action potentials elicited by depolarizing current pulse (300 ms, 150 pA) from neurons (n = 8) in the absence (Control) and presence of 100 nM VIP (VIP) and following washout of the neuropeptide (Wash). The asterisk denotes significant difference (p < 0.01).*
The transient component was observed in the absence of extracellular Ca\(^{2+}\) but was blocked by ryanodine (10 \(\mu\)M) or depletion of the Ca\(^{2+}\) stores by caffeine or thapsigargin. These results suggest that the transient component is due to VPAC mobilization of intracellular Ca\(^{2+}\) and, more specifically, due to Ca\(^{2+}\) release from ryanodine-sensitive Ca\(^{2+}\) stores. In pancreatic and submandibular gland cells, VPAC receptor activation promotes Ca\(^{2+}\) oscillations via a phospholipase C/IP\(_3\) cascade (59). Thus, in those secretory cells, VIP is probably increasing [Ca\(^{2+}\)]\(_i\) by evoking Ca\(^{2+}\) release from IP\(_3\)-sensitive Ca\(^{2+}\) stores. Although rat intracardiac neurons also contain IP\(_3\)-sensitive stores (49), our results with ryanodine and 2-APB suggest that a phospholipase C-IP\(_3\)-IP\(_3\)-receptor pathway is not involved in the VPAC receptor-induced increases in [Ca\(^{2+}\)]\(_i\) seen in these cells.

Unlike the transient component, the VPAC-mediated sustained elevations in [Ca\(^{2+}\)]\(_i\) were abolished by removal of extracellular Ca\(^{2+}\), suggesting that they are due to calcium entry through the plasma membrane. In various cell types, including αT3–1 gonadotrophs (60) and HIT-T15 insulinoma cells (61), VPAC\(_2\) receptor activation has been reported to increase [Ca\(^{2+}\)]\(_i\) by enhancing Ca\(^{2+}\) entry through plasma membrane voltage-gated Ca\(^{2+}\) channels. However, previous studies have shown that VIP does not alter the biophysical properties of high

![Image](http://www.jbc.org/)

**Fig. 8. PACAP-induced changes in intracardiac neuroexcitability are inhibited by the VPAC antagonist [N-Ac-Tyr\(^1\),p-Phe\(^3\)]GRF-(1–29).** A, action potentials evoked from a single intracardiac neuron by depolarizing current pulse (300 ms, 150 pA) in the absence (Control) and presence of 100 nM PACAP-27 (PACAP), 100 nM YF-GRF, or 100 nM YF-GRF and 100 nM PACAP-27 (PACAP-27 + YF-GRF). B, scatter plot of mean resting membrane potential (RMP) ± S.E. C, bar graph of the average number of action potentials ± S.E. elicited by depolarizing current pulse (300 ms, 150 pA) for neurons (\(n = 7\)) studied under the same conditions as described in A and following washout of the peptides (Wash). The asterisks denote significant difference from control (\(p < 0.01\)).

### Table 1

**Effects of extracellular Ca\(^{2+}\) conditions and various inhibitors on the PACAP-induced changes in membrane potential and action potential firing in response to a 150-pA current pulse**

All neurons recorded under Ca\(^{2+}\)-free, ryanodine, YF-GRF, and M65 conditions were first shown to respond to PACAP-27 alone (data not shown).

| Treatment          | Membrane potential (mV) | Action potential firing | Observations (n) |
|--------------------|-------------------------|-------------------------|------------------|
|                    | Control | Drug  |                    | Control | Drug  |                    |                  |
| PACAP 2.5 mM Ca\(^{2+}\) | -52.0 ± 0.6 | -47.7 ± 0.7* | 54                   | 3.2 ± 0.3 | 6.9 ± 0.5* | 42                   |
| Ca\(^{2+}\)-free     | -50.9 ± 1.0 | -51.9 ± 1.8 | 6                | 3.6 ± 0.6 | 3.7 ± 1.1 | 4                |
| Ryamodine           | -51.4 ± 1.3 | -52.3 ± 1.3 | 5                | 2.0 ± 0.6 | 2.3 ± 0.9 | 3                |
| M65                 | -52.2 ± 2.7 | -52.8 ± 2.3 | 7                | 2.0 ± 0.6 | 1.7 ± 0.3 | 3                |
| YF-GRF              | -50.8 ± 1.7 | -51.0 ± 1.5 | 3                | 3.0 ± 0.6 | 3.3 ± 0.3 | 3                |
| VIP                 | -47.3 ± 1.7 | -44.5 ± 1.5* | 8                | 2.8 ± 0.5 | 3.2 ± 0.6 | 8                |
| Maxadilan           | -50.9 ± 1.9 | -51.9 ± 1.7 | 4                | 3.5 ± 1.2 | 3.8 ± 1.1 | 3                |
| Caffeine            | -55.3 ± 2.1 | -55.9 ± 2.0 | 4                | 3.5 ± 1.0 | 3.5 ± 1.2 | 4                |

\(\text{\ast}\) Significant difference (\(p < 0.01\)).
voltage-activated calcium channels in intracardiac neurons (22). Furthermore, VPAC-mediated modulation of voltage-gated Ca\(^{2+}\)/H\(_{11001}\) channels appears to involve a cAMP/protein kinase A-dependent mechanism (60, 61), but in intrinsic cardiac neurons, the activator of adenylate cyclase, forskolin (100 \(\mu\)M), failed to mimic the effects of PACAP and VIP on [Ca\(^{2+}\)]\(_i\) (data not shown). Moreover, PACAP and VIP depolarize intracardiac neurons to a membrane potential of approximately -45 mV, which is negative to the threshold for activation of voltage-gated Ca\(^{2+}\) channels in these cells (42). Thus, the influx of Ca\(^{2+}\) observed following VPAC receptor stimulation is unlikely to be mediated by Ca\(^{2+}\) entry through such Ca\(^{2+}\) channels. Given that the sustained component is only observed following Ca\(^{2+}\) release from the ryanodine-sensitive stores and that it may also be produced by depletion of these stores by caffeine, it is probably mediated by store-operated channels. This conclusion is supported by our observation that the sustained elevation in [Ca\(^{2+}\)], produced by PACAP is blocked by the transient receptor potential channel antagonist 2-APB. Depletion of caffeine/ryanodine-sensitive Ca\(^{2+}\) stores has previously been shown to evoke capacitive Ca\(^{2+}\) entry in both excitable and nonexcitable cells (62, 63).

We have now shown that, in addition to increasing [Ca\(^{2+}\)], VIP and PACAP enhance neuronal excitability in intrinsic cardiac neurons. However, the effects on excitability produced by these neuropeptides are not identical. These differences may shed light onto possible distinct roles for the two neuropeptides in the regulation of intracardiac neurons and thus of the cardiovascular system. Bath application of PACAP or VIP results in a depolarization of intrinsic cardiac neurons. Whereas PACAP has previously been shown to depolarize guinea pig intracardiac neurons, VIP had no effect on the resting membrane potential of those cells (19). The observation that VIP depolarizes intracardiac neurons and that VPAC-specific antagonists block PACAP-induced depolarizations suggests that VPAC receptors directly influence neuroexcitability in rat intracardiac neurons. VPAC receptor activation has been shown to depolarize thalamic relay and medial pontine reticular formation neurons by 2–3 mV (6, 64), which is similar to the VIP-induced depolarizations reported here (2.4 ± 0.4 mV).

The observation that maxadilan, a PAC\(_1\) receptor-specific agonist (45), does not evoke a depolarization of intracardiac neurons or increase action potential firings suggests that PAC\(_1\) receptors do not have a direct effect on neuroexcitability in these cells. PAC\(_1\) receptor activation, however, potentiates VPAC receptor-induced depolarizations. Evidence for this conclusion is provided by two observations reported here: 1) PACAP depolarizes neurons to a greater extent than VIP, and 2) the PAC\(_1\) receptor antagonist, M65, depressed PACAP-induced depolarizations. Furthermore, increases in action potential firing in response to depolarizing current pulses were only observed under conditions in which both PAC\(_1\) and VPAC receptors were stimulated (i.e. when PACAP was used as the agonist and neither PAC\(_1\) nor VPAC receptor-specific antagonists were applied). Thus, simultaneous PAC\(_1\) and VPAC receptor stimulation elicits a synergistic
enhancement of neuroexcitability and produces changes in the active membrane properties that are not seen with stimulation of either receptor alone.

The VPAC-mediated increases in \([\text{Ca}^{2+}]_i\) appear to be critical for the enhanced neuroexcitability of intracardiac neurons by PACAP. PACAP failed to depolarize intracardiac neurons and to increase action potential firing when applied in the presence of ryanodine, when the intracellular stores were depleted with caffeine, or in the absence of extracellular \([\text{Ca}^{2+}]_i\). It is interesting to note that caffeine had no direct effects on neuroexcitability (Table I), and thus \([\text{Ca}^{2+}]_i\) release from intracellular stores is necessary but not sufficient to promote enhanced neuroexcitability. Muscarinic receptor activation has been shown to depolarize intracardiac neurons and to augment action potential firing in these cells (42). Our studies and those of others (49) have shown that muscarinic receptor activation mobilizes \([\text{Ca}^{2+}]_i\) from intracellular stores in intracardiac neurons. Although inhibition of the \(K^+\) conductance, \(I_{K}\), appears to be a major factor contributing to muscarinic receptor-induced neuroexcitability in these neurons (42), it would be of interest to determine whether muscarine-induced increases in \([\text{Ca}^{2+}]_i\), also play a role in the enhanced neuroexcitability and thus if mobilization of intracellular \([\text{Ca}^{2+}]_i\) is a mechanism by which various excitatory neurotransmitters regulate the electrical properties of intrinsic cardiac neurons.

In conclusion, the present study demonstrates the first evidence of VPAC receptor-mediated regulation of intracellular \([\text{Ca}^{2+}]_i\) in neurons and shows that by increasing \([\text{Ca}^{2+}]_i\), VPAC receptors enhance neuroexcitability. Furthermore, our results demonstrate that the heterogeneity in PAC1 and VPAC receptors observed in intracardiac neurons has significant implications for the effects of the neuropeptides on cellular function. The ability of PACAP to depolarize the neurons and increase action potential firing is dependent on PACAP stimulation of both PAC1 and VPAC receptors. VIP, by acting exclusively on VPAC receptors, evokes a depolarization of lesser magnitude than PACAP and fails to increase action potential firing. The ability of PACAP and VIP to differentially influence the electrical activity of intracardiac neurons may also help explain why these related neuropeptides have distinct effects on the cardiovascular system. Whereas both PACAP and VIP have been shown to dilate coronary arteries and to produce positive chronotropic effects (8, 10, 65), PACAP, but not VIP, evokes pronounced negative chronotropic and negative inotropic effects that are mediated by activation of intrinsic cardiac neurons (10).

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VPAC Receptor Modulation of Neuroexcitability in Intracardiac Neurons: DEPENDENCE ON INTRACELLULAR CALCIUM MOBILIZATION AND SYNERGISTIC ENHANCEMENT BY PAC1 RECEPTOR ACTIVATION
Wayne I. DeHaven and Javier Cuevas

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