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Short Communication

PACAP-INDUCED DEPOLARIZATIONS IN HAMSTER SUBMANDIBULAR GANGLION NEURONS

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Abstract

In this study, we have investigated the effects of pituitary adenylate cyclase-activating polypeptide (PACAP) on in vitro hamster submandibular ganglion neurons using the conventional intracellular recording technique. PACAP (10 µM) induced slow depolarizations in approximately 70% of tested cells. PACAP-induced depolarizations were approximately 10 mV in the peak amplitude, and their durations were approximately 10 min. The slow depolarizations were accompanied by a decrease in membrane conductance \( g_m \) at the initial phase and an increase in \( g_m \) at the peak phase. Membrane input resistance increased by 14.8 ± 2.2% (mean ± S.E., max.) of the resting value at the initial phase and decreased by 30.8 ± 4.3% (max.) at the peak phase. Anodal break spikes were elicited at the initial phase during PACAP-induced depolarization. In one neuron, anodal break spikes were elicited at the peak. Spikes which followed the anodal break spike were also elicited at 4 Hz in the initial phase during the slow depolarizations. The decrease in \( g_m \) was probably produced by an inhibition of calcium conductance and an inhibition of slow \( Ca^{2+} \)-activated K⁺ channels, while the increase in \( g_m \) might have been produced by an activation of nonselective cation channels. The slow depolarizations by PACAP might be mediated by a membrane-delimited signal transduction cascade involving G protein in the submandibular ganglion neurons.

Key words: Pituitary adenylate-cyclase activating polypeptide—Submandibular ganglion—Slow depolarization—\( g_m \) increase—\( g_m \) decrease

INTRODUCTION

Vasoactive intestinal polypeptide (VIP) is a synaptic transmitter of the submandibular ganglion. Pituitary adenylate cyclase-activating polypeptide (PACAP) belongs to a family of structurally and biologically related peptides including VIP, secretin, glucagon, peptide histidine isoleucin, and growth hormone-releasing hormone⁷,1¹, and has an N-terminal sequence that shares 68% amino acid homology with VIP¹². It possesses 1,000 times the potency of VIP in activating adenylate cyclase in rat pituitary cells in culture⁷. VIP receptors belong to a subfamily of PACAP receptors. Intravenous injection of PACAP (PACAP 1-38)
in the rat in the presence of adrenoceptor blockers and atropine evokes secretion of saliva from the major salivary glands\textsuperscript{2).} When comparing the secretory responses of VIP with those of PACAP in the rat, the effectiveness of PACAP was the same or less than that of VIP. However, when comparing the effect of these peptides on the vascular response of the submandibular glands, PACAP was more effective than VIP in decreasing the vascular resistance and in increasing the blood flow\textsuperscript{2).}

Suzuki\textsuperscript{9) investigated effects of VIP on in vitro hamster submandibular ganglion (SMG) neurons. In 80\% of the neurons, VIP induced slow depolarizations accompanied by a decrease in membrane conductance of $K^+$ ion ($g_{K}$). Recently we found that PACAP-induced depolarizations were accompanied by a decrease in membrane conductance ($g_m$) at an initial phase and an increase in $g_m$ at a peak phase. In this study, we have investigated the effects of PACAP on in vitro submandibular ganglion neurons using the conventional intracellular recording technique.

**MATERIALS AND METHODS**

Experiments were conducted according to the guidelines for the treatment of experimental animals at Tokyo Dental College. An isolated submandibular ganglion was superfused continuously with oxygenated Krebs solution (30°C) at 2 ml/min. Intracellular recordings were conducted using glass microelectrodes filled with 3M KCl (input resistance; 30–60 MΩ). Penetration of the glass microelectrode was achieved under visual control with Zeiss Nomarski optics (10× ocular; 25.8× objective, UD40). PACAP was applied at concentration of 10 μM by pressure ejection from a pipette with a tip 5 μm in inner diameter. The micropipette is positioned 20–30 μm away from a target cell. Thirty pulses of air pressure (2 kg/cm$^2$, 50 msec) were delivered to the pipette at 1 Hz through a pressure ejection system. Cellular responses were recorded using a strip chart recorder. For estimating the membrane input resistance of each ganglion cell, hyperpolarizing currents (0.1–0.2 nA) were applied for 50 msec at 0.1 Hz through the recording electrode by means of a balanced bridge circuit. The membrane potential of the ganglion cell was similarly changed by injection of direct currents (0.1–0.5 nA) using the balanced bridge circuit. The preganglionic nerve was stimulated through a suction electrode at a frequency of once a minute.

The ionic composition of Krebs solution was as follows (mM): 136 NaCl, 5 KCl, 2.5 CaCl$_2$, 1 MgCl$_2$, 12 NaHCO$_3$, 1.2 NaH$_2$PO$_4$ and 11 glucose. PACAP (1-32) was purchased from Peptide Institute Inc.

**RESULTS**

PACAP-induced depolarizations were generated in 11 out of tested 20 cells (69\%) (shown in Fig. 1). The resting membrane potential was $-57.4 \pm 3.5$ mV (mean ± S.E.). The maximum amplitude of PACAP-induced depolarization was $10.3 \pm 1.4$ mV. Its duration was $9.7 \pm 0.7$ min. The membrane input resistance (R$_m$) during depolarization increased by $14.8 \pm 2.2$% (max.) of the resting value in

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure1.png}
\caption{PACAP-induced depolarizations recorded from two cells}
\end{figure}

Upper trace: A slow depolarization was induced by ten ejections of PACAP (10 μM) at 1 Hz. The resting membrane potential (resting $E_m$) was $-64$ mV. R$_m$ increased in the initial phase and decreased in the peak phase. In the initial phase, anodal break spikes were evoked at 0.1 Hz, and spikes which followed the anodal break spike were evoked at 4 Hz.

Lower trace: In another neuron, a slow depolarization was induced by thirty ejections of PACAP (10 μM). In this neuron, two anodal break spikes were evoked in the peak phase accompanied by a decrease in R$_m$. Fifty msec hyperpolarizing pulses in the peak phase were followed by subthreshold depolarizations due to the anodal break response. The resting $E_m$ was $-92$ mV.
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the initial phase, and decreased by 30.8 ± 4.3% (max.) in the peak phase. Anodal break spikes were evoked in the initial phase during PACAP-induced depolarizations. In one neuron, anodal break spikes were also elicited in the peak phase as shown in Fig. 1, lower trace. Spikes which followed the anodal break spike were often elicited at 4 Hz in the initial phase during PACAP-induced depolarizations. PACAP potentiated the amplitude of fast EPSPs evoked during PACAP-induced depolarization by 55% in typical cases (n = 2; data not shown).

DISCUSSION

VIP (10 μM) induces slow depolarizations in 80% of tested neurons in hamster SMG6). In this study, PACAP also induced slow depolarizations accompanied by both a decrease and an increase of g_{m} in approximately 70% of tested neurons. Studies in neuronal tissue have shown that VIP activates a Na⁺-selective cation current, inhibits three distinct g_{K}(Ca), a background g_{K}, an inward rectifier g_{K}, and an inhibition of slow g_{K}(Ca) was induced via stimulation of adenylate cyclase, while g_{Cation} was activated6). In addition, an inhibition of high threshold I_{Ca} by VIP and PACAP may potentiate the inhibition of slow g_{K}(Ca) through a decrease in Ca²⁺ entry5). PACAP (1 μM) inhibits the L- and N-type Ca²⁺ channels of in vitro hamster SMG neurons4). The inhibition in the total high threshold I_{Ca} by VIP was characterized by voltage-independency mediated by G protein activation. Thus, an increase in R_m during the initial phase of PACAP-induced depolarization was presumably produced by an inhibition of slow Ca²⁺-activated K⁺ (SK) channels in SMG neurons, while a decrease in R_m during the peak phase of PACAP-induced depolarization might be induced by activation of nonselective cation channels, which is presumably mediated by second messenger signals8,10). The activated protein kinase A may be associated with an activation of nonselective cation channels8).

The receptor subtype that mediates VIP- and PACAP-induced calcium channel modulation in hamster SMG neurons is unclear. VIP and PACAP induced slow depolarizations in hamster SMG neurons, and the rank order of the potency was PACAP > VIP5). Hamster SMG neurons may possess VPAC1 receptors as well as PAC1 receptors, taking into account the VIP- and PACAP-potentiation of nicotinic ACh-evoked current in parasympathetic neurons5). VIP and PACAP have recently been shown to potentiate a nicotinic ACh-evoked current via G protein activation in the SMG neuron5). Elevation of intracellular cAMP levels has been proposed as the mechanism by which VIP and PACAP potentiated ACh-evoked currents in chick ciliary ganglion neurons5). The potentiation of ACh-evoked currents by VIP and PACAP might be mediated by a membrane-delimited signal transduction cascade initiating from the PTX-sensitive G_{o} protein, such as that found in rat intracardiac and submandibular ganglion neurons5). Members of the PACAP/VIP receptor family have been shown to couple to a variety of G proteins, including G_{s}, G_{q/11}, G_{o}, and G_{11,1}1,5). The identity of the G protein for inhibition of VIP- and PACAP-induced high threshold Ca²⁺ channels remains to be investigated.

REFERENCES


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