Effects of PACAP on morphologically identified myenteric neurons in guinea pig small bowel

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Christofi, F. L., and J. D. Wood. Effects of PACAP on morphologically identified myenteric neurons in guinea pig small bowel. Am. J. Physiol. 264 (Gastrointest. Liver Physiol. 27): G414–G421, 1993.—Intracellular microelectrodes were used to examine the actions of putative adenylate cyclase-activating peptide (PACAP) on morphologically identified myenteric neurons and glial cells of the guinea pig small bowel. PACAP-27 and PACAP-38 evoked excitatory responses in 96% of afterhyperpolarizing (AH)/type 2 neurons. The half-maximal concentration for PACAP-27 was 1.5 nM. The responses consisted of membrane depolarization in association with increased input resistance, suppression of afterhyperpolarizing afterpotentials, and repetitive spike discharge. Forskolin mimicked the action of PACAP in all AH/type 2 neurons. PACAP excited 36% of S/type 1 neurons. Most of the AH/type 2 neurons had Dogiel II morphology, whereas the S/type 1 neurons were uniaxial with morphology characteristics of Dogiel I or filamentous neurons. No glial cells responded to PACAP. A selective A1 adenosine receptor agonist blocked the excitatory action of PACAP, and this was reversed by a selective A1 antagonist. The results suggest that excitatory PACAP receptors and inhibitory adenosine A1 receptors are linked to adenylate cyclase in AH/type 2 myenteric neurons.

PITUITARY ADENYLATE CYCLASE-ACTIVATING PEPTIDE (PACAP) is a new brain-gut peptide that occurs in two bioactive forms, PACAP-27 and PACAP-38 (20, 22, 23). Immunocytochemical (34), immununocytochemical (34), autoradiographic (30), and motility studies (18) support the involvement of PACAP in neuroregulation in the guinea pig digestive tract. In other species, including humans, differences have been reported in the distribution of PACAP immunoreactive fibers (34), in the sites of action of PACAP, as well as in the types of responses evoked by PACAP (18, 29, 31). In the guinea pig, PACAP induces neurogenic contractions of longitudinal muscle in the small intestine, mediated by both cholinergic and noncholinergic nerves (18). Denervation studies in guinea pigs and rats indicated that PACAP-immunoreactive nerve fibers in the small intestine belong to the intrinsic nervous system.

Receptors for PACAP are thought to be linked by G proteins to stimulate adenylate cyclase (9, 23). PACAP-27 and PACAP-38 stimulate adenosine 3',5'-cyclic monophosphate (cAMP) formation in anterior pituitary cell cultures of neurons and astrocytes (19, 22, 35). In gastrointestinal tissues other than the small intestine, the action of PACAP is mediated by stimulation of adenylate cyclase (24, 29). However, involvement of adenylate cyclase in intestinal neurons has not been investigated.

Results of electrophysiological studies suggest that signal transduction for slow synaptic excitation in afterhyperpolarizing (AH)/type 2 neurons involves second messenger function of cAMP (25–27). Biochemical studies confirmed stimulation of cAMP formation by forskolin in isolated myenteric ganglia (42). Other studies suggested that adenosine interacts with inhibitory adenosine A1 receptors linked to adenylate cyclase in AH/type 2 neurons to suppress slow synaptic excitation, as well as the responses to slow excitatory postsynaptic potential (EPSP) mimetics (9, 26, 27, 44).

The primary aim of the present study was to use conventional intracellular recording techniques to examine the actions of PACAP on the excitability of morphologically identified myenteric neurons. A secondary aim was to determine the effects of adenosine A1 receptor stimulation on excitatory responses to PACAP. A preliminary report has been published as an abstract (9).

METHODS

Adult male guinea pigs, weighing 300–500 g, were killed by a blow to the head and subsequent exsanguination. This method was approved by the Institutional Laboratory Animal Care and Use Committee of The Ohio State University. The segment of ileum was pinned flat with the mucosal side up to Sylgard 184 encapsulating resin (Dow Corning, Midland, MI) in a dissection dish containing ice-cold Krebs solution. Fine forceps were used to remove the mucosa, submucosal plexus, and inner muscle layers and to expose the myenteric plexus on the underside of the longitudinal muscle layer. A 2.0 × 1.0 cm segment of the preparation was then pinned to Sylgard in a 2.0 ml recording chamber. The tissue was superfused with Krebs solution warmed to 37°C and gassed with 95% O2/5% CO2 (pH 7.3–7.4), at a rate of 10–15 ml/min. The composition of the Krebs solution was (in mM) 120 NaCl, 6 KCl, 2.5 CaCl2, 1.2 MgCl2, 1.55 NaH2PO4, 14.4 NaHCO3, and 11.5 glucose.

The myenteric ganglia were visualized with Nomarski differential interference contrast optics and epi-illumination. Ganglia were immobilized with 100 µL L-shaped stainless steel wires placed on either side of the ganglion, perpendicular to the longitudinal muscle axis, as described previously (41). In some experiments, it was necessary also to include 100 nM nicotine in the Krebs solution to prevent muscle movements from dislodging the intracellular electrode.

Transmembrane electrical potentials were recorded with conventional intracellular microelectrodes filled with 3 M KCl and having resistances of 60–120 MΩ. The preamplifier (M767, World Precision Instruments, New Haven, CT) had bridge circuitry for intraneuronal injection of electrical current. All data were recorded on magnetic tape for later analysis. Fast and slow EPSPs were evoked by electrical shocks (0.1–20 Hz) applied focally to interganglionic connectives from an etched tungsten electrode (tip diam 20 µm).

In most experiments the microelectrodes were filled with 4% biocytin in 2 M KCl containing 0.05 M tris(hydroxymethyl)-aminomethane buffer (pH 7.4). Resistances of the electrodes were 80–190 MΩ. The same electrodes were used to inject the marker dye biocytin by the passage of hyperpolarizing current (0.4–0.8 nA for 5–10 min) into the impaled neurons.

Impaled neurons were classified as AH/type 2 neurons, S/type 1 neurons, or nonspiking (inexcitable) cells using the criteria defined previously (17, 37, 39). In most cases only one
neuron per ganglion was studied electrophysiologically and pharmacologically and also injected with biocytin. This avoided confusion in distinguishing more than one neuron in the same ganglion.

At the end of each experiment, the anal end of the preparation was marked and fixative was added directly to the disposable recording chamber left overnight at 4°C. The fixative contained 2% formaldehyde plus 15% of a saturated solution of picric acid. The preparations were cleared in three changes of dimethyl sulfoxide and three 10 min washes with phosphate-buffered saline. The preparations were reacted with avidin coupled to horseradish peroxidase, carried through a diaminobenzidine color-developing reaction, and then dehydrated in alcohol (2). The preparations were mounted in Canada balsam and examined with an Olympus microscope equipped with Hofmann modulation contrast optics. All biocytin-injected neurons were characterized according to shape, cell diameter, number of long and short processes, and occurrence of retraction bulbs on long processes. The neurons were classified according to a previous report (13). All biocytin-injected neurons were photographed and categorized independently by each of us.

The neurons were exposed to PACAP-27 (5 µM), PACAP-38 (5 µM), forskolin (1 mM), and other pharmacological substances by N2 pressure microejection (Picospitzer, General Valve, East Hanover, NJ) from 10-µm diameter glass micropipettes. The micropipettes contained fast green dye for visualization of the distribution of the ejected substances in the chamber. Some of the substances were also applied in the superfusion solution. PACAP was superflushed for either a 30-s interval or until the peak response was observed. Both methods of application produced responses of similar magnitude. Cumulative dose-response data were obtained for PACAP at 5-min intervals for four to seven doses in each neuron. In some cases the recorded neuron was repeatedly exposed (i.e., 2-5 trials) to 50 nM PACAP at 15- to 20-min intervals to test for tachyphylaxis.

Two different protocols were used to expose the neurons to the adenosine analogue 2-chloro-N6-cyclopentyl adenosine (CCPA). In the first, CCPA was applied for 5-10 min in the superfusion solution after establishing control responses to PACAP, and PACAP was then reexamined in the presence of the adenosine A1 agonist. In the second, CCPA was applied during the peak depolarization produced by PACAP to determine if CCPA would abort the response once underway. The selective A1 antagonist 8-cyclopentyl-1,3-dimethylxanthine (CPT) was applied in the superfusion solution for 10-15 min before a second application of CCPA to evaluate the receptor specificity of the A1 agonist.

Data analysis. Neuronal input resistance was estimated from ohmic current-voltage plots as described previously. Pooled data are presented as the means ± SE. Analyses of variance, followed by posttests were used to calculate statistical significance between groups; corrected Bonferroni P values for multiple comparisons between groups were calculated using the INSTAT software package (Anderson-Bell, Parker). A spline function in Slidewrite graphics (Advanced Graphics Software, Sunnyvale, CA) was used to draw dose-response curves. The apparent half-maximal concentration (EC50) for PACAP depolarization of the membrane potential was extrapolated from the curve.

Materials. The adenosine analogues CCPA and CPT were purchased from Research Biochemicals (Natick, MA). PACAP-38 and PACAP-27 were obtained from Bachem (Torrance, CA). Hexamethonium, H2O-soluble form of forskolin, fast green dye, and biocytin were purchased from Sigma Biochemical (St. Louis, MO). Stock solutions of PACAP (0.5-0.1 mM), CCPA (1 mM), CPT (1 mM), and forskolin (1 mM) were all prepared in distilled water and diluted into Krebs solution.

Fig. 1. Excitatory action of pituitary adenylate cyclase-activating peptide (PACAP)-38 on afterhyperpolarizing (AH)/type 2 myenteric neuron in guinea pig small intestine. A: application of PACAP by pressure microejection evoked membrane depolarization associated with enhanced excitability and increased input resistance. Increased amplitude of electrotonic potentials produced by intraneuronal injection of hyperpolarizing current reflected increased input resistance. B: second application of PACAP 20 min later evoked excitatory response consisting of slowly activating depolarization and spike discharge in absence of intracellular injection of hyperpolarizing current pulses. Both responses were evoked by 50-80 pressure pulses of 5 µM PACAP. C: morphology of neuron was Dogiel type II. One process formed retraction bulbs where it was severed during dissection of preparation.
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Fig. 3. Excitatory action of PACAP- on AH/type 2 myenteric neuron of guinea pig small intestine. A: excitatory response to 100-ms micropressure pulse of 5 μM PACAP. This neuron did not discharge spikes to depolarizing pulses before PACAP application. B: excitatory response to 100-ms pulse of 1 mM forskolin. C: morphology of neuron was Dogiel type II.

Fig. 2. Comparative excitatory action of PACAP-27 and forskolin in AH/type 2 neuron of guinea pig small intestine. A: excitatory response to 100-ms micropressure pulse of 5 μM PACAP. This neuron did not discharge spikes to depolarizing pulses before PACAP application. B: excitatory response to 100-ms pulse of 1 mM forskolin. C: morphology of neuron was uniaxonal filamentous type.

Ten nonspiking cells, which did not respond to PACAP-27 or -38, were identified as glial cells when examined microscopically. When glial cells were injected, part or all of the ganglion was stained because of dye-coupling of the glia. The effect clearly delineated the ganglia (Fig. 4, K and L). PACAP-responsive Dogiel type II neurons had in most cases smooth ovoid cell bodies and multiple long processes projecting in the circumferential direction (Figs. 1, 2, and 4). In the majority of Dogiel type II neurons (74%), one or more long processes had retraction bulbs (Fig. 1). In two experiments where two cells were studied in the same ganglion, it was evident that two AH/Dogiel type 2 neurons with their cell bodies only 5–10 μm apart were both excited by PACAP.

Responsive Dogiel type I neurons had a single long process that ran for short distances in the circumferential direction and then projected mostly in the distal direction and could be followed for distances up to 10 mm. Lamellar dendrites were a prominent feature of these cells (Fig. 4, G–J). These neurons represented S/type 1 and nonspiking electrophysiological behavior. It was sometimes observed that two uniaxonal neurons within 5-μm proximity to each other were both excited by PACAP (Fig. 4J).

Responsive neurons with numerous filamentosus dendrites (Figs. 3 and 4) had a single long process, usually running distally. In some neurons (40%) the long process ended in a retraction bulb. One such neuron had an interrupted long process projecting in the circumferential direction. Another neuron in this class, which had its long process in the oral direction, did not respond to PACAP.

Forskolin. Activation of adenylate cyclase with forskolin mimicked the action of PACAP-27 or -38 in all of 12 AH/Dogiel type II neurons (Fig. 2). However, 100–1,000 μM forskolin were needed in the microejection pipette compared with only 2.5–5 μM PACAP to produce responses with equal intensity. Focal stimulation (3–15 Hz, 1–6 s) of interganglionic connectives evoked slow EPSPs in 10 AH/Dogiel type II neurons that also responded to PACAP.

Eight of the AH/type 2 neurons (14.5%) exposed to PAC-AP-27 or PACAP-38 by microapplication, responded with a transient 3 to 10 mV hyperpolarization lasting 2–6 s followed by the depolarizing response. The amplitude of the response increased with the duration of the microejection pulse. In one nonspiking cell, PAC-AP-38 caused a dose-dependent hyperpolarization (0.1–500 nM), associated with a decrease in input resistance. The peak hyperpolarization was 14 mV. In a different nonspiking cell, PACAP-38 evoked a transient hyperpolarization, followed by a depolarization, and repeated exposures did not cause any desensitization; the cell never became excitable. These responsive nonspiking cells were not stained successfully.

Morphology. The morphology of the cells exposed to PACAP-27 or PACAP-38 was identified in 55 of 60 injected cells, and these were classified according to their shape (13). The data are summarized in Table 1. PACAP excited multipolar Dogiel type II neurons (30%), uniaxonal Dogiel type I neurons (30%), and uniaxonal neurons with numerous filamentous dendrites (70%). Most PACAP-responsive Dogiel type II neurons (96.2%) had AH/type 2 electrophysiological behavior or converted to AH/type 2 behavior with PACAP. Neurons with filamentous dendrites belonged to all three electrophysiologically identified neuronal cell types.

The tissue for 10–60 min. Time for recovery during wash-out was directly related to the dose. The response to 50 nM PACAP-27 was resistant to desensitization (n = 10) with repeated exposures at 15- to 20-min intervals.

Of 25 nonspiking neurons, 20 became excitable and converted to AH/type 2 behavior after application of PACAP (Fig. 2). The five unresponsive cells received fast or slow synaptic input or were identified morphologically as neurons. Excitatory responses to PACAP also occurred in 4 of 11 neurons with S/type 1 electrophysiological characteristics. No effect was observed in 10 nonspiking cells identified morphologically as glia.

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PACAP ON MYENTERIC NEURONS

Table 1. Morphology of electrophysiologically identified myenteric neurons excited by PACAP

<table>
<thead>
<tr>
<th>Morphology</th>
<th>n (%)</th>
<th>Processes</th>
<th>Dimensions, μm</th>
<th>Swellings (Neurons)</th>
<th>PACAP Excitation</th>
<th>Electrical Behavior</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dogiel II</td>
<td>30</td>
<td>3.9±0.3</td>
<td>0.6±0.2</td>
<td>41.6±2.2</td>
<td>18.4±6</td>
<td>19</td>
</tr>
<tr>
<td>Dogiel I</td>
<td>12</td>
<td>1</td>
<td>0.9±0.3</td>
<td>23.3±2.3</td>
<td>15.4±1.1</td>
<td>3</td>
</tr>
<tr>
<td>Filament</td>
<td>10</td>
<td>1</td>
<td>12.9±0.9</td>
<td>26.7±1.8</td>
<td>14.3±1.2</td>
<td>4</td>
</tr>
</tbody>
</table>

Dimensions of Dogiel type II neurons were significantly greater than those in Dogiel type I and filamentous neurons (P < 0.0001). Short (minor) axis of Dogiel type II neurons was significantly greater than that of Dogiel type I (P = 0.018) or filamentous neurons (P = 0.0015). Number of long axonal processes for Dogiel II neurons was greater than that of other cell types (P < 0.0001). Number of short filamentous processes for filamentous neurons was greater than in other cell types (P < 0.001). Dogiel II (P = 0.0032), and of filamentous neurons (P < 0.0001) was significantly greater than short axes for each neuronal type. Swellings, retraction bulbs at ends of severed projections out of ganglia. Dimensions, long (major) axes and short (minor) axes of cell soma. PACAP, pituitary adenylate cyclase-actuating polypeptide; AH, afterhyperpolarizing.

Qualitatively, the excitatory actions of PACAP-38 and PACAP-27 were the same. PACAP-27 was also used to determine the potency in exciting the neurons. PACAP-27 caused a dose-dependent increase in membrane depolarization in AH/type 2 neurons with Dogiel II morphology (Figs. 5 and 6). The threshold dose for increased excitability and depolarization was 0.1 nM. The apparent EC50 concentration was 1.5 nM.

Adenosine A1 analogues. The A1 selective adenosine receptor agonist CCPA (250 nM) abolished the excitatory action of 500 nM PACAP-27 (n = 10) in AH/type 2 neurons. If CCPA was superfused before exposure to PACAP, it prevented its effect (Fig. 7). When CCPA was superfused during the peak effect of PACAP-27, the response was quickly and completely aborted in 2–3 min (Figs. 5 and 7) in 70% of neurons. By comparison, 20–60 min were required for recovery during washout in Krebs solution (n = 9). In the remaining 30% of neurons, only partial recovery occurred with CCPA (i.e., 60–80%).

CCPA prevented the increase in cell input resistance produced by PACAP-27 (n = 10, Fig. 8). The inhibition produced by 250 nM CCPA was reversible on application of 2.5–10 μM of the A1 selective antagonist CPT (Fig. 7).

DISCUSSION

Previous reports indicated that PACAP-27 or PACAP-38 produced a neurogenic contraction of the isolated guinea pig ileum (18). Our results show that PACAP has a potent excitatory action on myenteric neurons of the guinea pig ileum that can account for the neurogenic contractile responses seen in organ bath studies.

Excitation by PACAP-27 was dose dependent and reversible with the postwash recovery time also showing dose dependence. The low nanomolar potency and efficacy (amplitude of depolarization, increase in input resistance, increase in frequency of spike discharge) displayed by PACAP is consistent with a physiological role in enteric neurotransmission. A small proportion of myenteric neurons was also inhibited by PACAP. Nevertheless, the predominant effect was excitation. The significance of the hyperpolarizing response remains unclear.

Neurons with Dogiel/type I, type II, and filamentous morphologies (13) were activated by PACAP. However, a different percentage of each cell population had PACAP receptors with the greatest proportion by far residing in the AH/type 2 population. In order from highest to lowest percentage, Dogiel type II > filamentous > Dogiel type I neurons. This may be an important mechanism for the selectivity of action of PACAP, given that these neurons are likely to subserve different functions in the enteric microcircuits. Presumably, only subpopulations of filamentous and Dogiel type I neurons express functional PACAP receptors, whereas virtually all Dogiel type II neurons have the receptors.

The neurogenic contraction to PACAP was reported to involve both a cholinergic and a noncholinergic component (18). The available evidence suggests that AH/type 2 neurons with Dogiel II morphology and S/type 1 neurons with Dogiel I morphology are interneurons, whereas S/type 1 neurons with either filamentous uniaxonal or Dogiel I morphology are motor neurons projecting to both longitudinal and circular muscle layers (2, 4, 13). Among the group of neurons identified in our study, the majority of AH/type 2 neurons responded to PACAP. This is consistent with other evidence that AH/type 2 neurons are interneurons responsible for excitatory drive and coordination of discharge of motor neurons to the intestinal effector systems (37, 40).

Circular muscle motor neurons were identified as having a single long process and either lamellar dendrites or numerous filamentous dendrites (4). Furthermore, the cell bodies of motor neurons that supply the circular muscle of the guinea pig ileum are in the myenteric plexus (1, 4, 33). Excitatory cholinergic motor neurons to the circular muscle are believed to be S/type 1 neurons with Dogiel I morphology (14). Aside from acetylcholine, substance P (36) is another putative transmitter for excitatory drive and coordination of discharge of motor neurons to the intestinal effector systems (37, 40).

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role of PACAP in the excitation of the interneuronal driver circuits to the motor innervation of the intestinal musculature.

Enteric glial cells have been shown to be dye coupled with passage of small molecular weight dyes occurring across gap junctions (16). The intracellular dye biocytin also has a small molecular weight and therefore crosses glial cell gap junctions. When nonspiking cells, which turned out to be glial cells, were injected, multiple glial cells were stained as a result of dye coupling. PACAP did not affect the resting membrane potential of the glial cells. In the brain astrocytic glia were shown to have receptors for PACAP (35), and activation of the receptors stimulated formation of cAMP (19).

Myenteric AH/type 2 neurons with Dogiel II morphology were suggested to be primary sensory neurons in the enteric nervous system (2, 12, 28) because they have long processes terminating in the mucosa that could detect mechanical stimuli (15). Smith et al. (32) were unable to demonstrate any effects in AH/type 2 neurons of mechanical stimulation at sites 15–30 mm from the neurons and suggested this to be added support for identification.
PACAP ON MYENTERIC NEURONS

Fig. 5. Dose dependence of PACAP excitatory action on AH/type 2 myenteric neuron of guinea pig small intestine. A-C: application of progressively increasing concentrations of PACAP for 30 s in superfusion solution. D: application of adenosine A1 agonist 2-chloro-N6-cyclopentyl adenosine (CCPA) reversed excitatory action of PACAP.

Fig. 6. Dose-response relationship for excitatory action of PACAP on AH/type 2 myenteric neurons of guinea pig small intestine. Estimated half-maximal concentration was 1.5 nM.

Fig. 7. Effects of selective adenosine A1 analogues on excitatory action of PACAP on AH/type 2 myenteric neuron in guinea pig small intestine. A: excitatory response to 100-ms micropressure pulse of 5 PM PACAP. B: application of 1 nM CCPA in superfusion solution aborted excitatory action of PACAP. C: continued presence of CCPA prevented excitatory response to micropulse of PACAP. D: excitatory response to PACAP was restored by addition of A1 antagonist 8-cyclopentyl-1,3-dimethylxanthine (10 nM) in presence of 1 nM CCPA.

Fig. 8. Current-voltage relationship in presence and absence of 250 nM PACAP- and PACAP together with 250 nM CCPA for AH/type 2 myenteric neuron in guinea pig small intestine. Increased slope in presence of PACAP reflected increased input resistance. Application of A1 agonist CCPA reversed effects of PACAP on input resistance, with slope of current-voltage plot equaling that of control before PACAP.

of this neuronal type as the primary sensory neurons of the intestine. Nevertheless, no excitation of AH/type 2 neurons by mechanical stimulation could be demonstrated at any site along the preparations studied by these authors.

It is significant relative to the sensory neuron hypothesis that essentially all AH/type 2 neurons were excited by PACAP and as high as 72% of the total number of these neurons had large bulbous expansions at the ends of long processes. These expansions are presumed to be retraction bulbs formed by the rescaling of the cut ends of damaged processes (11), at their exit from the ganglion to other layers. In a few cases in which both the ganglion and the responsive Dogiel type II neuron were stained, it was evident that one or more long processes left the ganglion in the direction of the circular muscle. Whether these extensions normally transmit outbound spike information from the cell body or permit the soma to receive inbound information is unknown, and the significance of the projections toward the circular muscle and submucous plexus is therefore unresolved. To be consistent with the sensory neuron hypothesis, the projections in the direction of the mucosa would need to transmit inbound spike information to the multipolar somal of the AH/Dogiel II neurons.

Our results are generally inconsistent with the spurious argument that AH/type 2 neurons are primary sensory neurons. It seems improbable that sensory neurons adapted for detection and accurate coding of mechano-, chemo-, or other sensory information would be subject to the dramatic changes in excitability produced by PACAP and other slow EPSP mimetics. AH/type 2 neurons have multipolar cell bodies with several long and extensively branched neurites (13, 15). The cell bodies of these neurons receive a variety of synaptic inputs including slow excitatory, slow inhibitory, and fast nicotinic synaptic responses (see Ref. 39 for a review). They possess excitatory receptors for at least 15 excitatory neurotransmitters, as well as receptors for at least eight substances that mimic slow synaptic inhibition in the cell body (39, 40). If these neurons are indeed primary sensory neurons, their functional morphology and electrical and synaptic behavior are highly unusual and scarcely, if at all, found in the animal kingdom (5).

PACAP has been reported to act by both cAMP-dependent (7, 19, 22, 30, 35) and independent mechanisms (6, 31), depending on the cell type in which the studies...
were done. Our findings are consistent with activation of adenylate cyclase by PACAP in AH/Dogiel type II neurons. Similarity of action of PACAP and forskolin are consistent with this. Furthermore, slow synaptic excitation, that is mimicked by PACAP, is known to occur exclusively in AH/type 2 neurons via a cAMP-dependent mechanism (25, 42). We found that the selective adenosine A1 receptor agonist CCPA abolished the effect of PACAP. This action was blocked by CPT, indicating that CCPA was acting at specific adenosine receptors. Adenosine receptors that are negatively coupled to the cyclase are known to be of the A1 subtype (37). Inhibitory adenosine receptors on cell somas of AH/Dogiel type 2 neurons have been identified as an A1 subtype (9). When considered in light of other results that show that adenosine inhibits adenylate cyclase activity in these cells (26, 27, 44), our findings suggest that PACAP may be coupled through G proteins to stimulate adenylate cyclase and the formation of intraneuronal cAMP.

It is of interest that PACAP also excited neurons other than AH/type 2. Presumably, a cAMP-independent mechanism is involved in the action of PACAP in the other neurons. Likewise, the hyperpolarization because of PACAP in a small subgroup of myenteric neurons is also presumed to operate through a different signaling mechanism.

As with a number of other substances that mimic slow synaptic excitation in enteric neurons, understanding of the physiological relevance of the neuronal action of PACAP in the integrated system is incomplete. Interpretation of significance can be made in terms of its mimicry of slow synaptic excitation. The general significance of slow synaptic excitation is thought to be a mechanism for provision of prolonged excitatory or inhibitory input at synapses on other neurons within the microcircuits and/or at neuroeffector junctions. Slow excitatory inputs also modulate the excitability of the cell bodies of the AH/Dogiel II neurons and thereby gate the transfer of spike information across the cell body from neurites arising from opposing poles of the cell (38–40). Kuwahara et al. (21) recently reported that application of PACAP either directly or indirectly led to excitation of secretomotor neurons and increased chloride secretion in preparations of guinea pig colonic mucosa. Whereas, Katsoulis et al. (18) reported contractile responses in the guinea pig small intestine. Neurosci. Lett. 118: 227-230, 1990.


