# Effect of Pituitary Adenylate Cyclase–Activating Peptide on Isolated Rabbit Iris Sphincter and Dilator Muscles

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**Purpose.** Pituitary adenylate cyclase–activating peptide (PACAP) is a sensory neuropeptide in the eye that is released by noxious stimuli and considered to be a mediator of the neurogenic ocular injury response, including miosis. The purpose of this study was to clarify the functional role of PACAP in iris sphincter and dilator muscles.

**Methods.** Iris sphincter and dilator muscles were isolated from rabbit eyes, and the effect of PACAP on mechanical responses of these muscles using isometric tension-recording methods was investigated.

**Results.** The iris sphincter responded to electric field stimulation with contractions composed of fast twitch and subsequent slow components. Both PACAP 27 and PACAP 38 enhanced the twitch response, but neither had an effect on the slow response. The effect of both PACAPs on the twitch response was dose dependent. Neither PACAP had an effect on the amplitude of contraction evoked by exogenously applied Ach. For the iris dilator muscle, PACAP 27 inhibited the contractions induced by field stimulation or phenylephrine, whereas PACAP 38 had no effect.

**Conclusions.** Both PACAP 27 and PACAP 38 enhance cholinergic transmission in sphincter muscle. The PACAP 27 induces relaxation of the dilator muscle by a direct effect on the muscle itself. The PACAP released during an ocular inflammatory response may induce miosis by the enhancement of cholinergic stimulation of the iris sphincter and by direct relaxation of the dilator muscles. (*Invest Ophthalmol Vis Sci.* 2002;43:780–783)

Pituitary adenylate cyclase–activating peptide (PACAP) is a novel neuropeptide with two molecular forms: one with 27 amino acid residues (PACAP 27) and one with 38 residues (PACAP 38).[1] Each has significant structural homology with vasoactive intestinal peptide (VIP).[2] There are two types of receptors for PACAP. One has a higher affinity for PACAP than for VIP; the other has a similar affinity for PACAP and VIP. The anterior uvea of the rabbit has mainly the latter type of PACAP receptors.[3]

PACAP immunoreactive nerve fibers have been identified in the central nervous system[4,5] as well as peripheral tissues including lung,[6] pancreas,[7] and gastrointestinal tract,[8] suggesting that PACAP acts as a neurotransmitter or neuromodulator. PACAP immunoreactive nerve fibers are also present in ocular tissues, including iris-ciliary body, choroid, cornea, and sclera.[9,10] Wang et al.[10] reported that the distribution pattern of PACAP-immunoreactive nerve fibers in the eye is similar to calcitonin gene-related peptide (CGRP) immunoreactivity, a known component of sensory C-fiber neurons. Tajti et al.[11] demonstrated the presence of CGRP, substance P (SP), and PACAP immunoreactivity in the human trigeminal ganglion. These results indicate that PACAP is a sensory neuropeptide in the eye.

Trigeminal nerve stimulation induces inflammatory responses in the rabbit eye.[12] These include vasodilation, breakdown of the blood–aqueous barrier, and miosis. Sensory nerve fibers are likely to play important roles in these responses, because SP and CGRP are released from such nerve terminals and evoke these responses.[13,14] PACAP is also a mediator of the neurogenic ocular injury response. Intravitreal administered PACAP causes breakdown of the blood–aqueous barrier, conjunctival hyperemia, and decreased pupil diameter of the rabbit eye.[10] Capsaicin, which causes release of SP from trigeminal sensory nerves in rabbits,[15] also releases CGRP and PACAP in the rabbit uvea in vitro.[16] Thus, it is likely that SP, CGRP, and PACAP coexist in sensory nerve fibers and are released by noxious stimuli.

In an attempt to clarify the functional role of PACAP on the iris smooth muscles, we isolated iris sphincter and dilator muscles from rabbit eyes and investigated the mechanical properties of these muscles, using isometric tension recording methods.

## Methods

### General

All animals were treated in accordance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research. Male albino rabbits weighing 2 to 3 kg were killed with an overdose of intravenous pentobarbital sodium (Abbott Laboratories, North Chicago, IL). The eyes were immediately enucleated and placed in Krebs solution composed of (in millimolar): NaCl, 94.8; KCl, 4.7; $MgSO_4$, 1.2; $CaCl_2$, 2.5; $KH_2PO_4$, 1.2; $NaHCO_3$, 25.0; and glucose, 11.7 and gassed with 95% $O_2$ and 5% $CO_2$. Under microscopic observation, ring-shaped iris sphincter muscle specimens (1 mm wide) and dilator muscle specimens (1 mm wide, 3–4 mm long) were prepared according to a method previously reported.[17,18]

### Isometric Tension Recording Experiments

The ends of each specimen were tied with silk thread and mounted vertically in a 1.5-mL organ bath. One end was connected to an isometric tension transducer (EF-601G; Nihon Koden Ltd., Tokyo, Japan), and the other end was secured to a hook at the bottom of the organ bath. The initial loads were 100 and 50 mg for sphincter and dilator, respectively. The organ bath was perfused continuously (0.17 mL/sec) with oxygenated Krebs solution warmed to 37°C, as we described previously.[19] Experiments were started after a 60-minute equilibration period. The responsiveness of each preparation was tested initially by application of 1 mM Ach for sphincter or 10 $\mu$M phenylephrine for dilator at least three times, to confirm that the same amplitude of contraction was observed each time. Specimens that did

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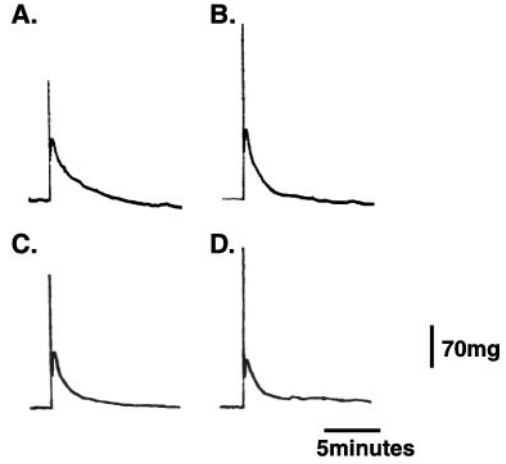


**FIGURE 1.** Iris sphincter muscle recordings of contractile responses evoked by 70 pulses of electrical field stimuli. Twitch and slow responses (**A**) before and (**B**) after 10 nM PACAP 27. Twitch and slow responses (**C**) before and (**D**) after 10 nM PACAP 38.

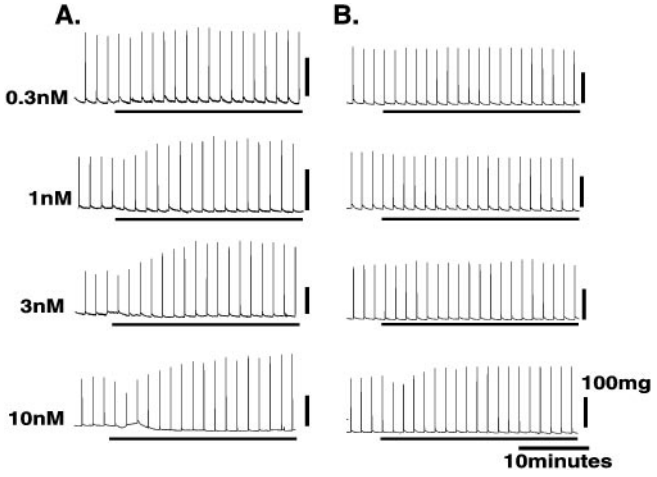


**FIGURE 2.** Iris sphincter muscle recordings of contractile responses evoked by five pulses of electrical field stimuli applied every 1.5 minutes. Effect of various concentrations of (**A**) PACAP 27 and (**B**) PACAP 38 on the mechanical responses during field stimulation. All four traces were obtained from the same specimens

not show the same amplitude of contractions in response to agonists were excluded from the study. After 1 to 2 hours of washout period, test drugs were added to the perfusing solution. Transmural electrical field stimulations were applied through a pair of platinum electrodes separated by 11 mm and placed in the organ bath, so that the current pulse would pass transversely across the tissue. Pulse stimuli of 10 V at 20 Hz were applied for 2.0 ms.

### Data Analysis

All data are expressed as the mean ± SD. Student's *t*-test was used for statistical evaluation of the differences between means. $P < 0.05$ was considered significant.

### Drugs and Chemicals

The following drugs and chemicals were used in this study: PACAP 27 and PACAP 38 (Peptide Institute, Inc., Osaka, Japan) and Ach, tetrodotoxin (TTX), and phenylephrine (all from Wako Chemical Inc., Osaka, Japan). Peptides were prepared in aliquots and stored at −30°C.

## RESULTS

The iris sphincter and dilator muscles, mounted in an organ bath, gradually relaxed to a steady tension during 60 minutes of equilibration. Muscle tone subsequently remained constant for several hours. The electrical field stimulus (70 pulses) evoked a biphasic contraction of the iris sphincter muscle characterized by an initial twitch followed by long-lasting slow contraction. For the dilator muscle, the same electrical field stimuli evoked only twitch contractions. These responses were abolished by pretreatment with TTX (0.1 $\mu$M), suggesting that the responses were neurogenic in origin (data not shown).

### Effect of PACAPs on the Mechanical Properties of Iris Sphincter Muscle

PACAP 27 and PACAP 38 (10 nM) enhanced the amplitude of twitch component of the sphincter contraction evoked by field stimulation but had no effect on the slow component (Fig. 1). This effect of PACAP 27 and PACAP 38 on the fast component of contraction evoked by field stimulation was investigated further. To inhibit the prolonged, slow component of contraction, field stimulation composed of 5 pulses was applied every 1.5 minutes. Both PACAP 27 and PACAP 38 enhanced in a dose-dependent manner the amplitude of the twitch contraction evoked by field stimulation (Figs. 2, 3). At 10 nM for each peptide, there was a transient inhibition of the contraction immediately after application (Fig. 2). The maximum enhancement of contraction evoked by field stimulation for 10 nM PACAP 27 and PACAP 38 was 41.1% ± 17.1% and 25.8% ± 11.9% ($n = 6$), respectively.

The effect of PACAP 27 and PACAP 38 on the amplitude of sphincter contraction evoked by field stimulation and 0.1 mM Ach were compared. Because the median effective concentration ($EC_{50}$) for contractile response to Ach in this tissue is approximately 0.3 mM,[20] a concentration of 0.1 mM Ach was chosen for this experiment. Both PACAPs enhanced the twitch contraction evoked by field stimulation but had no effect on the Ach-induced contraction (Fig. 4, Table 1). These results indicate that the PACAP-dependent increase of the cholinergic responses were due to enhancement of prejunctional cholinergic transmission, not enhancement of postjunctional Ach sensitivity.

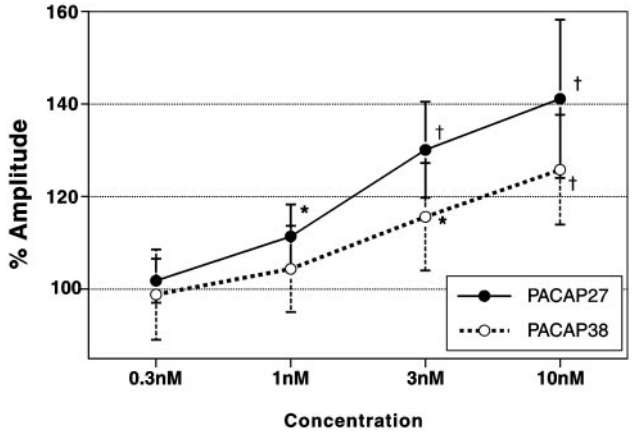


**FIGURE 3.** Dose–response relationship between PACAP concentration and the amplitude of contractions evoked in sphincter muscle by field stimulation (five pulses). The mean of the last five contractions before application of PACAPs was defined as the relative amplitude of 100%. The amplitude of contraction after the application of PACAPs was defined as mean of the five contractions, beginning 15 minutes after application. $^{*}P < 0.05$ and $^{\dagger}P < 0.01$

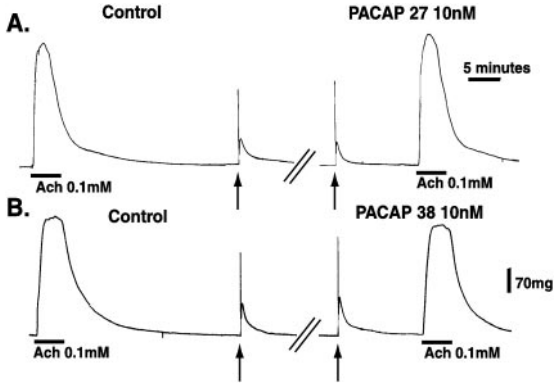


**FIGURE 4.** The effect of (**A**) PACAP 27 and (**B**) PACAP 38 on the contraction evoked by 0.1 mM Ach and field stimulation (*arrow*, 70 pulses) in the sphincter muscle.

## Effect of PACAPs on the Mechanical Properties of Iris Dilator Muscle

The effects of PACAP on the contraction evoked by field stimulation of dilator muscle were also determined. Neither PACAP had an effect on the basal tone of the dilator muscle. PACAP 27 reduced in a dose-dependent manner the amplitude of twitch contraction evoked by field stimulation (Figs. 5A, 5C). The maximum reduction in amplitude, achieved with 10 nM PACAP 27, was 42.0% ± 9.7%. PACAP 38 did not significantly change the amplitude of contraction at any concentration tested (Figs. 5B, 5C).

PACAP 27 also induced a dose-dependent relaxation of the dilator muscle that was precontracted by 10 $\mu$M phenylephrine (Fig. 6A, Table 2). However, PACAP 38 did not have such an effect (Fig. 6B, Table 2). The results indicate that PACAP 27 but not PACAP 38 directly induced relaxation of the dilator muscle.

## DISCUSSION

The twitch and slow contractions evoked by field stimulation in rabbit iris sphincter muscles result from the activation of cholinergic and SP-ergic nerve fibers, respectively.[21] The present investigation showed that both PACAP 27 and PACAP 38 enhanced twitch contraction, but had no effect on slow contraction evoked by field stimulation. Because PACAP had no effect on the contraction evoked by exogenously applied Ach in this tissue, we conclude that PACAPs enhances cholinergic transmission in sphincter muscle. Because SP-ergic slow contraction was not affected by PACAP (up to the concentration of 10 nM), the prejunctional action of PACAP seems to affect only cholinergic nerves, not SP-ergic nerves. Prejunctional action of PACAP is also reported in the guinea pig tenia coli.[22] However, our data are not consistent with previous data showing that 1 $\mu$M PACAP 27 and PACAP 38 had no effect on electrically evoked contractions in rabbit iris sphincter.[10] Although the cause of this discrepancy is not clear, a likely possibility is that the electrical stimulation settings may affect the results. Preliminary experiments showed that the PACAP-dependent enhancement of twitch contraction evoked by field stimulation was more apparent with the 5-pulse stimulus than with the 70-pulse. Because the amplitude of contraction by the 70-pulse stimulus was nearly maximum, there may be little room for greater contractions. We have shown the enhancement of the contraction evoked by field stimulation from concentrations as low as 1 nM PACAP 27, which is in the range of physiological relevance. However, the physiological relevance of transient inhibition of contraction immediately after application of both peptides in the concentration of 10 nM is not clear.

**TABLE 1.** Amplitude of Contraction Evoked by 0.1 mM Ach in Sphincter Muscle

| | Control Tension (mg) | +PACAP Tension (mg) |
|---|---|---|
| PACAP 27 | 192.4 ± 70.8 | 188.7 ± 77.0 |
| PACAP 38 | 216.3 ± 81.5 | 211.3 ± 82.2 |

Dose of PACAPs was 10 nM ($n$ = 6 for each).

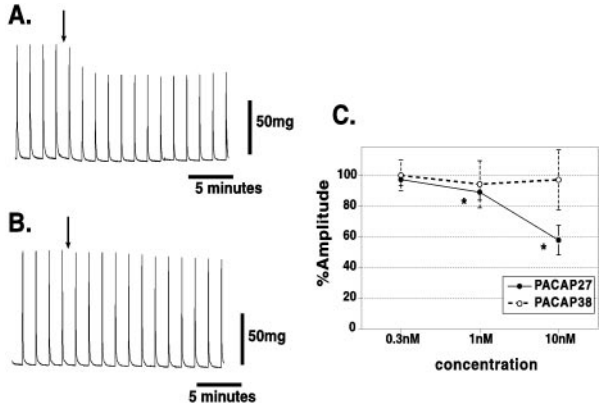


**FIGURE 5.** Iris dilator muscle recordings of contractile responses evoked by field stimulation with 50 pulses every 1.5 minutes *Arrows*: application of 10 nM (**A**) PACAP 27 and (**B**) PACAP 38. (**C**) Dose–response relationships between PACAP concentration and the amplitude of contractions evoked in dilator muscle by field stimulation (50 pulses). The mean of the last five contractions before application of PACAPs was defined as relative amplitude of 100%. The amplitude of contraction after the application of PACAPs was defined as the mean of the five contractions beginning 5 minutes after application. $^*P < 0.01$.

To our knowledge, this is the first report of the effect of PACAPs on iris dilator muscle. In contrast to the effect on iris sphincter, PACAP 27 inhibited the dilator muscle contractions evoked by both field stimulation and exogenously applied phenylephrine. The indicates that PACAP had mainly a direct relaxant effect on the dilator muscle and had little effect on adrenergic transmission. PACAP causes smooth muscle relaxation through the nitric oxide pathway in opossum internal anal sphincter[23,24] or by increasing the cAMP level in tenia of the guinea pig cecum.[25] VIP, which has significant structural homology with PACAP, also relaxes dilator muscle.[26]

Thus, PACAPs could induce miosis through two different mechanisms. One is the enhancement of cholinergic transmission in sphincter muscle, and the other is the direct relaxant effect on the dilator muscle. Intravitreal injection of PACAP induces miosis in rabbit, which supports our findings.[10]

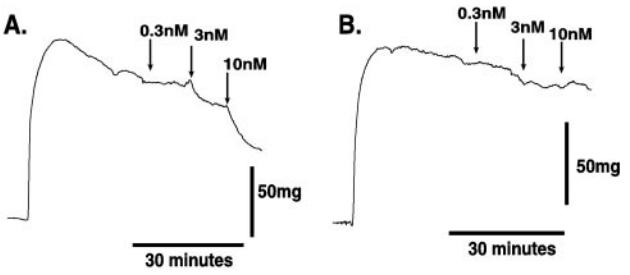


**FIGURE 6.** Concentration-dependent effect of (**A**) PACAP 27 and (**B**) PACAP 38 on the mechanical properties of iris dilator muscle precontracted with 10 $\mu$M phenylephrine.

**TABLE 2.** Amplitude of Contraction Evoked by 10 $\mu$M Phenylephrine in Dilator Muscle

| | Control Tension (mg) | +PACAP Tension (mg) |
|---|---|---|
| PACAP 27 | 94.5 ± 15.9 | 76.7 ± 10.7* |
| PACAP 38 | 116.1 ± 12.1 | 112.8 ± 12.3 |

Dose of PACAPs was 10 nM ($n = 6$ for each).
*$P < 0.01$

Trigeminal nerve stimulation induces inflammatory responses including miosis in the rabbit eye.[12] SP, CGRP, and PACAP all exist in trigeminal nerve fibers and are involved in these responses.[13–16] SP induces miosis through direct contractile effect on the sphincter muscle without effect on the dilator.[19] In addition, we have reported that CGRP also induces miosis through direct relaxation in the dilator muscle without effect on the sphincter.[18] We conclude in the present study that PACAPs induce miosis through enhancement of cholinergic transmission in sphincter muscle and by relaxation of dilator muscle. Therefore, the miosis that occurs during an ocular inflammatory response may result, at least in part, from neuropeptides released from the trigeminal nerve. These neuropeptides, SP, CGRP, and PACAP, all induce miosis at different sites of action.

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