Fig. 4. Scanning electron micrograph of what appears to be a dividing cell in the corneal endothelium of the kitten. Such cellular conformations are relatively common in the kitten but have not been observed in the adult cat (X2,000). The pronounced pleomorphism observed in the kitten endothelium has also been observed in newborn (6-day-old) rabbit corneal endothelium. The reason for this pleomorphism is unknown. A possible explanation is that mitotic activity combined with the rapid enlargement of the cornea during the postnatal period imposes a continuous requirement for readjustment of cells within the monolayer. Such a continuous readjustment would result in a less ordered cellular arrangement.

Key words: corneal endothelium, cat.

References

Exerts its stimulant effect, prejunctionally, ie, on the nerve terminals, this peptide probably contributes to the control of accommodation as a modulator of cholinergic neurotransmission between short ciliary nerves and the ciliary muscle. Invest Ophthalmol Vis Sci 24:250-253, 1983

Immunohistochemical investigations12 have provided evidence that vasoactive intestinal peptide (VIP) and substance P (SP) are distributed in intraocular tissues. SP causes atropine-resistant potent miosis,3 while VIP is much less a miotic.4,5 It was recently reported that VIP mediated the ocular vasodilation caused by facial nerve stimulation.6 Whether or not these peptides cause an increase in aqueous humor protein concentration and the development of anterior chamber flare has been studied mostly by intracameral injection of these peptides, and the so-obtained are controversial.4,5,7,8 The presence of VIP in the ciliary muscle1 raises the question of whether and how this peptide participates in the control of excitation of ciliary muscle. The effects of VIP on the motor function of ciliary muscle have apparently not been documented. VIP has a wide variety of actions,9,10 including production of relaxation of smooth muscle (intestine,10 trachea,10 and canine antral muscle11). We have now carried out studies on the neuronal stimulation of the isolated ciliary muscle tissue in an attempt to elucidate the possible regulatory role of VIP in accommodation.

**Materials and methods.** Bovine eyes were obtained at a slaughterhouse 5 min after enucleation and were immediately placed in an oxygenated modified Krebs solution. Experiments were begun within 30 min. Twenty-five muscle strips (5 mm wide and 6 mm long) were prepared under a binocular microscope by dissecting the ciliary body from the scleral spur, while taking care to avoid damage to the nerve-muscle tissues.

To investigate the mechanical responses, the tissue was mounted in a 0.5 ml organ bath through which 36°C Krebs solution gassed by 95% O₂: 5% CO₂ flowed continuously (1.5 ml/min). Stimulations were applied supramaximally with an electronic stimulator (MSE-3R, Nihon Kohden Ltd., Tokyo). Tensions that developed were measured under isometric conditions by means of a force displacement transducer (FD pickup, TB612T-Nihon Kohden Ltd., Tokyo), with a load of 100–150 mg.

The composition of the Krebs solution was as follows (mM): Na⁺, 137.4; K⁺, 5.9; Mg²⁺, 1.2; Ca²⁺, 2.5; Cl⁻, 134; H₂PO₄⁻, 1.2; HCO₃⁻, 1.5.5, and glucose 11.5. VIP (Peninsula Laboratories, Inc., San Carlos, Ca.) was dissolved in Krebs solution to a concentration of 10⁻⁶ M, stored at −80°C, and used within 2 weeks. The following drugs were used: carbachol chloride, L-isoproterenol hydrochloride, DL-norepinephrine, atropine sulfate, eserine, DL-propranolol hydrochloride, and tetrodotoxin. All drugs were from Sigma Chemical Co., St. Louis, Mo. To observe the direct effects of the drugs, the strips were exposed to these agents for at least 5 min. The concentration of agents used indicates the final concentration, achieved in about 20 sec after start of change of the solutions.

**Results.** One hour after incubation of the isolated muscle strips, relaxation occurred and a stable tone was maintained. A short ciliary nerve connected to the ciliary muscle was placed on paired electrodes, in the air, and the tissue stimulated. In occasional experiments, transmural field stimulation was carried out, and here the maximal contraction was usually larger than that given by isolated nerve stimulation. The pulse duration used (0.2–0.5 msec) was sufficient to stimulate the nervous element, but was too short to stimulate the muscle cell directly. A single shock to the nerve provoked a twitch-like contraction. Repeated stimulation produced a summation of contractions. The evoked contractions, whether stimulated singly or repetitively, were increased by adding eserine to the Krebs solution (Fig. 1a), whereas addition of atropine completely suppressed the evoked responses (Fig. 1b). Application of 10⁻⁶ M tetrodotoxin had the same effect as atropine. Tetrodotoxin suppresses nerve activity but does not affect the smooth muscle itself.12 Propranolol (10⁻⁷–5 × 10⁻⁶ M) or phentolamine (5 × 10⁻⁷–10⁻⁵ M) had no effects on the evoked contractions.

Figure 2 shows that the contractile amplitude due to electrical stimulation was increased by addition of VIP (a). The potentiation by VIP rapidly followed after initiation of the drug application, reached a
2 min

Fig. 2, a and b. a, Effects of VIP on contractile responses to field stimulation (0.5 msec duration, 5 Hz, 10 pulses) at 30 sec intervals. Evoked contractions were potentiated by application of $10^{-7}$ M VIP. b, Effects of VIP on contractile responses to electrical stimulation and carbachol. Potentiation due to nerve stimulation (0.5 msec, 10 Hz, 20 pulses) was generated by $10^{-7}$ M VIP. Electrical stimulation and administration of carbachol are indicated by dots and lines, respectively.

maximum in about 20 sec, and was then sustained. After washing with normal Krebs, the response promptly returned to a control value. VIP enhanced the contractile response at all stimulus frequencies. On the other hand, when electrical stimulation was replaced by administration of carbachol, the carbachol-induced contraction was not altered in amplitude, under conditions of a prior administration of VIP (b). Acetylcholine had the same effects as carbachol, in the presence of eserine.

The dose-response curve is illustrated in Figure 3. The enhancement of evoked contractions by VIP was dose dependent between $10^{-8}$ and $10^{-6}$ M, while lower doses ($10^{-10}-10^{-8}$ M) had no effects on the evoked contraction (a). Exogenous VIP did not significantly lower the resting tension (b). Similarly, VIP did not produce a significant relaxation, even when the preparation was contracted with carbachol. The enhancing effect of VIP was not influenced either by propranolol ($10^{-7}-10^{-5}$ M) or phentolamine ($5 \times 10^{-7}-10^{-5}$ M); however, the evoked contraction was abolished by application of $5 \times 10^{-7}$ M atropine.

In the bovine choroidal strip containing intrinsic nerves, there were no changes in tension evoked by nerve stimulation, either before or after application of VIP ($10^{-7}-10^{-6}$ M).

Discussion. VIP, an octacosapeptide with structural similarity to secretin and to glucagon, was originally considered as a gut hormone candidate. However, a recent immunohistochemical study has demonstrated that nerves with VIP immunoreactivity (VIP-ergic nerves) are located in the posterior third of the ciliary muscle. Furthermore, it has been reported that electrical stimulation of adrenergic nerves apparently has no VIP-releasing effect, while cholinergic stimulation (vagal and pelvic nerves) leads to a significant increase in the release of VIP. Thus, VIP-related nerves seem to function under conditions of a particular relation to the cholinergic nervous system, although exogenous VIP may differ in action from acetylcholine.

We found that the bovine ciliary muscle is innervated by the cholinergic system. VIP potentiated the contractile response of ciliary muscle elicited by nerve stimulation, and this enhanced contraction was abolished by atropine. As adrenergic agonists and blockers did not alter these effects, VIP probably plays a facilitative role in cholinergic neuromuscular transmission.

The tension of the bovine choroidal strip with intrinsic nerves and without the ciliary muscle remained preserved, both before and after the application of VIP. Therefore, the possibility that the alterations were due to choroidal blood vessels can be excluded, although VIP does possess potent vasodilatory effects.

As the response to exogenous acetylcholine was not potentiated by VIP, this peptide does not increase the postsynaptic sensitivity to acetylcholine. Exogenous VIP alone did not have a direct effect on the muscle; therefore, all of our evidence taken together suggests...
that this peptide enhances the acetylcholine release from the nerve terminals and may be an excitatory modulator, rather than a neurotransmitter between nerve and muscle.

VIP generally inhibits the mechanical activity of smooth muscle,9–11 probably without affecting electrophysiologic properties of this muscle membrane.11 There is no evidence that VIP potentiates the neurotransmission prejunctionally in smooth muscle tissues, although this peptide does stimulate bicarbonate secretion of the pancreas, which is cholinergically innervated.15 On the other hand, in the canine ciliary muscle, VIP also enhanced the release of acetylcholine (unpublished data).

Thus, while SP has a direct effect on the iris,3 VIP seems to potentiate the release of acetylcholine in the ciliary muscle tissue.

Key words: VIP, cholinergic transmission, bovine ciliary muscle, accommodation.

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From the Department of Ophthalmology, Yamaguchi University School of Medicine, Kogushi 1144, Ube 755, Japan. Submitted for publication February 18, 1982. Reprint requests: Ryo Suzuki, MD, Department of Ophthalmology, Yamaguchi University School of Medicine, Kogushi 1144, Ube 755, Japan.

References


Enzymatic Disaggregation of the Infected Rat Cornea

Paul R. Badenoch, John J. Finlay-Jones,* and Douglas J. Coster

Analysis of cell populations in the cornea may be performed rapidly and accurately employing the technique of enzymatic disaggregation. To illustrate this method normal rat corneas and corneas infected 24 and 48 hours previously with Staphylococcus aureus were disaggregated in a solution containing pancreatin and collagenase. The cells released were counted and identified morphologically. These results were compared to cell counts made from histologic sections. Over 95% of the corneal cells were viable after the disaggregation and leukocytes obtained from the infected corneas retained their phagocytic capacity. This approach allows sensitive analysis of cell populations in a wide range of corneal conditions, including infection and allograft rejection. Invest Ophthalmol Vis Sci 24:253–257, 1983.

The potential for corneal destruction resides in the invading cells such as neutrophils in microbial keratitis1 and lymphocytes in allograft rejection.2 The analysis of the cell population in diseased corneas is essential for the understanding of these and other pathologic processes.

Published techniques for the quantitation of the cellular component of corneal inflammation often