Vasoactive Intestinal Polypeptide and the Innervation of the Human Lacrimal Gland

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• **Vasoactive intestinal polypeptide (VIP) is a biologically active neuropeptide found in both the peripheral and the central nervous systems. Previous studies have shown that VIP-like immunoreactive nerves are present in the uveal tissues of the human eye. The distribution of VIP-like immunoreactivity of the human lacrimal gland and sphenopalatine ganglion was studied. A lacy network of VIP-like immunoreactive nerve fibers was found in the lacrimal gland and was predominantly located along the basilar surface of the acinar epithelium and in the interstitial connective tissue of the gland. This pattern of innervation was nearly identical to the distribution of cholinesterase-positive fibers in human lacrimal glands. The VIP-like immunoreactive cell bodies were found throughout the sphenopalatine ganglion obtained at autopsy. The distribution of VIP-like immunoreactive nerves in the human lacrimal gland and sphenopalatine ganglion was generally similar to that described in mammalian and avian systems, although some differences were noted. Vasoactive intestinal polypeptide may represent an important cotransmitter or neuromodulator for the facial parasympathetic nerves that supply the eye and the lacrimal gland.**

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Vasoactive intestinal polypeptide (VIP) is a biologically active neuropeptide found in the peripheral nerves of the gut, exocrine glands, blood vessels, and in the perikarya of some autonomic ganglia. Vasoactive intestinal polypeptide may have several functions, including vasodilation, relaxation of smooth muscle, and stimulation of exocrine secretion. The theory that VIP may function as a neurotransmitter is based on its localization in neurons and synaptic vesicles and its release after neural stimulation; furthermore, exogenous application of VIP mimics neural stimulation.[1-3] Since previous animal studies have shown that the VIP may play an important role in exocrine secretion, we carried out immunocytochemical studies to determine if VIP is also present in the nerve fibers of the human lacrimal gland and neurons of the sphenopalatine ganglion.

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MATERIALS AND METHODS

Sphenopalatine ganglia were obtained at autopsy. After the brain was removed using standard neuropathological techniques, the orbital roof, the soft tissues of the orbital apex, and the roof of the foramen rotundum were removed, exposing the orbital floor and pterygopalatine fossa. The contents of the pterygopalatine fossa were excised en bloc with an oscillating saw. Using a binocular microscope, the sphenopalatine ganglion was dissected from the bone. The lacrimal gland was excised through the roof of the orbit.

Ten lacrimal glands and sphenopalatine ganglia were obtained from six men and four women (age range, 40 to 73 years). Cause of death included ovarian carcinoma, brain metastasis, lung cancer, breast cancer, cardiomyopathy, sepsis, encephalitis, and cardiac arrest. Eight of the patients had no history of neurological dysfunction; two patients with neurological impairment had no premortem history of ocular or lacrimal abnormalities, dysautonomia, or peripheral sensory disturbances. All tissues were obtained within 24 hours after the time of death; in six cases, tissue was retrieved within three to 12 hours of death. Two additional specimens of the lacrimal gland were obtained following orbital exenteration, performed in one case for a choroidal melanoma with extrascleral intraconal extension, and in the other case for a sebaceous cell carcinoma of the lower eyelid that had spread to the inferior orbit. All specimens were obtained only after appropriate informed consent was obtained for unrestricted autopsy and orbital exenteration, respectively.

Specimens were immersion fixed in 4% phosphate-buffered paraformaldehyde (pH, 7.4) with 0.1 mol/L of sodium periodate and 0.1 mol/L of lysine (PLP) at 4°C. The effects of fixation time were studied in several specimens by fixing portions of the same tissue block for variable periods. Postmortem specimens were fixed for 4, 8, and 24 hours; postsurgical specimens were fixed for two and four hours. Tissue specimens were then washed overnight in 30% sucrose-0.1 mol/L of phosphate-buffered saline solution (PBSS) at 4°C. Frozen sections (12 μm) of the ganglion and the gland were thaw-mounted on gelatin-coated slides, dried at room temperature, and then stored at −20°C.

Two immunohistochemical methods were employed: fluorescein isothiocyanate (FITC) and avidin-biotin immunoperoxidase.

Polyclonal rabbit antiserum to porcine VIP (Immunonuclear Corp, Stillwater, Minn) was diluted 1:1000 in PBSS and 0.25% Triton X-100 (Sigma, St Louis). In a moist chamber, thawed tissue sections were incubated with VIP antiserum overnight at room temperature. After washing with 1% bovine serum albumin in PBSS for one hour, sections were coated with the secondary antisera (goat anti-rabbit IgG, Boehringer-Mannheim, Indianapolis) that were conjugated to FITC for one hour, washed in PBSS, and then coverslipped using glycerol buffer media.

A commercially available immunoperoxidase avidin-biotin method (Vectastain ABC Kit, Vector Laboratories, Burlingame, Calif) was also used. Endogenous peroxidase activity was quenched for ten minutes in 0.3% hydrogen peroxide in methyl alcohol and then washed with PBSS. Tissue sections were then incubated in a moist chamber with VIP antiserum (1:1000 in PBSS-0.25% Triton X-100) for 24 hours and then washed in PBSS. Sections were exposed to biotinylated affinity-purified, rabbit anti-immunoglobulin solution for two hours and washed again in PBSS. Avidin and biotinylated horseradish peroxidase (Vectastain ABC reagent) was applied to the tissue sections for two hours. After washing, sections were incubated in a freshly made solution that consisted of equal volumes of 0.02% hydrogen peroxide (pH, 7.2) in distilled water and 0.1% diaminobenzidine in 0.1 mol/L of TRIS (pH of 7.2) for up to four to five minutes and then washed in PBSS. Sections were dried and then coverslipped with mounting medium (Pro-Texx, American Scientific, Chicago).

Two controls were used with both immunohistochemical methods. In the first control, primary antiserum was reacted overnight with an excess of the pure VIP (10^{-6} mol/L) before being applied to the tissue sections. In the second control, secondary antiserum was applied without primary antiserum. Both controls were simulta-

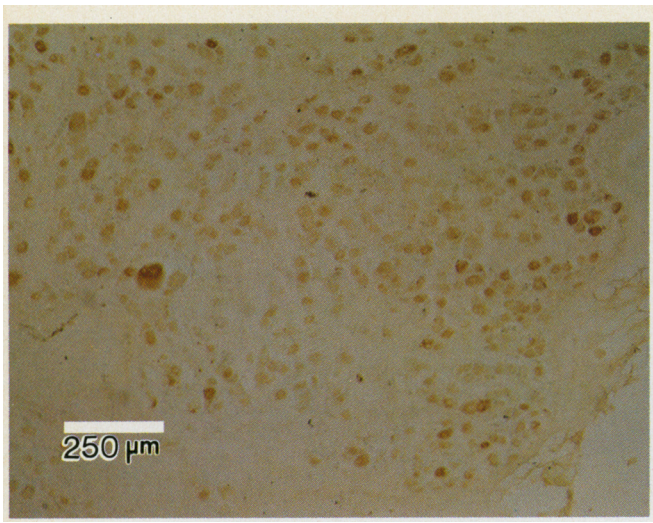


Figure 1.

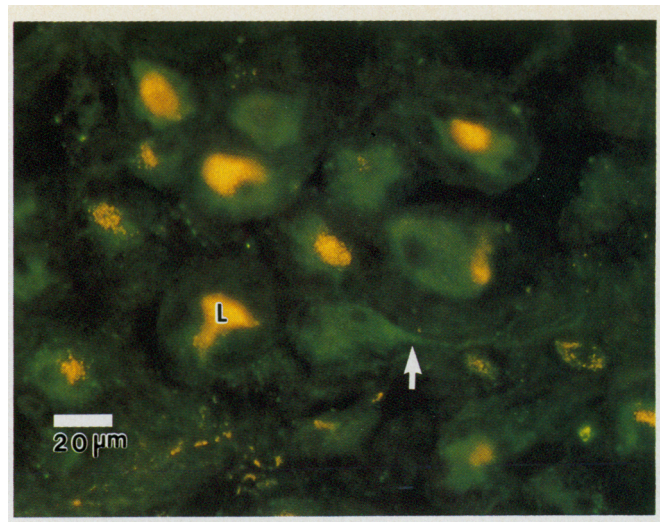
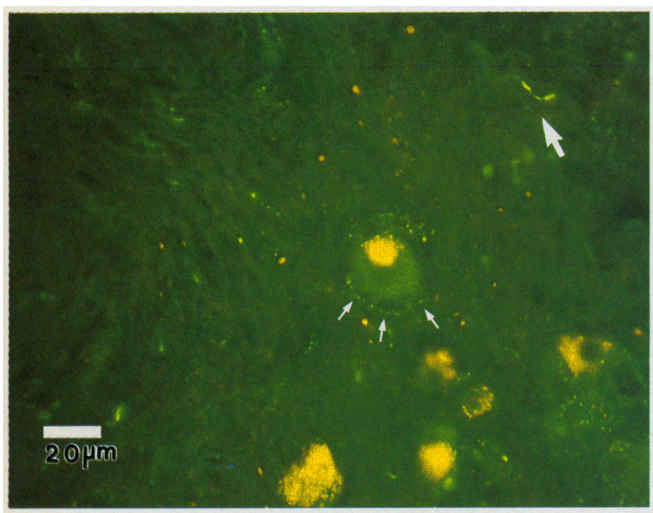


Figure 2.



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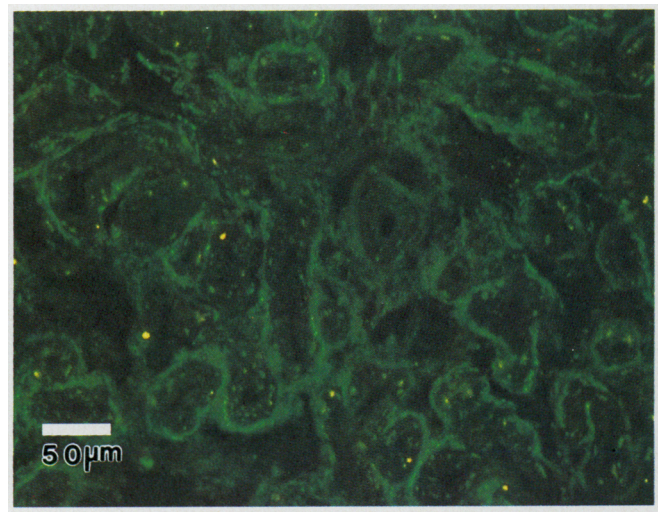
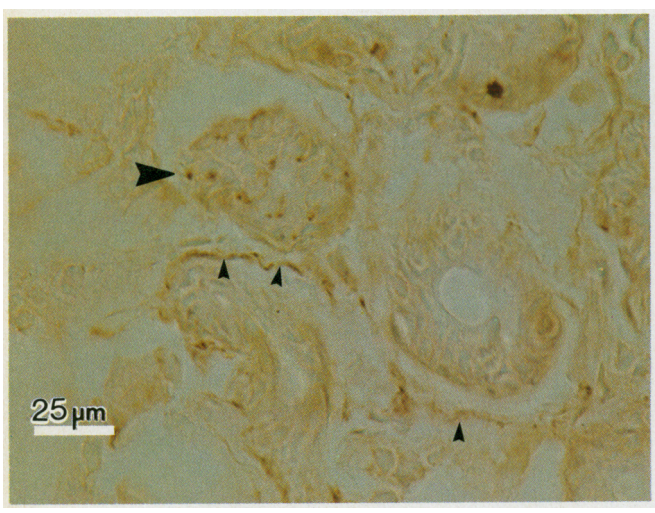


Figure 4.



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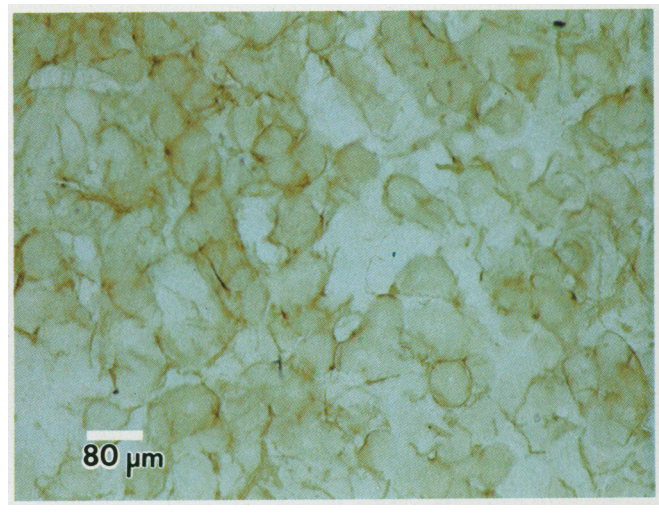

Figure 6.

neously processed with all experimental reactions. The VIP-like immunoreactive specificity was considered positive only if controls exhibited an absence of labeling in the presence of immunoreactivity in the experimental tissue sections. Slides were viewed and photographed with an epifluorescence compound microscope (Zeiss).

Fragments of the postsurgical lacrimal glands were also studied for the presence of acetylcholinesterase using the Coupland-Holmes technique.[4]

RESULTS

The majority of cells within the sphenopalatine ganglion exhibited a variable degree of intracytoplasmic VIP-like immunoreactivity (VIP-LI) that occasionally extended from the perikarya into cell processes (Figs 1 and 2). Some of the sphenopalatine ganglion cells also showed a beaded halo of VIP-LI around the perikarya (Fig 3). Varicose VIP-LI nerve fibers were present throughout the ganglion.

Fig 1.—Numerous vasoactive intestinal polypeptide-like immunoreactive cell bodies in human sphenopalatine ganglion (immunoperoxidase, original magnification ×60).

Fig 2.—Vasoactive intestinal polypeptide-like immunoreactive neurons (fluorescein isothiocyanate, green) in human sphenopalatine ganglion. One cell (arrow) shows label extending out along cell process (×400). Yellow autofluorescence labeled L is lipofuscin (see "Results" section).

Fig 3.—Fluorescein isothiocyanate-labeled vasoactive intestinal polypeptide (VIP)-like immunoreactive cell body from human sphenopalatine ganglion demonstrating beaded halo of VIP-like immunoreactivity on its outer surface (small arrows). Isolated VIP-like immunoreactive fiber shown by large arrow (×400).

Fig 4.—Fluorescein isothiocyanate-labeled human lacrimal gland shows lacy network of vasoactive intestinal polypeptide-like immunoreactive nerve fibers along basilar surface of epithelial acini and terminal tubule (×200).

Fig 5.—Several lacrimal acini. Vasoactive intestinal polypeptide-like immunoreactive fiber is shown in fibrous interstitium (small arrowheads). Acinus (large arrowhead) displays number of punctate vasoactive intestinal polypeptide-like immunoreactive deposits that appear to be located in intercellular spaces between epithelial cells (see "Results" section) (immunoperoxidase, original magnification ×400).

Fig 6.—Human lacrimal gland showing cholinesterase-positive fibers surrounding many epithelial acini. Note similarities in location between these fibers and vasoactive intestinal polypeptide-like immunoreactive nerve fibers shown in Fig 4 (acetylcholinesterase, original magnification ×100).

Both FITC and immunoperoxidase techniques were equally sensitive in detecting VIP-LI in the sphenopalatine ganglion, although tissue preservation, morphology, and immunoreactivity varied considerably among patients. Those specimens obtained within 12 hours of death (fixed with PLP for four or eight hours) yielded more immunoreactivity than those obtained between 12 and 24 hours post mortem. Many cells in the ganglion displayed an intense yellow autofluorescence caused by a degenerative intracellular pigment (lipofuscin). The autofluorescence of lipofuscin was easily distinguished from the green immunofluorescence (Fig 2).

With both FITC and immunoperoxidase methods, VIP-LI fibers were present throughout the postsurgical specimens of the lacrimal gland; however, immunoreactive nerve fibers were more easily identified in the FITC-processed glands. The lacrimal gland displayed a fine lacy network of VIP-LI varicose nerve fibers that surrounded the basilar surface of the acinar epithelium (Fig 4). Their position relative to the basement membrane could not be determined in most cases; however, punctate immunoreactive deposits, presumably representing terminal axonal swellings, were occasionally observed in the intercellular spaces between epithelial cells (Fig 5). The VIP-LI nerve fibers were also evident in the interstitial connective tissue of the gland, but these fibers were rarely observed to be surrounding blood vessels. Nerve fibers could sometimes be followed from the fibrous interstitium into the periacinar regions of the gland. Occasionally, VIP-LI fibers were present along small segments of the terminal tubules (Fig 4).

The postsurgical lacrimal glands exhibited intense VIP-LI, especially those specimens immersed in fixative for brief periods (two hours). In contrast, we were unable to demonstrate reliably VIP-LI fibers in most of the lacrimal glands that were obtained post mortem, although several specimens contained scattered, isolated VIP-LI fibers around some of the epithelial acini. The postmortem lacrimal glands appeared to undergo a greater degree of autolysis than the ganglia or the postsurgical glands.

The distribution of cholinesterase-positive fibers was similar to the distribution of VIP-LI nerve fibers in the gland. These fibers formed a dense network in the fibrous interstitium and in regions that surrounded epithelial acini (Fig 6).

COMMENT

Radioimmunoassay and immunohistochemical studies carried out in chickens,[5] rats,[6,7] rabbits,[8] cats,[7,9,10] guinea pigs,[6,7] and nonhuman primates[7] have shown VIP-LI fibers in the harderian gland, lacrimal gland, choroid, ciliary body, and iris. In the mammalian lacrimal gland and avian harderian gland, VIP-LI fibers are located along the basilar surface of the acinar epithelial cells, in the fibrous interstitium, and along blood vessels. The density of lacrimal VIP-LI nerve fibers at different locations may vary among different species. For example, avian glands exhibit a large number of VIP-LI nerve fibers in the fibrous interstitium along ducts and blood vessels,[5] whereas rodents display VIP-LI fibers that predominantly surround epithelial acini.[7] Most of the cell bodies in the feline pterygopalatine ganglion display VIP-LI.[1,10,11] Several groups[12-14] have reported that VIP-LI nerve fibers are also present in human eyes obtained both at autopsy[13,14] and following enucleation.[12,13] The VIP-LI fibers can be found in the choroid, iris, ciliary body, and trabecular meshwork. The present study extends these observations in humans by showing VIP-LI fibers in the lacrimal gland and neurons of the sphenopalatine ganglion.

Although the neurons of the brainstem reticular formation that are specifically responsible for lacrimation have not been clearly distinguished from those neurons that subserve nasal secretion and salivation, it is clear that parasympathetic preganglionic fibers exit the brain stem with the facial nerve in the nervus intermedius. These fibers travel with the greater superficial petrosal and vidian nerves to synapse on cell bodies of the sphenopalatine ganglion. Contrary to the commonly held view that postganglionic parasympathetic fibers follow the zygomatic and lacrimal nerve to the lacrimal gland, Ruskell[15-18] has shown that postganglionic fibers pass directly through the rami orbitales, to the lacrimal gland, the choroidal vasculature, and the anterior uvea.[19]

The results of the present study confirm earlier reports that have described the distribution of cholinesterase-positive fibers in the human lacrimal gland.[20,21] We also found that the preponderance of *both* cholinesterase-positive fibers and VIP-LI fibers are found along the bases of the epithelial acini and in the fibrous interstitium. The similarities in the distribution of the facial parasympa-

thetic and VIP-LI innervation of the uvea, lacrimal gland, and sphenopalatine ganglion are consistent with studies showing that VIP colocalizes with acetylcholine in axon terminals and cell bodies.[9,22,23] While sympathectomy has no effect on the presence of VIP-LI, ablation of the sphenopalatine ganglion completely eliminates *both* cholinergic and VIP-LI fibers in the lacrimal gland and choroid.[8,10,11,24,25] Physiological studies have shown that VIP can stimulate lacrimal protein secretion from lacrimal gland fragments—an effect that is atropine resistant and additive to that of carbachol.[26] Lundberg et al[22] have suggested that VIP may, by vasodilation, potentiate salivary gland fluid secretion induced by acetylcholine. Thus, it is possible that VIP represents a cotransmitter for the cholinergic neurons of the sphenopalatine ganglion.

The distribution of orbital and ocular VIP-LI in humans is, in most respects, similar to its distribution in mammalian species, although there are several important differences. The first difference is that perivascular VIP-LI fibers are distinctly uncommon in the human lacrimal gland. It is possible that VIP, in humans, stimulates lacrimation by acting directly on the epithelial acini as suggested by Dartt et al.[26] However, a sampling error cannot be ruled out in view of the small number of surgical specimens studied. The second feature, not previously observed in animal studies, is the presence of immunoreactive halos that surround some of the perikarya. It is unclear if these immunoreactive halos represent (preganglionic?) axonal terminations, sequestered intracellular VIP, or bystanding immunoreactive nerve fibers.

The use of postmortem human tissue inevitably raises questions concerning the suitability of this material for neuropeptide studies. The degree to which premortem factors such as age, cause of death, medications, and postmortem changes from autolysis, fixation time, and neuropeptide degradation interfere with the detection of VIP-LI is still unknown. Nonetheless, radioimmunoassay studies indicate that some neuropeptides are relatively stable post mortem.[27-29] Moreover, several studies using immunocytochemical techniques have detected VIP-LI nerve fibers in eyeballs obtained at autopsy.[13,14] The present study shows that VIP-LI cell bodies can be demonstrated in postmortem human sphenopalatine ganglia, particularly when they are fixed within 12 hours of death; however, the longer the postmortem interval, the greater the degree of autolysis in the ganglion. In comparison, the lacrimal gland seems to autolyze much more rapidly than the ganglion, which probably explains why VIP-LI nerve fibers were best demonstrated in postsurgical specimens. The fact that the lacrimal gland autolyzes more rapidly than the ganglion may be due to high levels of certain degradative enzymes in the lacrimal gland (eg, lysozyme and various proteases) and to the relative absence of such enzymes in neural tissue.

The functional implications of VIP-LI nerves in the human lacrimal gland and sphenopalatine ganglion are presently unknown; however, preliminary studies in rabbits that have shown that topically administered VIP stimulates tear secretion[23] suggest that VIP or an analogue may, in the future, have a therapeutic role in the treatment of various dry-eye syndromes.

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