Neuropeptide Y-like Immunoreactivity Localizes to Preganglionic Axon Terminals in the Rhesus Monkey Ciliary Ganglion

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PURPOSE. To characterize neuropeptide distribution in the ciliary ganglion of rhesus monkeys (Macaca mulatta).

METHODS. Cryostat tissue sections of fixed rhesus monkey ciliary, pterygopalatine, superior cervical, and trigeminal ganglia were incubated with antisera to neuropeptide Y (NPY), calcitonin gene-related peptide (CGRP), substance P (SP), vasoactive intestinal peptide (VIP), tyrosine hydroxylase (TH), and dopamine-β-hydroxylase (DBH). Antibody binding was visualized by indirect immunofluorescence.

RESULTS. NPY-like immunoreactive (LI) nerve terminals surrounded 80% of ciliary ganglion cells, but ciliary ganglion cell somata were unstained. NPY-LI cells were present in the superior cervical ganglion, in which almost all cells were TH- and DBH-LI, and in the pterygopalatine ganglion, in which almost all cells were VIP-LI. Because neither TH, DBH, nor VIP immunoreactivity was detected in nerves contacting ciliary ganglion cells, the NPY-LI input to ciliary neurons does not likely derive from the autonomic ganglia. The trigeminal ganglion, another potential source, had no NPY-LI neurons. CGRP- and SP-LI axons from the nasociliary nerve traversed the ciliary ganglion; a small number of varicose axons were distributed among ganglion cells and rarely surrounded cell somata. Most ciliary ganglion cells were TH-LI, but only a few were DBH-LI.

CONCLUSIONS. Based on these patterns of peptide immunoreactivities, the NPY-LI nerve fibers investing ciliary ganglion cells in the rhesus monkey are most likely preganglionic axon terminals of mesencephalic parasympathetic neurons. Although the origin and function of these NPY-LI nerves remains to be established, the present finding adds to the remarkable diversity of neuropeptide immunoreactivity so far identified in preganglionic and postganglionic cells of the ciliary ganglion in different species of birds and mammals, including primates. (Invest Ophthalmol Vis Sci. 1998;39:227-232)
Animals in Ophthalmic and Vision Research. While under deep general anesthesia, all animals were perfused transcardially after perfusion, were immersed either in 4% paraformaldehyde in 0.1 M phosphate buffer; the eyes and brains, used by other investigators for unrelated experiments, were unavailable for study. The ganglia, removed approximately 16 to 20 hours after perfusion, were immersed either in 4% paraformaldehyde solution for 24 hours or in Zamboni’s solution for 24 or 48 hours and then were transferred to 0.1 M phosphate buffer containing 30% sucrose for 24 hours. Cryostat tissue sections, 16- to 20-µm thick, were cut and thaw mounted on gelatin-coated slides.

Tissue sections were incubated with one of three different rabbit polyclonal antisera raised against porcine NPY (Incstar, Stillwater, MN; Peninsula Laboratories, Belmont, CA; Affiniti Research Products, Manhead, Exeter, UK) and diluted 1:500 in 0.05 M phosphate-buffered saline containing 0.3% Triton X-100. All three NPY antisera showed the same quality and occlusion of the eye (four animals) from shortly after birth; one mature 18-year-old monkey was not part of any ocular study. This investigation adhered to the tenets of the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research. While under deep general anesthesia, all animals were perfused transcardially with 0.9% NaCl solution followed by 4% paraformaldehyde in 0.1 M phosphate buffer; the eyes and brains, used by other investigators for unrelated experiments, were unavailable for study. The ganglia, removed approximately 16 to 20 hours after perfusion, were immersed either in 4% paraformaldehyde solution for 24 hours or in Zamboni’s solution for 24 or 48 hours and then were transferred to 0.1 M phosphate buffer containing 30% sucrose for 24 hours. Cryostat tissue sections, 16- to 20-µm thick, were cut and thaw mounted on gelatin-coated slides.

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The proportion of ciliary ganglion cells surrounded by NPY-LI cell processes was estimated from counts of all somata receiving labeled and unlabeled inputs in a series of non-overlapping photographs of a single section from each ganglion. Approximately 250 cells were counted in each section. Except where specifically noted, the observations described are based on examinations of ganglia ipsilateral to the control phakic or nonoccluded eyes.

RESULTS

The ciliary ganglia in this study showed the same anatomic features and neural connections described previously for rhesus monkeys. Preganglionic parasympathetic nerves enter the ganglion at its attachment to the oculomotor nerve at the origin of the branch to the inferior oblique muscle. The ganglion also is joined by two or three fine branches of the nasociliary nerve carrying both sensory and sympathetic axons that are thought to traverse the ganglion without synapse and to exit with postganglionic parasympathetic axons in the multiple ciliary nerves supplying the eye. Many fine nerves (rami orbitales) originating from the pterygopalatine ganglion lie in proximity to the ciliary ganglion and could join the ganglion.

In all ciliary ganglia examined, the majority of cell somata were surrounded by a dense array of NPY-LI nerve processes. Tangential sections passing through the perisomatic region showed that the NPY-LI processes consisted of fine fibers often terminating in bulbous expansions. Some smooth nerve fibers coursing among the ganglion cells also were positively stained. All ganglion cell somata were NPY negative. The proportion of ciliary neurons surrounded by NPY-LI nerve processes averaged 80% (range, 55%-96%) in ganglia innervating the untreated eyes of the nine young monkeys; counts from three ganglia innervating aphakic eyes gave the same value (average, 80%; range, 66%-93%). In the two ganglia from one untreated mature monkey, the proportions were 66% and 76%. NPY immunoreactivity was not detected in cross-sections of the inferior ramus of the oculomotor nerve or in longitudinal sections of the parasympathetic root supplying the ganglion.

The localization of NPY immunoreactivity to nerve processes surrounding ciliary ganglion cells contrasts notably with its localization in the other ganglia supplying the rhesus monkey eye. We found NPY immunoreactivity in the postganglionic neurons and not in preganglionic processes of both the sympathetic superior cervical ganglion and the parasympathetic pterygopalatine ganglion. These observations are fully consistent with other reports. In the superior cervical ganglion, approximately half of the cell somata were NPY-LI; almost all cells were TH positive (Figs. 2A, 2B) and DBH positive (data not shown). In the pterygopalatine ganglion, approximately half of the cells were NPY-LI and almost all cells were VIP-LI (Figs. 2C, 2D). Neuropeptide Y immunoreactivity was not detected in sensory neurons of the trigeminal ganglion, but CGRP-LI and SP-LI cells were present (data not shown).

In the ciliary ganglion, the NPY-LI nerve processes surrounding ganglion cells were not immunoreactive for TH or DBH, the adrenergic marker enzymes that colocalize with NPY in postganglionic sympathetic neurons (Fig. 3). However, almost all the cholinergic ciliary ganglion cells (>90%) displayed TH immunoreactivity (Fig. 3A), and rare ciliary ganglion cells (<1%) were immunoreactive for DBH (Figs. 3B, 3C). Small bundles of NPY-, TH-, or DBH-LI nerve fibers, presumably postganglionic sympathetic axons, were present in nasociliary nerve branches entering the ganglion.
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**FIGURE 1.** Neuropeptide Y (NPY) immunoreactivity in the ciliary ganglion. (A) Most ganglion cell somata are surrounded by NPY-like immunoreactive (LI) nerve processes. Where the perisomatic region is sectioned tangentially, a complex array of stained fibers and bulbous profiles is visible (arrow). Small granules in the ganglion cell nuclei are nonspecifically stained. (B) A small proportion of ciliary neurons scattered throughout the ganglion is not invested with NPY-LI nerve processes (arrows). Some thick immunoreactive nerve fibers (arrowheads) course within the ganglion. (C) In sections incubated with preabsorbed antiserum, staining of nerve processes is absent, but nonspecific staining of granular structures in the cell nuclei persists (arrows). Magnification bar, 50 μm.

VIP immunoreactivity, which coexists with NPY immunoreactivity in pterygopalatine ganglion cells, was not detected in any neurons of the ciliary ganglia (data not shown). VIP-LI varicose nerve fibers, always seen around adjacent blood vessels, provided evidence of effective immunohistochemical staining.

CGRP and SP immunoreactivity, identified in sensory neurons of the trigeminal ganglion, localized to axons of nasociliary nerve branches joining the ciliary ganglion and in bundles of axons traversing the ganglion (Fig. 4). In addition, a small number of isolated varicose nerve fibers immunoreactive to either peptide were diffusely distributed throughout the ganglion; rarely, these varicose fibers partially or completely surrounded a ciliary ganglion cell (Fig. 4). No ciliary ganglion cell somata stained positively for either CGRP or SP.

**DISCUSSION**

The present results demonstrate dense networks of NPY-LI nerve fibers surrounding approximately 80% of rhesus monkey ciliary ganglion cells. Unilateral aphakia or occlusion of the eye in the young monkeys studied did not cause a difference in the distribution of NPY immunoreactivity between ipsilateral and contralateral ciliary ganglia. Demonstration of the same immunohistochemical staining pattern in the ciliary ganglia of an untreated monkey indicates that the unilateral ocular procedures in the experimental monkeys did not induce expression of NPY-like immunoreactivity in both ganglia.

The distribution of NPY-LI nerve fibers, clustered densely around most ciliary ganglion cell somata, suggests they are terminals of preganglionic parasympathetic neurons located in the mesencephalon. NPY-LI axons were not detected in the oculomotor nerve supplying the ganglion, but it is possible that the amount of peptide in preterminal axons is below the level detectable with immunohistochemical analysis. In support of a mesencephalic origin, the NPY-positive nerves investing ciliary ganglion cells clearly differ from NPY-positive nerves that join or potentially join the ganglion through connections from the superior cervical, pterygopalatine, or trigeminal ganglia. Superior cervical ganglion cells immunoreactive for NPY are also immunoreactive for TH and DBH, enzymes present in all postganglionic sympathetic neurons. Although bundles of sympathetic axons joining the ciliary ganglion are NPY-, TH-, and DBH-LI and many ciliary ganglion cell somata are TH-positive, the NPY-LI nerves surrounding ciliary ganglion cells are TH and DBH negative. Many pterygopalatine neurons also are NPY-LI, but almost all are immunoreactive for VIP, indicating extensive, if not complete, coexistence of the two immunoreactivities in these pterygopalatine neurons. Because we found no VIP immunoreactivity in the ciliary ganglion, a major projection of NPY-positive nerves from the pterygopalatine ganglion to ciliary ganglion cells seems unlikely. No NPY immunoreactivity...
FIGURE 2. Immunoreactivity to neuropeptide Y (NPY) and other markers in the superior cervical ganglion (SCG) and pterygopalatine ganglion (PPG). (A) In the SCG, NPY-like immunoreactive (LI) granules are present in the perikaryal cytoplasm of approximately 50% of the sympathetic ganglion cells, and (B) almost all cells are tyrosine hydroxylase (TH) positive. (C) NPY-LI granules are present in approximately half of the parasympathetic ganglion cells of the PPG, whereas (D) approximately 90% of the cells demonstrate varying levels of vasoactive intestinal peptide (VIP) immunoreactivity. Magnification bar, 50 μm.

FIGURE 3. Tyrosine hydroxylase (TH) and dopamine-β-hydroxylase (DBH) immunoreactivity in the ciliary ganglion. (A) Nearly all ciliary ganglion cells are TH positive with staining varying from bright to dim. Rare, dim cells were considered negative. No staining is evident in the perisomatic nerve processes, although many TH-positive smooth nerve fibers course between the cell somata. (B, C) DBH immunoreactivity is detected only in a few scattered ganglion cells. Magnification bar, 50 μm.
was identified in sensory neurons of the trigeminal ganglion in these rhesus monkeys, a finding consistent with observations of others in cynomolgus monkeys and in the Japanese macaque and excluding a sensory origin for the NPY-positive terminals in the ciliary ganglion.

A major NPY-LI preganglionic input has not been identified in previous immunohistochemical studies of the ciliary ganglion of any species, including that of cynomolgus monkey and humans. In cynomolgus monkeys, however, van der Werf observed NPY-LI nerve fibers surrounding some ciliary ganglion cells and assumed that they originated from the ptg-galapatic ganglion; an alternative origin from preganglionic mesencephalic neurons seems equally possible based on the present results in rhesus monkey. NPY-LI nerve fibers in the human ciliary ganglion are extremely rare and are not in contact with ciliary ganglion neurons. Besides NPY immunoreactivity, other differences are evident between primates in the immunohistochemistry of the ciliary ganglion with respect to both neural somata and nerve fiber staining. Although one study reported SP immunoreactivity in approximately 60% and 40% of ciliary neurons of the cynomolgus monkey and the cat, respectively, this localization had not been detected in either animal by other investigators. Approximately 30% of ciliary ganglion cell somata have been reported to be VIP-LI in the cynomolgus monkey. In comparison, cell somata in the ciliary ganglion of rhesus monkeys and humans are negative for all neuropeptides examined, including SP, VIP, CGRP, and NPY. One consistent characteristic of primates is the presence of many TH-positive–DBH-negative ciliary ganglion cells. In rhesomolagus, and Japanese macaque monkeys, these cells comprise 70% to 90% of the ganglion cell population; in the human ciliary ganglion, 25% of cell somata are TH positive and DBH negative. The function of TH in cholinergic ciliary neurons of primates and of other mammals remains unknown.

Differences also occur in the nerve fiber localization of neuropeptides within the ciliary ganglion. Although bundles of sensory CGRP- and SP-LI nerves from the nasociliary nerve enter and traverse the rhesus monkey ganglion, we rarely found CGRP- or SP-positive varicose axons surrounding ciliary neurons. In cynomolgus monkeys, SP- but not CGRP-LI varicose nerve fibers invest a small proportion of ciliary ganglion cells. In the human ciliary ganglion, 18% of cells are contacted by SP-LI varicose axons and 12% by CGRP-LI axons, almost all of which were also SP positive. Some of the sensory axons passing through the ciliary ganglion could give rise to the fine varicose nerves that contact the ganglion cells in these species; alternatively, the CGRP- and SP-LI perisomatic nerve fibers may originate from another source.

Immunohistochemical studies of neuropeptide distribution in the primate ciliary ganglion are still limited in number, and the discrepancies in results may be attributed in part to technical problems. The neuropeptides identified in neuronal elements of the ciliary ganglion in lower mammals, however, vary so markedly from one species to another that the observed diversity among primates may be valid. Although the distribution of specific neuropeptides in the preganglionic input to the ciliary ganglion of birds has been well demonstrated in both the ganglion and the Edinger-Westphal nucleus, neuropeptides in the mesencephalic neurons projecting to the ciliary ganglion of primates or other mammals have not been described. Localization of candidate neuropeptides, including NPY, in these neurons may clarify the functional organization of the autonomic pathway regulating pupillary movement and accommodation.

References


