Regulatory peptides in the eye

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The eye is designed to provide the retina with protection and nutrition as well as a focussed image. The present review concentrates on regulatory peptides in nerves to the supportive structures of the eye. The retina itself will be discussed but briefly. To understand the innervation of the eye requires an overview of ocular physiology and anatomy.

1. Structure of the eye

The eye is made up of three coats: a tough outer collagenous coat of cornea or sclera; an intermediate vascular coat, the uvea; and an inner epithelial coat, highly differentiated by region. The transparent and convex cornea not only contributes to the form of the eye but also serves as a powerful lens. A multicomponent tear film secreted by the lacrimal gland and by small glands in the eyelids wets and lubricates the corneal surface. A rich vascular system supplies the junction of cornea and sclera, the limbus, and provides nutrition to the cornea. Just deep to the corneo-scleral limbus are the filtration tissues responsible for the drainage of aqueous humor from the anterior chamber of the eye into the limbal blood vessels. The secretion of aqueous humor generates intraocular pressure and maintains the shape of the eye.

The densely pigmented middle or uveal coat contains many specialized structures. Anteriorly within the iris are the sphincter and dilator muscles to regulate pupillary diameter. Just behind the iris, the ciliary body contains the ciliary muscles and the ciliary processes. Acting through zonules connected to the equatorial region of the lens, the ciliary muscle controls accommodation. The innermost part of the ciliary body is thrown up into heavily vascularized, epithelial-lined radial projections termed ciliary processes; these secrete the aqueous humor. In combination, the secretion of aqueous humor by the ciliary processes and the resistance to its drainage in the chamber angle regulate the internal pressure of the eye. The aqueous humor also provides nutrition to the lens and cornea. Posteriorly, the uvea is called the choroid. A heavily vascularized tissue, it provides blood flow so vastly in excess of the nutritional requirement of the retina that it not only guarantees a wide margin of safety but also controls the retinal temperature within strict limits.

The third or epithelial layer of the eye has remarkable regional differences. Its two epithelial layers result from the invagination of the optic vesicle during embryogenesis. At the front of the eye, one layer differentiates into the iris dilator muscle cells; the other is heavily pigmented and lines the back surface of the iris. As the two cell layers continue back onto the ciliary body, they are responsible for secretion of aqueous humor. Thereafter, the outer cell layer continues as the retinal pigmented epithelium while the inner differentiates into the neurosensory retina. The retinal pigmented epithelium separates the choroidal circulation and the neurosensory retina. It provides nutrition to the outer retinal layers, mediates photoreceptor renewal and constitutes an essential part of the blood-retinal barrier. The mature retina contains three layers of neurons, the outermost having photosensitive outer segments. Signalling is influenced greatly by laterally integrated neurons in the middle layer, termed horizontal and amacrine cells. In keeping with the optical needs of the eye, all pigment cells, the pigmented epithelia as well as the melanocytes within the uveal coat, absorb light to reduce the random reflection and thus prevent glare.

II. Sources of peripheral innervation to the eye

The peripheral nerves to the eye derive from several sources. The parasympathetic innervation is the most complex. All postganglionic nerves of the ciliary ganglion provide the eye with important parasympathetic supply to the ciliary and iris muscles. The pterygopalatine ganglion, from which some postganglionic nerve fibers enter the orbit as rami orbitales, provides parasympathic innervation chiefly to the choroid[87]. In addition, microganglia and ganglion cells are scattered within the orbit and are even found occasionally within the eye itself[16,71,105,112]. The number and location of these neurons vary among species and even among individuals within a species, but they are located in such places as the third nerve near the ciliary ganglion, the optic nerve sheath, the ciliary nerves near their penetration into the sclera, the choroid and the iris[53,106]. Sympathetic nerves to the eye are supplied almost exclusively from the ipsilateral superior cervical ganglion. Sensory nerves to the eye derive from the trigeminal ganglion. Those from the first division enter the orbit within the ophthalmic nerve. The maxillary nerve also makes a contribution to the sensory component of the ciliary nerves in monkeys[86] and most likely in man as well[4].

III. Cholinergic innervation to the eye

The thiocholine technique for acetylcholinesterase[50], though indirect, remains the only histochemical method used to date for the demonstration of cholinergic nerves in the eye[24,59]. Because this technique demonstrates the enzyme that metabolizes acetylcholine instead of the neurotransmitter itself, the method is indirect. Immunohistochemical techniques using antisera against both choline acetyltransferase and acetylcholine itself have been developed and promise greater specificity, but these newer methods have not yet been applied successfully to peripheral ocular nerves. The issue of the specificity of the thiocholine technique is complex. Perhaps it is best simply to assert that it has proved to have practical value in ocular studies. Specifically, findings with it correlate well with physiological responses and biochemical measurements of choline acetyltransferase[72]. On this basis, it is believed to provide an accurate description of cholinergic innervation within the eye. Interestingly, the thiocholine technique works especially well in albino animals, particularly the albino rabbit. The presence of melanin in tissue sections tends to dampen the histochemical reaction for cholinesterase and thus limits the effectiveness of this method for pigmented eyes.

In the cornea, nerve bundles show acetylcholinesterase activity, chiefly in the middle and anterior stroma and in the basal layers of the epithelium where in favorable tissue section multiple fine beaded fibers are seen. With regard to the drainage angle for aqueous humor, the friability of cryostat-cut sections in this region makes it difficult to preserve tissue relationships. To add to the difficulty, considerable species variation has been observed in density of cholinergic nerves to this area[59]. In spite of these problems, in the chamber angle of rabbit for instance, a moderate number of cholinesterase reactive nerves have been seen. For monkey and cat, however, only occasional reactive fibers are visualized; no studies have been reported in man.

For the ciliary body, the issue of innervation of the ciliary muscle must be separated from that to the ciliary processes;

innervation of the former relates to accommodation; of the latter, to aqueous humor formation. For the ciliary muscle, the density of acetylcholinesterase enzyme is so great that individual nerve fibers can be distinguished only at short incubation times. For the ciliary processes, pigmentation is important. Cholinesterase positive nerve fibers are found in each and every ciliary process of the albino rabbit; but in pigmented rabbits, rats and monkeys a careful search must be undertaken to visualize a few nerve fibers in the same region. This apparent discrepancy most likely is explained by melanin dampening of the histochemical reaction rather than a true difference in innervation density.

For the iris the thiocholine technique reveals a rich network of cholinesterase positive nerve fibers in the sphincter muscle, a parallel to the ciliary muscle. In the dilator muscle, however, the reaction indicates far fewer nerve fibers but importantly a dual innervation to a tissue once presumed to be innervated exclusively by the sympathetic nerves.

In the choroid[24,42], histochemical studies have revealed cholinesterase positive nerve fibers surrounding large and medium sized arterioles. Nerve fibers also run within the stroma and on occasion are seen at the choriocapillaris.

IV. Adrenergic innervation to the eye

Of all the components of the ocular innervation, the adrenergic is the most extensively studied. Since the introduction of histofluorometric method for tissue localization of catecholamines[34], numerous reports have appeared on diverse species[25,26,59]. The adrenergic nerves within the eye derive from sympathetic nerve fibers originating in the ipsilateral superior cervical ganglion[30]. While variations among and even within species have been recorded, some general observations can be made.

As a rule innervation is plentiful to blood vessels within the eye, but the retina presents an exception. A dense adrenergic innervation to the central retinal artery drops markedly as the vessel enters the globe[57]. A few adrenergic autonomic nerve fibers are seen surrounding blood vessels on and near the optic nerve head within the eye. The rest of the retinal vasculature is devoid of adrenergic innervation[57]. Some have taken exception and dispute this observation[36], but the disagreement may reflect species differences.

In the ciliary body, the ciliary muscle has a modest adrenergic innervation that takes part in the relaxation of accommodation. Each ciliary process ordinarily contains a visible array of adrenergic nerves, providing the anatomical counterpart for the adrenergic sensitivity of its epithelial function. A sparse but definite innervation serves the junction of choroid and sclera at the lamina fusca.

In the iris, the radially directed dilator muscle is heavily innervated and readily responsive to catecholamines. As already noted, the thiocholine technique gives presumptive evidence for dual, albeit unbalanced, innervation. The scant adrenergic innervation to its antagonist, the pupillary sphincter, taken with the results of cholinesterase staining, thus indicates a dual and reciprocal adrenergic/cholinergic innervation to each of the intraocular muscles.

Surprisingly, melanocytes within the eye are innervated[29,44] although outside the eye this is a rarity. Especially on the anterior surface of the iris, melanocytes have a plentiful adrenergic innervation and are dependent on it for the maintenance of their pigment content. Iris tyrosinase activity drops within days of superior cervical denervation[58]. Alternatively, topical application of the α-adrenergic blocker thymoxamine but not the β-adrenergic blocker timolol leads to a pale iris in juvenile rabbits, an observation that confirms adrenergic dependency while defining an α-adrenergic receptor mechanism[77].

The density of adrenergic innervation to the aqueous humor outflow apparatus varies widely among species. It is generally plentiful in animals such as guinea pig and rabbit. In humans it is sparse but usually present in the young[25,26]. In most instances it cannot be seen in eyes from older individuals, but this observation must be qualified by an age-related increase in autofluorescence. If meshwork innervation truly is diminished or lacking in aged humans, the observation has potential importance in understanding the age-related occurrence of ocular hypertension and glaucoma.

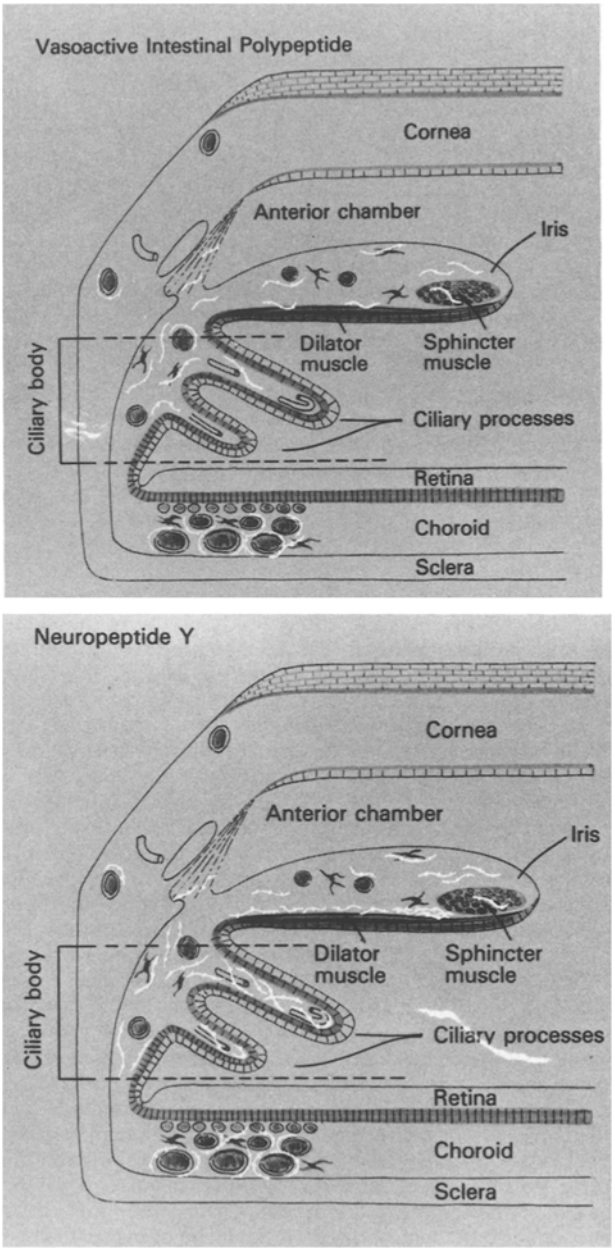

Figure 1. Schematic illustrations of the comparative distribution of two types of peptidergic nerve fibers in the human eye. For precise descriptions, see text and references. Top: Nerve fibers immunoreactive to vasoactive intestinal polypeptide represent a sub-population of parasympathetic nerve fibers. They occur with greatest density in the choroid but are also found more anteriorly in the eye. Bottom: Nerve fibers immunoreactive to neuropeptide Y, likely representing a substantial portion of sympathetic nerve fibers, are distributed differently within the eye. While also occurring in high density in the choroid, a richer anterior segment distribution is evident for nerve fibers containing this neuropeptide. Particularly prominent is the innervation to the ciliary processes and to the iris dilator muscle.

Distribution of peptide-containing peripheral nerves in the mammalian eye

Neuropeptide	Corneal innervation	Intraocular muscle innervation			Vascular and tissue innervation						
		Iris sphincter	Iris dilator	Ciliary muscle	Limbal blood vessel	Drainage angle	Iris blood vessels	Ciliary body blood vessels	Ciliary processes	Choroid	Melanocytes
VIP	0	+	+	+	++	+	+	++	+	+++	+
Neuropeptide Y	0	+	+++	++	++	+	+	++	+++	+++	+
Substance P	++	+	+	++	++	++	+	++	++	++	+
CGRP	++	+	±	++	++	+	+	++	++	++	+

The distribution of other neuropeptides found in peripheral ocular nerves, peptide histidine isoleucine, cholecystokinin and enkephalin, have not been described in sufficient detail to include in this summary. See text. Arbitrary of 0 to 3+, generalized from published reports.

Variability among species is even more pronounced for the adrenergic innervation of the cornea. In rabbit and guinea pig, the adult cornea harbors a moderate but predictable adrenergic innervation, most of it anteriorly disposed. In monkey and man, histochemically demonstrable adrenergic corneal innervation is for practical purposes absent; only rarely are nerve fibers seen and then mostly at the junction of cornea and sclera. Interestingly, a remarkable adrenergic innervation is found during embryonic development of the cornea in primates[31], suggesting that the rare nerve fiber visible in the adult is vestigial.

V. Neuropeptides localized to peripheral ocular nerves

Many neuropeptides have already been identified in ocular nerves by the use of immunohistochemical techniques. The potential for unrecognized cross-reactivity justifies the suffix 'like immunoreactive' (-LI) in describing peptidergic nerves; in a few instances, confirming biochemical isolation has been performed for the antigen in ocular nerves.

Because of the diversity of tissues and the regional differences in function within individual coats of the eye, highly detailed descriptions of the distribution of peptidergic nerves are required both for accuracy and for understanding (fig. 1). Among eyes of different mammalian species, the distribution of nerve fibers containing individual neurotransmitters and neuropeptides tends to be reasonably similar. The chief differences concern nerve density. The table contains a general summary of the distribution of peptidergic nerves most extensively studied to date in the mammalian eye. The precise details for particular species are provided in the cited immunohistochemical references.

Vasoactive intestinal polypeptide

Vasoactive intestinal polypeptide (VIP) was the first neuropeptide localized to ocular autonomic nerves[117], and ocular nerves containing this peptide are an important part of the parasympathetic system to the eye (figs 2 and 3). A rich VIP-LI innervation supplies ocular blood vessels, most prominently in the choroid[112,117,122]. More recently a non-vascular VIP-LI innervation has also been recognized as a forward extension with supply that includes the ciliary muscle, both iris muscles, and the chamber angle[71,90,95,105,123]. A pterygopalatine origin for choroidal vascular nerves has been established in rabbit, cat and monkey[87] and has been shown to be the source of the VIP-LI nerve fibers in the cat choroid[117]. VIP immunoreactivity also occurs in a few ciliary ganglion and accessory ciliary neurons of rat[53], and in uveal ganglion cells of guinea pig[112] and man[71,105]. Whether nerves supplying specific structures within the eye thus originate from one or more particular parasympathetic sources is not fully known. Nor is the matter readily clarified. An orbital parasympathetic denervation of the eye is complicated by the mixed nature and overlapping paths of many nerves in this region. For instance, sympathetic and sensory fibers pass through the ciliary ganglion in many species. While the rat is an exception to this generalization, the ciliary ganglion of this species contains only a limited proportion of the parasympathetic ganglion cells within the orbit[53]. Studies undertaken so far to ascertain the relative contribution and intra-

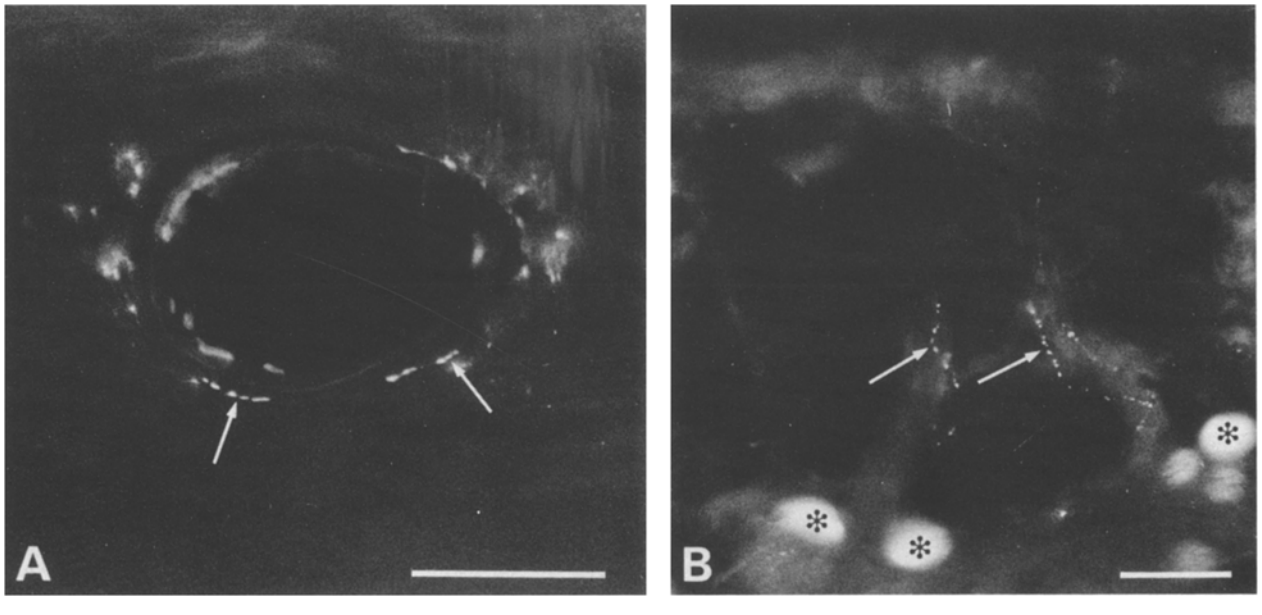

Figure 2. Vasoactive intestinal peptide-like immunoreactive nerve fibers in the corneo-scleral limbus. (Fluorescence micrographs; bars, 50 μm). *A* Immunoreactive nerve fibers (arrows) surround a superficial limbal blood vessel in the rhesus monkey eye. *B* Immunoreactive nerve fibers (arrows) lie in the cat ciliary cleft through which aqueous humor drains. Chromatophores (*) in the cat uvea show intense autofluorescence.

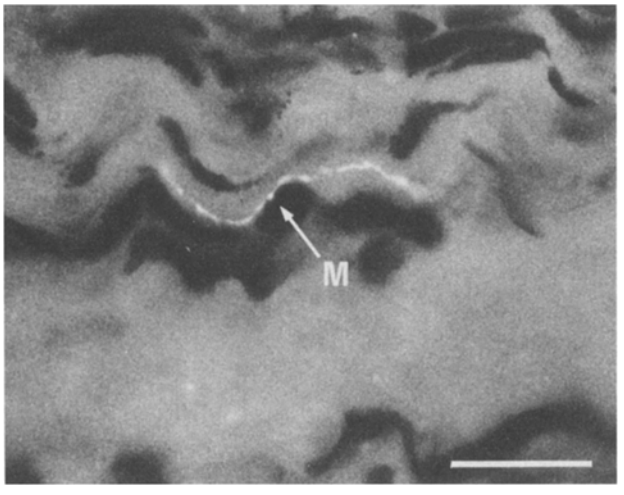

Figure 3. Nerve fibers immunoreactive to individual neuropeptides have been observed to drape around melanocytes in the uvea. Illustrated is a vasoactive intestinal polypeptide-like immunoreactive nerve fiber in apposition to a melanocyte (M) within the posterior ciliary body of the bovine eye (Fluorescence micrograph; bar, 25 μm).

ocular contribution from different parasympathetic sources by selective denervation have not yielded definitive results.

Peptide histidine isoleucine

Peptide histidine isoleucine (PHI) co-localizes with VIP in many peripheral autonomic neurons[7,33,84]. This neuropeptide has marked structural similarities to VIP and in fact derives from the same precursor molecule[109]. Immunoreactivity to PHI has been observed both in the guinea pig choroid[84] and in the rat iris[9]. In the rat iris, the distribution of PHI-LI nerve fibers closely parallels that of VIP-LI nerves, suggesting co-localization. No more detailed studies of the distribution of PHI in the eye have appeared to date.

Neuropeptide Y

A peptide of the pancreatic polypeptide family occurs in ocular nerves[14,45,96,102,110] and now has been identified biochemically as neuropeptide Y (NPY) in rat and guinea pig[104]. The distribution of nerves immunoreactive for NPY closely mirrors the distribution of sympathetic nerves containing catecholamines as revealed by histofluorometric methods (fig. 4). Notable differences include the absence of NPY from corneal nerves and a lower density of NPY-LI nerves in the ciliary body and around anterior uveal vessels. Following superior cervical ganglionectomy, most of NPY-LI ocular nerves disappear from the ipsilateral eye just as do the adrenergic[14,110], and radioimmunoassay levels of the peptide fall[1]. Unlike the observations with histofluorometric techniques, however, some choroidal nerve fibers persist[14,110]. Based on these observations, it is possible that a proportion of NPY-LI nerves originate elsewhere. As described below, however, superior cervical ganglionectomy induces non-sympathetic neurons with ocular projections to express a NPY-LI antigen[11]; on this basis, the few NPY-LI nerve fibers observed shortly after sympathectomy could as well represent the initial steps in this repair process.

Neuropeptides in ocular sensory nerves

Three neuropeptides have been identified to date in ocular sensory nerves originating in the trigeminal ganglion (figs 5 and 6): substance P (SP)[70,98,103,112-116], calcitonin gene-related peptide (CGRP)[62,67,101,111] and a peptide of the cholecystokinin-gastrin (CCK) family[9,54,81,100]. Only for substance P has biochemical identification been performed[94]. Whether the newly discovered tachykinin, substance K[65], also occurs in ocular sensory nerves is not known. The evidence for the occurrence in the eye of CGRP and CCK rests solely on immunohistochemical identification. The validity of identification of these peptides by immunohistochemistry is discussed at length in the cited manuscripts. Based on analysis of trigeminal ganglia, SP-LI and CCK-LI immunoreactivity has been observed primarily in small trigeminal ganglion cells; CGRP immunoreactivity is present in small but also in larger trigeminal neurons.

About 20% of trigeminal ganglion cells are immunoreactive to SP, about 40% are immunoreactive to CGRP[61,91,111], and about 2% are immunoreactive to CCK[55]. Their pattern of co-localization is complex. Based on co-localization studies in the trigeminal ganglion of rat[61,91,111] and guinea pig[55], essentially all SP-LI cells also are immunoreactive to CGRP. Consistent with their greater number, many CGRP-LI cells are negative for SP. For SP and CCK in the guinea pig trigeminal ganglion, some immunoreactive cells are positive for only one of these peptides and some are positive for both[54]. While not directly shown, it seems reasonable to suspect that some trigeminal ganglion cells are immunoreactive to all three peptides. Since many trigeminal cells are not reactive to any of the three, further reports are expected as new peptides become recognized.

The pattern of co-localization observed in ocular nerve fibers parallels in many ways the observation in trigeminal ganglion. By the use of simultaneous immunohistochemical labeling techniques, nerve fibers immunoreactive to both SP and CCK[54] or to both SP and CGRP[37,67] have been observed in the eye. Although not studied in the eye, it is even now known that the latter two can coexist in the same secretory vesicle[38]. Consistent with the co-localization pattern of SP and CCK in the trigeminal ganglion, nerve fibers have been observed in the eye that are immunoreactive to both or to just one of these peptides[54]. In co-localization studies with CGRP and SP, ocular nerve fibers immunoreactive to CGRP but negative for SP are observed, also consistent with the pattern in the trigeminal ganglion[67].

Enkephalin

Fine nerve fibers immunoreactive to leu-enkephalin have been observed in the eye of the rat[10] and the guinea pig (Kuwayama and Stone, unpublished observations) by immunohistochemical techniques. They present a novel problem. Standard selective denervations do not affect their integrity[10]. It is possible that they arise from accessory intraorbital microganglia or from neurons intrinsic to the uvea. Without known connection, their origin is speculative.

VI. Intraganglionic organization

By intraocular injection of probes of retrograde axoplasmic transport, it is possible to identify neurons both in the superior cervical ganglion and the trigeminal ganglion that project to the eye[2]. In the case of the superior cervical ganglion, neurons projecting to the eye are distributed in the caudal portion of the ganglion. No specific transmitter-related histochemistry has yet been reported on these cells. For the trigeminal ganglion in guinea pig, the specific issue of intraganglionic origin of cells projecting to the eye has been addressed[55]. They are located within the anteromedial region of the ophthalmic division of the ganglion[2]. When studied by immunohistochemical techniques, there is no recognizable somatotopical organization to peptidergic neurons containing SP, CCK or CGRP, either for the ganglion as a whole or for cells specifically projecting to the eye. When the proportion of neurons immunoreactive for each peptide and projecting to the eye is compared to the immunoreactive neurons of the ganglion as a whole, proportions are similar[55]. Because of the diversity of innervated intraocular structures

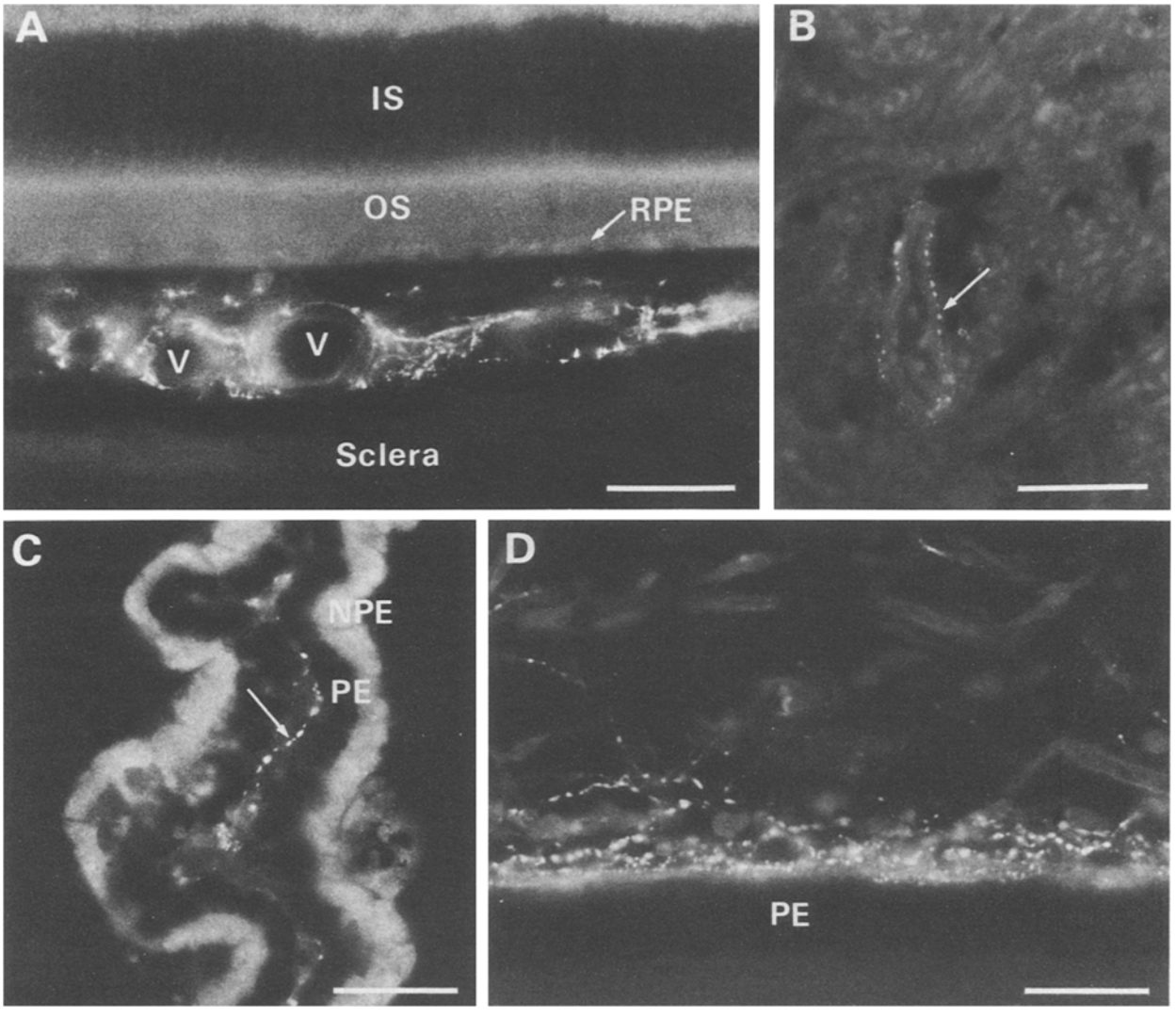

Figure 4. Neuropeptide Y-like immunoreactive nerve fibers supply both vascular and non-vascular structures in the eye. (Fluorescence micrographs; bars, 50 μm). *A* In the albino rat choroid, a rich innervation is present and is clearly related to the vascular supply. IS, photoreceptor inner segment; OS, photoreceptor outer segment; RPE, retinal pigmented epithelium; V, blood vessel in choroid. *B* An immunoreactive nerve fiber (arrow) surrounds an arteriole in the ciliary body of the rhesus monkey. *C* An immunoreactive nerve fiber (arrow) within the ciliary process of the cat. At the light microscopic level, it is not possible to resolve whether immunoreactive nerve fibers in this structure relate primarily to the epithelial layer or to the blood vessels within the core of the process. NPE, non-pigmented epithelial layer; PE, pigmented epithelial layer. *D* A rich innervation lies along the dilator muscle in the rhesus monkey iris. Nerve fibers also are seen more anteriorly in the stroma. PE, pigmented epithelial layer.

and because retrograde tracers label neurons non-selectively, such techniques may not adequately select neurons of specific functional subtype. Alternatively, a complex and perhaps variable correlation between neuropeptide content and sensory function may exist[60]. Because additional neuropeptides are likely to be found in the eye, the ocular sensory innervation might still have distinguishing properties not currently evident.

VII. Interactions of parasympathetic, sympathetic and sensory nerves

The eye has proved a usable model for demonstrating the plasticity of the peripheral nervous system, particularly with respect to neuropeptides. For instance, two changes occur to the ocular innervation following sympathectomy besides the apparent loss of adrenergic nerves. First, unilateral sympathectomy results in a gradual enhancement of sensory neuropeptides in the ipsilateral eye judged both by immunohistochemistry and by radioimmunoassay[17,49,54,88,120,128]. Second, nonsympathetic nerve fibers in the eye gradually develop tyrosine hydroxylase and NPY immunoreactivity, each of which is considered a 'sympathetic' marker[11]. Yet despite the presence of tyrosine hydroxylase immunoreactivity in sympathectomized eyes, there is no evidence of catecholamine synthesis. Some nerve fibers induced to express tyrosine hydroxylase or neuropeptide Y derive from the ciliary ganglion; it has not been possible to identify the source of all such nerve fibers[11].

In total, these observations indicate a complex interaction of parasympathetic, sympathetic and sensory nerves and substantiate prior observations on the inductive plasticity of neurotransmitters/neuromodulators in peripheral nerves as studied in other systems. The full functional implications of these interactions are not currently known, but the eye continues a useful end organ for their elucidation.

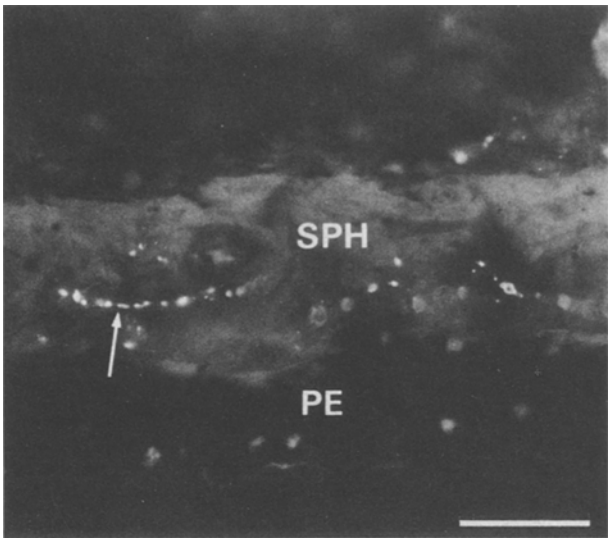

Figure 5. A substance P-like immunoreactive nerve fiber (arrow) in the sphincter muscle (SPH) of the human iris. Despite the widespread distribution throughout the eye of substance P-like immunoreactive nerve fibers, the only established ocular effect of this peptide is miosis in selective species. Despite the innervation present, substance P seems inactive to the human iris sphincter muscle.[121] PE, pigmented epithelial layer (Fluorescence micrograph; bar, 50 µm).

VIII. Conspicuously absent neuropeptides

Considering the diversity of tissues within the eye and the observations on the trigeminal ganglion discussed above, one might expect the neuropeptides of the eye to represent fully the neuropeptides in the ganglia that serve it. Interestingly, however, this is not the case. Several neuropeptides have been described in the relevant peripheral ganglia that have not been found in peripheral ocular nerves. Some, such as somatostatin, have been sought by several groups. Although present in retina, there are no reports of its existence in ocular peripheral nerves. Other neuropeptides, such as corticotropin-releasing factor, are so newly described in peripheral ganglia[92] that they may not have been specifically studied by ophthalmic researchers as yet.

IX. Physiological functions

Physiology trails behind immunohistochemistry in the eye. Despite the large number of neuropeptides described by immunohistochemical and biochemical techniques, relatively little is known about the physiological function of these peptides.

One of the most extensively studied neuropeptides is VIP. It acts as a vasodilator in the eye[76], consistent with this function elsewhere in the body. VIP also enhances prejunctional cholinergic transmission in the ciliary muscle[107]. Both iris muscles relax under its influence[40,41,108]. VIP stimulates adenylate cyclase in the rabbit iris ciliary body[73], suggesting a role in the regulation of aqueous humor formation. VIP also stimulates the production of cyclic AMP by cultured retinal pigmented epithelial cells[51], an observation that suggests it could help regulate the retinal environment. VIP localizes to retinal amacrine cells[13] as well as autonomic nerves of the choroid. Since the retinal pigmented epithelium lies between sensory retina and choroid, either or both sources of VIP might be involved in the regulation of retinal pigmented epithelial function.

Despite the rich distribution of NPY-LI ocular nerves and the well-established and varied physiologic functions for this peptide, little physiologic work has been performed with it in the eye. NPY augments the contractile effects of l-phenylephrine on the iris dilator but has no direct effect on the muscle[83]; this observation is consistent with the rich innervation of the iris dilator by nerves containing this neuropeptide as well as the concomitant adrenergic innervation. No other physiologic reports have appeared to the present. For ocular sensory nerves, a role in mediating the ocular injury response is now clear, at least in rabbit[8]. Severe mechanical or chemical trauma induces a four-part response in the eye, consisting of miosis, vasodilation, breakdown of the blood-aqueous barrier with protein leakage and elevated intraocular pressure. Both neurogenic and prostaglandin-mediated mechanisms have been identified, the relative contribution of each depending on the type of stimulus[23]. For the ocular reaction to chemical injury, a major role for the sensory innervation is widely accepted[15]. Sensory denervation or prior treatment either with anesthetics or tetrodotoxin inhibit the reaction to chemical injury[15], while direct stimulation of the trigeminal ganglion elicits its four components[82]. Moreover, individual neuropeptides appear responsible for individual components of the ocular injury response. Substance P is a potent miotic[93,116] and is released into the eye after trigeminal nerve stimulation[6]. When applied to the eye, it stimulates the atropine-resistent miosis which occurs in the neurogenic ocular injury response. CGRP is known to be a potent vasodilator[12]; in rabbits, it induces breakdown of the blood-ocular barriers and a rise in intraocular pressure but does not cause miosis[120]. It thus has been suggested that CGRP acts jointly with SP in the eye after injury, each eliciting different components of the ocular injury response[120]. This is a reasonable hypothesis in view of the co-localization of both neuropeptides in many ocular nerves and leads to a unified theory for understanding the response of the rabbit eye to mechanical and chemical trauma. A species qualification is required for this attractive hypothesis, however. The miotic response to substance P shows great species variability. Although maximal in the rabbit, it is not observed in species such as cat, dog and man[121]. Perhaps as the full array of neuropeptides is discovered in ocular sensory nerves, these species differences will become understandable. Possibly, the principle will remain but the participant peptides will differ. It is important to realize that a function for most of the substance P-LI and CGRP-LI nerves in the eye is unknown. While corneal nerve fibers in a general sense serve a nociceptive role, important other possibilities remain unexplored. For instance, substance P has potent effects on fluid flow in the kidney[39]. While substance P has been found in nerves to

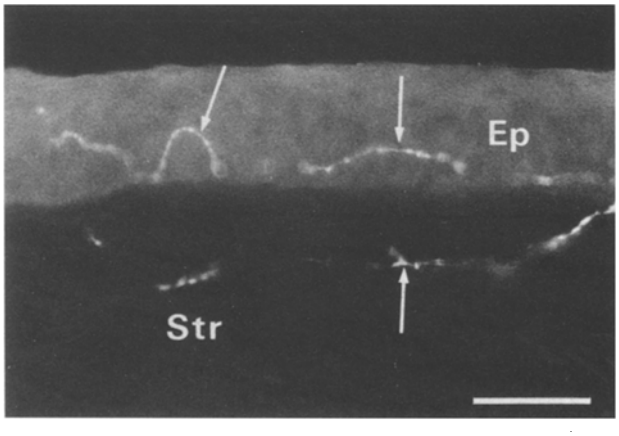

Figure 6. Calcitonin gene-related peptide-like immunoreactive nerve fibers (arrow) are present in the epithelium (Ep) and anterior stroma (Str) of the rat cornea. (Fluorescence micrograph; bar, 50 µm).

the ciliary process, there have been no studies of possible secretory effects for substance P in the eye. For CCK, the third sensory neuropeptide already discovered in the eye, no physiologic studies have yet been reported in this organ.

Clues to possible ocular functions of enkephalins are known. Systemic opioids induce remarkable effects on pupillary diameter without inflammatory signs, and they can affect intraocular pressure. For the pupil either miosis or mydriasis is observed, depending on the species[74]. Everyone is familiar with the police examination of suspected drug addicts for a pin-point pupil. This response is thought to be mediated primarily by the central nervous system[74]. However, morphine applied topically to the human eye[35] or injected into the rabbit eye induces a unilateral miosis[21], indicating a peripheral action as well. In this regard, enkephalin inhibits the cholinergic and SP-ergic in vitro responses of the electrically stimulated rabbit sphincter muscle but does not affect the sphincter response to exogenously applied carbachol or SP, suggesting the presence of opioid receptors on cholinergic and SP iris nerves[119]. Dynorphin, on the other hand, augments the cholinergic but inhibits the SP-ergic responses in this system[118]. As far as intraocular pressure is concerned, intraocular injection of morphine or D-ala-met-enkephalinamide in rabbits lowers it[22]. Moreover, morphine or heroin addicts have lower intraocular pressure than normal; and topical instillation of morphine to the eye lowers intraocular pressure in glaucoma patients[22]. Opioids appear to affect intraocular pressure by enhancing the drainage of aqueous humor from the eye[22]. In view of the enkephalin-like immunoreactive nerves now known to be present, further studies are justified to clarify fully the local effects of the opioid peptides in the eye.

X. Non-neuronal biologically active peptides

Ocular physiology is replete with reports of the effects of hormones on ocular function[46]. By and large, the hormones in question have not been found in ocular nerves and are not thought to be produced locally; the observed effects are assumed to be humoral. Yet the matter is unsettled. Paracrine cells have not been identified in the uvea. Using an antibody to neuron-specific enolase, however, it has been proposed that cells of the diffuse neuroendocrine system do exist in the trabecular meshwork of the monkey[99]. To date, no biologically active peptide or neurotransmitter has been identified within these cells.

In the same vein, high levels of atrial natriuretic peptide recently have been found in the anterior uvea[97]. The cellular localization in the eye of this peptide has yet to be reported. Intriguingly, receptors to it have been identified on the pigmented epithelium of the ciliary body[5,66,85]. Although the physiologic function for atrial natriuretic peptide in the eye presumably relates to the secretion of aqueous humor, this connection remains to be established.

IX. Retina

The image of the outside world received by the rods and cones of the eye is signalled to the visual areas of the brain by the retinal ganglion cells. Between the photoreceptors at the depth of the retina and the ganglion cells near its surface lay three distinct classes of neurons – horizontal cells, bipolar cells, and amacrines. As the links between photoreceptor and ganglion cell, they are responsible for the coherent translation and processing of rod and cone impulses. Some idea of the complexity of their task is gained when it is recalled that within the retina diverse types of physiological information are processed: the ganglion cells relay information about shape, color, contrast, and light level in an economic and accurate manner. To a great extent this task is facilitated by the intervention of the interneurons-horizontal cells at the outer plexiform layer at the photoreceptor/bipolar cell border and amacrine cells at the inner plexiform layer at the bipolar/ganglion cell border. Amacrine cell subtypes have long been thought to contain specific neurotransmitters[27]; cholinergic[75], dopaminergic[28] or serotonergic[78] subtypes have been recognized, among others.

More recently, immunohistochemical and biochemical studies have revealed the presence of neuropeptides in the retina[13], chiefly within specific types of amacrine cells (fig. 7); in the anuran retina, neuropeptides also appear to be present in ganglion cell processes within the optic nerve[52]. While the detailed discussion of neuropeptides in the retina is beyond the scope of the present review, some general comments can be made to provide a framework for readers working outside this area. Consistent with the considerable species variability in retinal structure and retinal function, species variability exists in the identity of neuropeptides within the retina and in

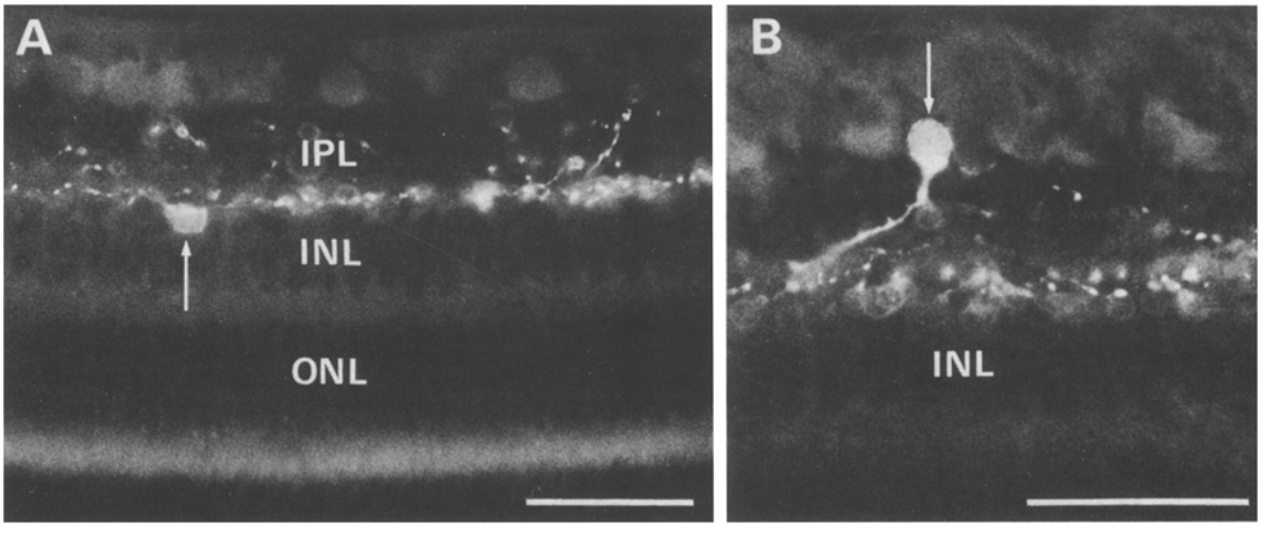

Figure 7. Vasoactive intestinal polypeptide-like immunoreactivity in the guinea pig retina. (Fluorescence micrographs; bars, 50 μm). *A* An immunostained amacrine cell (arrow) lies adjacent to the inner plexiform layer (IPL). Some of the nerve fibers in this layer are immunoreactive as well. *B* Sometimes, an immunoreactive amacrine cell (arrow) is placed in the ganglion cell layer. INL, inner nuclear layer; ONL, outer nuclear layer.

the number, subtype and distribution of amacrine cells containing specific neuropeptides[13]. Amacrine cells containing many different neuropeptides have been recognized within the vertebrate retina, including substance P, enkephalin, neurotensin, neuropeptide Y, somatostatin, glucagon, vasoactive intestinal polypeptide, and cholecystokinin. As in brain, co-localization of individual neuropeptides with other neuropeptides[47, 48, 63, 64, 125] or with classical neurotransmitters[125–127] is recognized.

Efforts are now underway to establish a physiological role for neuropeptides in the retina[19]. Light or dark adaptation influences neuropeptide levels[32, 43, 68, 69]. Individual neuropeptides also have been shown to influence specific retinal cells[18, 20, 56, 124]. Neuropeptides cause the selective release of retinal neurotransmitters[3], and potassium depolarization leads to neuropeptide release[79]. Receptor mechanisms are being identified utilizing second messengers such as the adenylate cyclase pathway[56, 80, 89, 124].

XII. Conclusions

The application of immunohistochemical techniques to the discovery of neuropeptides in the eye has indicated a complexity to the peripheral innervation of the eye that was unimagined only a few years ago. Not only have neuropeptides been identified within parasympathetic, sympathetic and sensory nerves to this organ, but patterns of neuropeptide and neurotransmitter co-localizations are now being described. Important interactions between the three divisions of the ocular peripheral innervation are now also evident. The classical conception of a push-pull ocular innervation, a cholinergic parasympathetic supply counterbalanced by an adrenergic sympathetic supply, probably remains valid but is vastly oversimplified. Because of the importance of the eye's vegetative physiology in clinical disorders, such as glaucoma, and because of the central role played by cholinergic and adrenergic agents in clinical pharmacology, understanding the role of potent biologically active peptides clearly will lead to enhanced understanding of ocular physiology and to new drug therapies. Because of the lag between immunocytochemical identification and physiological definition, our knowledge of the functional role of neuropeptides within the eye is considerably behind what we know of its neuroanatomy. Neuroanatomical studies disclose a complexity to the ocular innervation that undoubtedly parallels a complexity of physiological function. It will be revealed as neuropeptide actions are delineated.

1 Allen, J.M., McGregor, G.P., Adrian, T.E., Bloom, S.R., Zhang, S.Q., Ennis, K.W., and Unger, W.G., Reduction of neuropeptide Y (NPY) in the rabbit iris-ciliary body after chronic sympathectomy. Exp. Eye Res. *37* (1983) 213–215.

2 Arvidson, B., Retrograde transport of horseradish peroxidase in sensory and adrenergic neurons following injection into the anterior eye chamber. J. Neurocyt. *8* (1979) 751–764.

3 Bauer, B., Ehinger, B., Tornqvist, K., and Waga, J., Neurotransmitter release by certain neuropeptides in the chicken retina. Acta ophthalm. *63* (1985) 581–587.

4 Beauvieux, J., and Dupas, J., Étude anatomo-topographique et histologique du ganglion ophtalmique chez l'homme et divers animaux. Archs Ophthalm. *43* (1926) 641–671.

5 Bianchi, C., Anand-Srivastava, M.B., De Léan, A., Gutkowska, J., Forthomme, D., Genest, J., and Cantin, M., Localization and characterization of specific receptors for atrial natriuretic factor in the ciliary processes of the eye. Curr. Eye Res. *5* (1986) 283–293.

6 Bill, A., Stjernschantz, J., Mandahl, A., Brodin, E., and Nilsson, G., Substance P: release on trigeminal nerve stimulation, effects in the eye. Acta physiol. scand. *106* (1979) 371–373.

7 Bishop, A.E., Polak, J.M., Yiangou, Y., Christofides, N.D., and Bloom, S.R., The distributions of PHI and VIP in porcine gut and their co-localisation to a proportion of intrinsic ganglion cells. Peptides *5* (1984) 255–259.

8 Bito, L.Z., Species differences in the responses of the eye to irritation and trauma: a hypothesis of divergence in ocular defense mechanisms, and the choice of experimental animals for eye research. Exp. Eye Res. *39* (1984) 807–829.

9 Björklund, H., Fahrenkrug, J., Sieger, Å., Vanderhaeghen, J.-J., and Olson, L., On the origin and distribution of vasoactive intestinal polypeptide-, peptide HI-, and cholecystokinin-like-immunoreactive nerve fibers in the rat iris. Cell Tiss. Res. *242* (1985) 1–7.

10 Björklund, H., Hoffer, B., Olson, L., Palmer, M., and Seiger, Å., Enkephalin immunoreactivity in iris nerves: distribution in normal and grafted irides, persistence and enhanced fluorescence after denervations. Histochemistry *80* (1984) 1–7.

11 Björklund, H., Hökfelt, T., Goldstein, M., Terenius, L., and Olson, L., Appearance of the noradrenergic markers tyrosine hydroxylase and neuropeptide Y in cholinergic nerves of the iris following sympathectomy. J. Neurosci. *5* (1985) 1633–1643.

12 Brain, S.D., Williams, T.J., Tippins, J.R., Morris, H.R., and MacIntyre, I., Calcitonin gene-related peptide is a potent vasodilator. Nature *313* (1985) 54–56.

13 Brecha, N.C., Eldred, W., Kuljis, R.O., And Karten, H.J., Identification and localization of biologically active peptides in the vertebrate retina, in: Progress in Retinal Research, vol. 3, chapt. 7, pp. 185–226. Eds N.N. Osborne and G. Chader. Pergamon Press, New York 1984.

14 Bruun, A., Ehinger, B., Sundler, F., Tornqvist, K., and Uddman, R., Neuropeptide Y immunoreactive neurons in the guinea-pig uvea and retina. Invest. Ophthalm. vis. Sci. *25* (1984) 1113–1123.

15 Butler, J.M., Unger, W.G., and Hammond, B.R., Sensory mediation of the ocular response to neutral formaldehyde. Exp. Eye Res. *28* (1979) 577–589.

16 Castro-Correia, J., Studies on the innervation of the uveal tract. Ophthalmologica *154* (1967) 497–520.

17 Cole, D.F., Bloom, S.R., Burnstock, G., Butler, J.M., McGregor, G.P., Saffrey, M.J., Unger, W.G., and Zhang, S.Q., Increase in SP-like immunoreactivity in nerve fibres of rabbit iris and ciliary body one to four months following sympathetic denervation. Exp. Eye Res. *37* (1983) 191–197.

18 Dick, E., and Miller, R.F., Peptides influence retinal ganglion cells. Neurosci. Lett. *26* (1981) 131–35.

19 Djamgoz, M.B.A., Downing, J.E.G., and Prince, D.J., Physiology of neuroactive peptides in vertebrate retina. Biochem. Soc. Trans. *11* (1983) 686–689.

20 Djamgoz, M.B.A., Stell, W.K., Chin, D.-A., and Lam, D.M.-K., An opiate system in the goldfish retina. Nature *292* (1981) 620–623.

21 Drago, F., Gorgone, G., Spina, F., Panissidi, G., Dal Bello, A., Moro, F., and Scapagnini, U., Opiate receptors in the rabbit iris. Naunyn-Schmiedeberg's Arch. Pharmak. *315* (1980) 1–4.

22 Drago, F., Panissidi, G., Bellomio, R., Dal Bello, A., Aguglia, E., and Gorgone, G., Effects of opiates on intraocular pressure of rabbits and humans. Clin. exp. Pharm. Physiol. *12* (1985) 107–113.

23 Eakins, K.E., Prostaglandin and non-prostaglandin mediated breakdown of the blood-aqueous barrier. Exp. Eye Res. *25* (1977) 483–498.

24 Ehinger, B., Ocular and orbital vegetative nerves. Acta physiol. scand. *67* (1966) 1–35.

25 Ehinger, B., Adrenergic nerves to the eye and to related structures in man and in the cynomolgus monkey (*Macaca irus*). Invest. Ophthalm. *5* (1966) 42–52.

26 Ehinger, B., A comparative study of the adrenergic nerves to the anterior eye segment of some primates. Z. Zellforsch. *116* (1971) 157–177.

27 Ehinger, B., Neurotransmitter systems in the retina. Retina *2* (1982) 305–321.

28 Ehinger, B., Functional role of dopamine in the retina, in: Progress in Retinal Research, vol. 2, pp. 213–232. Eds N.N. Osborne and G.J. Chader. Pergamon Press, Oxford 1983.

29 Ehinger, B., and Falck, B., Innervation of iridic melanophores. Z. Zellforsch. *105* (1970) 538–542.

30 Ehinger, B., Falck, B., and Rosengren, E., Adrenergic denervation of the eye by unilateral cervical sympathectomy. Albrecht v. Graefes Arch. klin. exp. Ophthalm. *177* (1969) 206–211.

31 Ehinger, B., and Sjöberg, N.-O., Development of the ocular adrenergic nerve supply in man and guinea pig. Z. Zellforsch. *118* (1971) 579–592.

32 Eriksen, E.F., and Larsson, L.-I., Neuropeptides in the retina: evidence for differential topographical localization. Peptides *2* (1981) 153–157.

33 Fahrenkrug, J., Bek., T., Lundberg, J.M., and Hökfelt, T., VIP and

PHI in cat neurons: co-localization but variable tissue content possible due to differential processing. Reg. Peptides *12* (1985) 21–34.
34 Falck, B., Hillarp., N.-Å., Thieme, G., and Torp, A., Fluorescence of catechol amines and related compounds condensed with formaldehyde. J. Histochem. Cytochem. *10* (1962) 348–354.
35 Fanciullacci, M., Boccuni, M., Pietrini, U., and Sicuteri, F., Search for opiate receptors in human pupil. Int. J. clin. pharmac. Res. *1* (1980) 109–113.
36 Fukuda, M., Presence of adrenergic innervation to the retinal vessels. A histochemical study. Jap. J. Ophtalm. *14* (1970) 1–7.
37 Gibbins, I.L., Furness, J.B., Costa, M., MacIntyre, I., Hillyard, C.J., and Girgis, S., Co-localization of calcitonin gene-related peptide-like immunoreactivity with substance P in cutaneous, vascular and visceral sensory neurons of guinea pigs. Neurosci. Lett. *57* (1985) 125–130.
38 Gulbenkian, S., Merighi, A., Wharton, J., Varndell, I.M., and Polak, J.M., Ultrastructure evidence for the coexistence of calcitonin gene-related peptide and substance P in secretory vesicles of peripheral nerves in the guinea pig. J. Neurocyt. *15* (1986) 535–542.
39 Gullner, H.G., Campbell, W.B., and Pettinger, W.A., Role of substance P in water homeostasis. Life Sci. *24* (1979) 2351–2360.
40 Hyashi, K., and Masuda, K., Effects of vasoactive intestinal polypeptide (VIP) and cyclic-AMP on the isolated sphincter pupillae muscles of the albino rabbit. Jap. J. Ophthalm. *26* (1982) 437–442.
41 Hyashi, K., Mochizuki, M., and Masuda, K., Effects of vasoactive intestinal polypeptide (VIP) and cyclic AMP on isolated dilator pupillae muscle of albino rabbit eye. Jap. J. Ophthalm. *27* (1983) 647–654.
42 Imai, K., Cholinergic innervation of the choroid. Ophthalm. Res. *9* (1977) 194–200.
43 Ishimoto, I., Millar, T., Chubb, I.W., and Morgan, I.G., Somatostatin immunoreactive amacrine cells of chicken retina: retinal mosaic, ultrastructural features, and light-driven variations in peptide metabolism. Neuroscience *17* (1986) 1217–1233.
44 Jacobowitz, D.M., and Laties, A.M., Direct adrenergic innervation of a teleost melanophore. Anat. Rec. *162* (1968) 501–504.
45 Jacobowitz, D.M., and Olschowka, J.A., Bovine pancreatic polypeptide-like immunoreactivity in brain and peripheral nervous system: coexistence with catecholaminergic nerves. Peptides *3* (1982) 569–590.
46 Kass, M.A., and Sears, M.L., Hormonal regulation of intraocular pressure. Surv. Ophthalm. *22* (1977) 153–176.
47 Katayama-Kumoi, Y., Kiyama, H., Emson, P.C., Kimmel, J.R., and Tohyama, M., Coexistence of pancreatic polypeptide and substance P in the chicken retina. Brain Res. *361* (1985) 25–35.
48 Katayama-Kumoi, Y., Kiyama, H., Manabe, R., Shiotani, Y., and Tohyama, M., Co-existence of glucagon- and substance P-like immunoreactivity in the chicken retina. Neuroscience *16* (1985) 417–424.
49 Kessler, J.A., Bell, W.O., and Black, I.B., Interactions between the sympathetic and sensory innervation of the iris. J. Neurosci. *3* (1983) 1301–1307.
50 Koelle, G.B., and Friedenwald, J.S., A histochemical method for localizing cholinesterase activity. Proc. Soc. exp. Biol. Med. *70* (1949) 617–622.
51 Koh, S.-W.M., and Chader, G.J., Elevation of intracellular cyclic AMP and stimulation of adenylate cyclase activity by vasoactive intestinal polypeptide and glucagon in the retinal pigment epithelium. J. Neurochem. *43* (1984) 1522–1526.
52 Kuljis, R.O., Krause, J.E., and Karten, H.J., Peptide-like immunoreactivity in anuran optic nerve fibers. J. comp. Neurol. *226* (1984) 222–237.
53 Kuwayama, Y., Grimes, P.A., Ponte, B., and Stone, R.A., Autonomic neurons supplying the rat eye and the intraorbital distribution of VIP-like immunoreactivity. Exp. Eye Res. (1987) in press.
54 Kuwayama, Y., and Stone, R.A., Cholecystokinin-like immunoreactivity occurs in ocular sensory neurons and partially co-localizes with substance P. Brain Res. *381* (1986) 266–274.
55 Kuwayama, Y., Terenghi, G., Polak, J.M., Trojanowski, J.Q., and Stone, R.A., A quantitative correlation of substance P-, calcitonin gene-related peptide- and cholecystokinin-like immunoreactivity with retrogradely labeled trigeminal ganglion cells innervating the eye. Brain Res. *405* (1987) 220–226.
56 Lasater, E.M., Watling, K.J., and Dowling, J.E., Vasoactive intestinal peptide alters membrane potential and cyclic nucleotide levels in retinal horizontal cells. Science *221* (1983) 1070–1072.
57 Laties, A.M., Central retinal artery innervation. Absence of adrenergic innervation to the intraocular branches. Archs Ophthalm. *77* (1967) 405–409.
58 Laties, A.M., Ocular melanin and the adrenergic innervation of the eye. Trans. Am. ophthalm. Soc. *72* (1974) 560–605.
59 Laties, A.M., and Jacobowitz, D., A comparative study of the autonomic innervation of the eye in monkey, cat, and rabbit. Anat. Rec. *156* (1966) 383–396.
60 Leah, J.D., Cameron, A.A., Kelly, W.L., and Snow, P.J., Coexistence of peptide immunoreactivity in sensory neurons of the cat. Neuroscience *16* (1985) 683–690.
61 Lee, Y., Kawai, Y., Shiosaka, S., Takami, K., Kiyama, H., Hillyard, C.J., Girgis, S., MacIntyre, I., Emson, P.C., and Tohyama, M., Coexistence of calcitonin-gene related peptide and substance P-like peptide in single cells of the trigeminal ganglion of the rat: immunohistochemical analysis. Brain Res. *330* (1985) 194–196.
62 Lee, Y., Takami, K., Kawai, Y., Girgis, S., Hillyard, C.J., MacIntyre, I., Emson, P.C., and Tohyama, M., Distribution of calcitonin gene-related peptide in the rat peripheral nervous system with reference to its coexistence with substance P. Neuroscience *15* (1985) 1227–1237.
63 Li, H.-B., Marshak, D.W., Dowling, J.E., and Lam, D.M.-K., Colocalization of immunoreactive substance P and neurotensin in amacrine cells of the goldfish retina. Brain Res. *366* (1986) 307–313.
64 Li, H.-B., Watt, C.B., and Lam, D.M.-K., The coexistence of two neuroactive peptides in a subpopulation of retinal amacrine cells. Brain Res. *345* (1985) 176–180.
65 Maggio, J.E., 'Kassinin' in mammals: the newest tachykinins. Peptides *6* (1985) 237–243.
66 Mantyh, C.R., Kruger, L., Brecha, N.C., and Mantyh, P.W., Localization of specific binding sites for atrial natriuretic factor in peripheral tissues of the guinea pig, rat, and human. Hypertension *8* (1986) 712–721.
67 Matsuyama, T., Wanaka, A., Yoneda, S., Kimura, K., Kamada, T., Girgis, S., MacIntyre, I., Emson, P.C., and Tohyama, M., Two distinct calcitonin gene-related peptide-containing peripheral nervous systems: distribution and quantitative differences between the iris and cerebral artery with special reference to substance P. Brain Res. *373* (1986) 205–212.
68 Millar, T.J., and Chubb, I.W., Substance P in the chick retina: effects of light and dark. Brain Res. *307* (1984) 303–309.
69 Millar, T.J., Salipan, N., Oliver, J.O., Morgan, J.G., and Chubb, I.W., The concentration of enkephalin-like material in the chick retina is light dependent. Neuroscience *13* (1984) 221–226.
70 Miller, A., Costa, M., Furness, J.B., and Chubb, I.W., Substance P immunoreactive sensory nerves supply the rat iris and cornea. Neurosci. Lett. *23* (1981) 243–249.
71 Miller, A.S., Coster, D.J., Costa, M., and Furness, J.B., Vasoactive intestinal polypeptide immunoreactive nerve fibres in the human eye. Aust. J. Ophthalm. *11* (1983) 185–193.
72 Mindel, J.S., and Mittag, T.W., Choline acetyltransferase in ocular tissues of rabbits, cats, cattle, and man. Invest. Ophthalm. *15* (1976) 808–814.
73 Mittag, T.W., and Tormay, A., Drug responses of adenylate cyclase in iris-ciliary body determined by adenine labelling. Invest. Ophthalm. vis. Sci. *26* (1985) 396–399.
74 Murray, R.B., Adler, M.W., and Korczyn, A.D., Minireview-The pupillary effects of opioids. Life Sci. *33* (1983) 495–509.
75 Neal, M.J., Cholinergic mechanism in the vertebrate retina, in: Progress in Retinal Research, vol. 3, pp. 191–212. Eds N.N. Osborne and G.J. Chader. Pergamon Press, Oxford 1983.
76 Nilsson, S.F.E., and Bill, A., Vasoactiove intestinal polypeptide (VIP): effects in the eye and on regional blood flows. Acta physiol. scand. *121* (1984) 385–392.
77 Odin, L., and O'Donnell, F.E. Jr., Adrenergic influence on iris stromal pigmentation: evidence for α-adrenergic receptors. Invest. Ophthalm. vis. Sci. *23* (1982) 528–530.
78 Osborne, N.N., Evidence for serotonin being a neurotransmitter in the retina, in: Biology of Serotonergic Transmission, pp. 401–430. Ed. N.N. Osborne. John Wiley & Sons Ltd., New York 1982.
79 Osborne, N.N., Patel, S., Terenghi, G., Allen, J.M., Polak, J.M., and Bloom, S.R., Neuropeptide Y (NPY)-like immunoreactive amacrine cells in retinas of frog and goldfish. Cell Tiss. Res. *241* (1985) 651–656.
80 Pachter, J.A., and Lam, D.M.-K., Interactions between vasoactive intestinal peptide and dopamine in the rabbit retina: stimulation of a common adenylate cyclase. J. Neurochem. *46* (1986) 257–264.
81 Palkama, A., Uusitalo, H., and Lehtosalo, J., Innervation of the anterior segment of the eye: with special reference to functional aspects, in: Neurohistochemistry: Modern Methods and Applications, pp. 587–615. Eds P. Panula, H. Paivarinta and S. Soinila. Alan R. Liss, Inc., New York 1986.

82 Perkins, E.S., Influence of the fifth cranial nerve on the intra-ocular pressure of the rabitt eye. Br. J. Ophthalm. *41* (1957) 257–300.
83 Piccone, M., Krupin, T., Wax, M., Stone, R., and Davis, M., Effects of neuropeptide Y on isolated rabbit iris dilator muscle. Invest. Ophthalm. vis. Sci. *27* Suppl. (1986) 355.
84 Polak, J.M., and Bloom, S.R., Regulatory peptides – the distribution of two newly discovered peptides: PHI and NPY. Peptides *5* (1984) 79–89.
85 Quirion, R., Dalpé, M., De Lean, A., Gutkowska, J., Cantin, M., and Genest, J., Atrial natriuretic factor (ANF) binding sites in brain and related structures. Peptides *5* (1984) 1167–1172.
86 Ruskell, G.L., Ocular fibres of the maxillary nerve in monkeys. J. Anat. *118* (1974) 195–203.
87 Ruskell, G.L., Facial nerve distribution to the eye. Am. J. Optom. Physiol. Optics *62* (1985) 793–798.
88 Schon, F., Ghatei, M., Allen, J.M., Mulderry, P.K., Kelly, J.S., and Bloom, S.R., The effect of sympathectomy on calcitonin gene-related peptide levels in rat trigeminovascular system. Brain Res. *348* (1985) 197–200.
89 Schorderet, M., Hof, P., and Magistretti, P.J., The effects of VIP on cyclic AMP and glycogen levels in vertebrate retina. Peptides *5* (1984) 295–298.
90 Shimizu, Y., Localization of neuropeptides in the cornea and uvea of the rat: an immunohistochemical study. Cell. molec. Biol. *28* (1982) 103–110.
91 Skofitsch, G., and Jacobowitz, D.M., Calcitonin gene-related peptide coexists with substance P in capsaicin sensitive neurons and sensory ganglia of the rat. Peptides *6* (1985) 747–754.
92 Skofitsch, G., Zamir, N., Helke, C.J., Savitt, J.M., and Jacobowitz, D.M., Corticotropin releasing factor-like immunoreactivity in sensory ganglia and capsaicin sensitive neurons of the rat central nervous system: colocalization with other neuropeptides. Peptides *6* (1985) 307–318.
93 Soloway, M.R., Stjernschantz, J., and Sears, M., The miotic effect of substance P on the isolated rabbit iris. Invest. Ophthalm. vis. Sci. *20* (1981) 47–52.
94 Stjernschantz, J., and Sears, M., Identification of substance P in the anterior uvea and retina of the rabbit. Exp. Eye Res. *35* (1982) 401–404.
95 Stone, R.A., Vasoactive intestinal polypeptide and the ocular innervation. Invest. Ophthalm. vis. Sci. *27* (1986) 951–957.
96 Stone, R.A., Neuropeptide Y and the innervation of the human eye. Exp. Eye Res. *42* (1986) 349–355.
97 Stone, R.A., and Glembotski, C.C., Immunoactive atrial natriuretic peptide in the rat eye: Molecular forms in anterior uvea and retina. Biochem. biophys. Res. Commun. *134* (1986) 1022–1028.
98 Stone, R.A., and Kuwayama, Y., Substance P-like immunoreactive nerves in the human eye. Archs Ophthalm. *103* (1985) 1207–1211.
99 Stone, R.A., Kuwayama, Y., Laties, A.M., and Marangos, P.J., Neuron-specific enolase-containing cells in the rhesus monkey trabecular meshwork. Invest. Ophthalm. vis. Sci. *25* (1984) 1332–1334.
100 Stone, R.A., Kuwayama, Y., Laties, A.M., McGlinn, A.M., and Schmidt, M.L., Guinea-pig ocular nerves contain a peptide of the cholecystokinin/gastrin family. Exp. Eye Res. *39* (1984) 387–391.
101 Stone, R.A., Kuwayama, Y., Terenghi, G., and Polak, J.M., Calcitonin gene-related peptide: occurrence in corneal sensory nerves. Exp. Eye Res. *43* (1986) 279–283.
102 Stone, R.A., and Laties, A.M., Pancreatic polypeptide-like immunoreactive nerves in the guinea pig eye. Invest. Ophthalm. vis. Sci. *24* (1983) 1620–1623.
103 Stone, R.A., Laties, A.M., and Brecha, N.C., Substance P-like immunoreactive nerves in the anterior segment of the rabbit, cat and monkey eye. Neuroscience *7* (1982) 2459–2468.
104 Stone, R.A., Laties, A.M., and Emson, P.C., Neuropeptide Y and the ocular innervation of rat, guinea pig, cat and monkey. Neuroscience *17* (1986) 1207–1216.
105 Stone, R.A., Tervo, T., Tervo, K., and Tarkkanen, A., Vasoactive intestinal polypeptide-like immunoreactive nerves to the human eye. Acta ophthalm. *64* (1986) 12–18.
106 Sunderland, S., and Hughes, E.S.R., The pupillo-constrictor pathway and the nerves to the ocular muscles in man. Brain *69* (1946) 301–309.
107 Suzuki, R., and Kobayashi, S., Vasoactive intestinal peptide and cholinergic neurotransmission in the ciliary muscle. Invest. Ophthalm. vis. Sci. *24* (1983) 250–253.
108 Suzuki, R., and Kobayashi, S., Different effects of substance P and vasoactive intestinal peptide on the motor function of bovine intraocular muscles. Invest. Ophthalm. vis. Sci. *24* (1983) 1566–1571.
109 Tatemoto, K., PHI–a new brain-gut peptide. Peptides *5* (1984) 151–154.
110 Terenghi, G., Polak, J.M., Allen, J.M., Zhang, S.Q., Unger, W.G., and Bloom, S.R., Neuropeptide Y-immunoreactive nerves in the uvea of guinea pig and rat. Neurosci. Lett. *42* (1983) 33–38.
111 Terenghi, G., Polak, J.M., Ghatei, M.A., Mulderry, P.K., Butler, J.M., Unger, W.G., and Bloom, S.R., Distribution and origin of calcitonin gene-related peptide (CGRP) immunoreactivity in the sensory innervation of the mammalian eye. J. comp. Neurol. *233* (1985) 506–516.
112 Terenghi, G., Polak, J.M., Probert, L., McGregor, G.P., Ferri, G.L., Blank, M.A., Butler, J.M., Unger, W.G., Zhang, S.Q., Cole, D.F., and Bloom, S.R., Mapping, quantitative distribution and origin of substance P- and VIP-containing nerves in the uvea of guinea pig eye. Histochemistry *75* (1982) 399–417.
113 Tervo, K., Tervo, T., Eränkö, L., Eränkö, O., and Cuello, A.C., Immunoreactivity for substance P in the Gasserian ganglion, ophthalmic nerve and anterior segment of the rabbit eye. Histochem. J. *13* (1981) 435–443.
114 Tervo, K., Tervo, T., Eränkö, L., Eränkö, O., Valtonen, S., and Cuello, A.C., Effect of sensory and sympathetic denervation on substance P immunoreactivity in nerve fibres of the rabbit eye. Exp. Eye Res. *34* (1982) 577–585.
115 Tervo, K., Tervo, T., Eränkö, L., Vannas, A., Cuello, A.C., and Eränkö, O., Substance P-immunoreactive nerves in the human cornea and iris. Invest. Ophthalm. vis. Sci. *23* (1982) 671–674.
116 Tornqvist, K., Mandahl, A., Leander, S., Lorén, I., Håkanson, R., and Sundler, F., Substance P-immunoreactive nerve fibres in the anterior segment of the rabbit eye. Cell Tiss. Res. *222* (1982) 467–477.
117 Uddman, R., Alumets, J., Ehinger, B., Håkanson, R., Lorén, I., and Sundler, F., Vasoactive intestinal peptide nerves in ocular and orbital structures of the cat. Invest. Ophthalm. vis. Sci. *19* (1980) 878–885.
118 Ueda, N., Muramatsu, I., and Fujiwara, M., Dual effects of dynorphin-(1–13) on cholinergic and substance P-ergic transmissions in the rabbit iris sphincter muscle. J. Pharmac. exp. Ther. *232* (1985) 545–550.
119 Ueda, N., Muramatsu, I., Hayashi, H., and Fujiwara, M., Effects of met-enkephalin on the substance P-ergic and cholinergic responses in the rabbit iris sphincter muscle. J. Pharmac. exp. Ther. *226* (1983) 507–511.
120 Unger, W.G., Terenghi, G., Ghatei, M.A., Ennis, K.W., Butler, J.M., Zhang, S.Q., Too, H.P., Polak, J.M., and Bloom, S.R., Calcitonin gene-related peptide as a mediator of the neurogenic ocular injury response, J. ocular Pharmac. *1* (1985) 189–199.
121 Unger, W.G., and Tighe, J., The response of the isolated iris sphincter muscle of various mammalian species to substance P. Exp. Eye Res. *39* (1984) 677–684.
122 Uusitalo, H., Lehtosalo, J., Palkama, A., and Toivanen, M., Vasoactive intestinal polypeptide (VIP)-like immunoreactivity in the human and guinea pig choroid. Exp. Eye Res. *38* (1984) 435–437.
123 Uusitalo, H., Lehtosalo, J.I., and Palkama, A., Vasoactive intestinal polypeptide (VIP)-immunoreactive nerve fibers in the anterior uvea of the guinea pig. Ophthalm. Res. *17* (1985) 235–240.
124 Watling, K.J., and Dowling, J.E., Effects of vasoactive intestinal peptide and other peptides on cyclic AMP accumulation in intact pieces and isolated horizontal cells of the teleost retina. J. Neurochem. *41* (1983) 1205–1213.
125 Watt, C.B., Li, H.-B., and Lam, D.M.-K., The presence of three neuroactive peptides in putative glycinergic amacrine cells of an avian retina. Brain Res. *348* (1985) 187–191.
126 Watt, C.B., Ying-yet, T.S., and Lam, D.M.-K., Interactions between enkephalin and GABA in avian retina. Nature *311* (1984) 761–763.
127 Weiler, R., and Ball, A.K., Co-localization of neurotensin-like immunoreactivity and ^{3}H-glycine uptake system in sustained amacrine cells of turtle retina. Nature *311* (1984) 759–761.
128 Zhang, S.Q., Terenghi, G., Unger, W.G., Ennis, K.W., and Polak, J., Changes in substance P- and neuropeptide Y-immunoreactive fibres in rat and guinea-pig irides following unilateral sympathectomy. Exp. Eye Res. *39* (1984) 365–372.

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