Neuropeptides in the Uveal Tract

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The Presence of Neuropeptides in Uveal Structures

The uveal tract is richly innervated by post-ganglionic parasympathetic fibres of 3rd and 7th Nerve origin, sympathetic nerves from the paravertebral ganglion chain and somatic sensory afferents derived from the ophthalmic division of the Trigeminal nerve. Our present understanding of the relevance of autonomic neurotransmission to vegetative physiology of the eye has been based largely on the known pharmacological actions of the classical transmitters, noradrenalin and acetylcholine, and the effects of various related mimetic and antagonistic drugs. Classically, sensory elements are considered to pass impulses centripetally without a determinant influence on the uvea. With the advent of sensitive and specific immunochemical methods, however, a number of biologically active polypeptides, known to exist in the enteric nerve plexi and the central nervous system, have been identified within both autonomic and sensory nerves subserving the uveal tract. Certain of these peptides have been shown to exert profound somatic physiological effects and in many cases have come to be considered as neuromediators coexistent with the classical transmitters in their respective nerves.

To date, five neuropeptides have been positively identified by radio-immunoassay (RIA) in extracts of ocular tissues and have been localised by immuno-histochemistry to nerve fibres within structures of the mammalian uveal tract. These include substance P, calcitonin gene-related peptide (CGRP) and an arginine-vasopressin-like peptide (AVP) within sensory fibres, VIP in parasympathetic fibres derived from the facial nerve and NPY found within the sympathetic supply.¹⁻⁹ Substance P and CGRP are now known to act conjointly mediating the miosis,

hypertension and aqueous flare induced by noxious stimulation. AVP and vasopressin-like peptides are known to influence the rate of aqueous formation¹⁰ while VIP is a potent vasodilator of great importance in the choroid and possibly in the ciliary muscle. Aspects of the localisation and relevance of these peptides are described.

The ocular injury response

Acute irritation of the eye causes immediate miosis as well as a transient rise of intraocular pressure (IOP) and disruption of the blood-aqueous barrier. The upset in aqueous dynamics is initiated by dilation of anterior uveal vessels and ciliary extravasation.¹¹⁻¹⁷ The response is rapid in onset and comparatively short in duration and involves neither humoral antibodies, circulating blood leukocytes nor the activation of tissue facultative inflammatory cells (macrophages, mast cells and fibroblasts). These transient changes represent the immediate defensive response of the ocular tissue to injury, analogous to the "Triple Response" of Lewis¹⁸ which occurs in skin following acute noxious stimulation. Vasodilation in the region surrounding an injury site and focal oedema (a weal) are two features considered to be mediated neurogenically within sensory elements subserving the skin, by the propagation of nerve impulses peripherally along small unmyelinated sensory afferents (antidromic vasodilation) and collateral and branched fibres (axon reflex).¹⁹⁻²⁰

That sensory fibres of the Vth Nerve can conduct impulses antidromically to the eye was first shown by Claud Bernard in 1958²¹ who observed a pronounced atropine-resistant miosis after rubbing the peripheral cut end of the Vth Nerve cut distal to the Gasserian ganglion, deducing that the response was independent of a

Fig. 1. Noxious stimulation causes an immediate ocular injury response

central pathway. Maurice²² noted that the miotic reaction was abolished if the peripheral fibres were allowed to degenerate and that stimulation of the central end of a sectioned nerve produced no effect, thereby confirming that sensory activation followed an antidromic and not a centripetal pathway. Raised IOP and a profound disruption of the blood-aqueous barrier were seen in similar experiments by Perkins.¹²

A number of substances have been proposed as the antidromic vasodilator in the eye including acetylcholine,²³ histamine,¹⁸ ATP²⁴ and bradykinin,²⁵ but their relevance as a sensory mediator in the anterior uvea has now been discounted. ATP has no acute actions when instilled into the rabbit anterior chamber,^{12,26} although it is released into aqueous humour upon Vth Nerve stimulation.^{26,27} Butler²⁶ failed to see any notable effects of histamine (up to 50 µg) or ATP (50 µg). Bradykinin, however, is a potent miotic and has a profound effect on rabbit IOP and BAB, but its actions are now thought to be mediated through a sensory neurogenic pathway.^{28,29} Prostaglandin has also been suggested to be the final antidromic chemostimulator³⁰⁻³² but this has also now been ruled out.^{13,28,32}

Antidromic vasodilation in the skin is now thought to be mediated by substance P and CGRP. These peptides are known to occur in sensory fibres of the skin³³ and to be released by antidromic stimulation and the action of noxious substances.³⁴ Exogenous substance P can induce flare and extravasation (weal³⁵⁻³⁷) either by a direct action or by releasing

histamine from mast cells^{38,39} or indirectly by releasing histamine from mast cells.^{34,40} We have accumulated a convincing body of evidence that in the eye CGRP and substance P, or similar tachykinin peptide, are the sensory transmitters which mediate antidromic vasodilation and the pupil constriction respectively in response to trauma. Substance P effects a miosis without any effect on ocular tension, while CGRP elicits vascular changes leading to raised IOP and breakdown in the BAB, without affecting the pupil size.⁹

(a) Substance P

Substance P is a tachykinin peptide which is mammalian neuronal tissue, acts as a neurotransmitter of primary sensory afferents exerting a diversity of central effects including the transmission of nociceptive impulses.⁴¹⁻⁴³ It has been demonstrated immunochemically in small cell bodies of trigeminal systems^{5,44,45} and in accordance with Davie's hypothesis is translocated to both the central and the peripheral somatic end. Substance P-like immunoreactive material can be found in the rabbit anterior uvea^{1,28,46,47} and in trigeminal nerve fibres in rabbit, rat, guinea pig, cat, monkey, man,^{2,4,47-53} and even the squirrel eye.⁵⁴ Substance P is released into aqueous humour of the rabbit by antidromic stimulation⁵⁵ or by intra-camerally infused hypertonic KCl, NaCl, capsaicin or bradykinin.^{56,57} Unilateral destruction of the Gasserian ganglion depletes both substance P-like immunoreactivity in extracts^{1,37} and its

immunostaining of nerve fibres in samples of anterior uveal tissues.^{4,58} The time course of depletion corresponds to degeneration of the peripheral fibres and to the decrease in sensitivity of the eye to injury. Likewise, chronic sensory denervation produced by treatment of neonatal rats with capsaicin⁵⁹ destroys peripheral unmyelinated sensory fibres and their terminals^{60,61} and prevents the appearance of substance P-like immunostaining in the iris-ciliary bodies.⁶²

Although substance P produces vasodilation and extravasation when injected intradermally,^{36,37,63} it has no apparent effect on the anterior uveal blood vessels or the BAB when infused intracamerally into the rabbit eye.^{28,47} Early studies did report a rise of IOP and disruption of the blood-aqueous barrier⁶⁴ but these effects are small and inconsistent²⁸ and have been attributed to increased resistance to outflow due to pupil block and iris bombé.⁶⁵⁻⁹

However, substance P does cause a strong constriction of the rabbit pupil which is resistant to muscarinic blockade, in both the intact and sensorily denervated rabbit eye.^{28,32,65-66} Excised rabbit or bovine iris sphincter muscle placed into an isolated organ bath is contracted by

nanomolar concentrations of substance P in the presence of both atropine and tetrodotoxin, indicating a direct action on the muscle, independent of any intermediate neuronal pathway or activation. (See Fig. 2.) Moreover, the response is essentially intact in tissue obtained from sensorily denervation eyes, indicating the presence of postsynaptic tachykinin receptors on the muscle, coupled to the myogenic apparatus through which the neuropeptide exerts a direct action.²⁹ Indeed, tachykinin peptides have been found to bind with high affinity to the plasma membrane fraction from rabbit iris-ciliary bodies⁶⁷ and to display pharmacokinetically a correspondingly high potency. Subjecting isolated rabbit iris sphincter tissues to electrical field stimulation evokes frequency-dependent contractions which are totally abolished by tetrodotoxin but only partly inhibited by atropine. That residual non-cholinergic component is abolished by sensory denervation suggests that it is due to the release of substance P from lengths of sensory fibre within the excised tissue.²

(b) *Calcitonin gene-related polypeptide (CGRP)*
CGRP is a polypeptide of 37 amino acids

Fig. 2. Neurogenic contraction of isolated rabbit or cow iris sphincter muscle can be demonstrated by electrical field stimulation which results from activation of muscarinic and tachykinin excitatory receptors^{9,67-69}

encoded within the calcitonin gene but expressed only in neuronal tissue.^{70,71} In the eye, CGRP-like immunoreactivity is restricted to fine unmyelinated and varicosed fibres found throughout the mammalian anterior uvea. Abundant CGRP-immunostaining fibres form a continuous plexus which associates closely with vascular elements of the iridial processes and the iris of the rat or guinea pig. The elements innervate the dilator muscle and extend into the pupil region where they end in a network of thin fibres investing the sphincter muscle.^{5,72} A few CGRP-containing fibres can be seen in the guinea pig ciliary processes, running parallel to the epithelium,⁷³ which suggests the possibility that sensory elements might exert an influence on aqueous formation. Stone and co-workers⁵¹ observed in several mammalian species a similar presence of substance P immunostaining fibres within the ciliary processes, in the vicinity of the epithelium. CGRP-like immunoreactivity has been localised to two cell types in the Gasserian ganglion, larger cell bodies which stain both for CGRP and substance P, and smaller cells that are positive only for CGRP.^{5,71,74} Sensory denervation induced by diathermic coagulation of this ganglion or by neonatal treatment with capsaicin, results in a substantial depletion of CGRP in tissue samples of iris ciliary-bodies, not unlike its effect on substance P levels^{5,9,72,75} (see Table I.) Wahlestedt and his co-workers⁷⁶ have reported that CGRP-like substance P is related into aqueous humour by mechanical or by electrical stimulation of the rabbit Vth Nerve. CGRP is known to be a potent vasodilator⁷⁷⁻⁷⁹ and when infused into the rabbit anterior chamber produces pronounced ciliary vasodilation and extravasation, a rise of IOP and a

breakdown in the BAB.⁹ The concentration of aqueous protein is elevated and intravenously injected fluorescein rapidly enters the anterior chamber together with dense plasmoid material⁹ (see Table II). The response of IOP to CGRP is also accompanied by a delayed but obvious consensual IOP response, not unlike that occurring after unilateral Vth Nerve stimulation.⁸⁰ Given alone it has no apparent action on the pupil⁹ but can increase the miotic effect of substance P.⁷⁶ The IOP increase, therefore, does not result from a pupil block but from vascular changes within the ciliary processes. Mediation of the ocular injury response appears, accordingly, to require the complementary effects of CGRP and substance P, both of which are derived from sensory nerves of trigeminal origin but which act primarily at different effector sites within the anterior uvea. Their actions, facilitated in certain situations by PG,¹³ add to co-ordinate the ocular changes which characterise the neurogenic injury response, substance P producing miosis and CGRP the ciliary vascular changes and subsequent alterations in aqueous humour dynamics (see Fig. 3).

(c) Denervation supersensitivity

Supersensitivity of the pupil to exogenous nor-adrenaline after chronic sympathetic denervation is a well known phenomenon which results from the disappearance of presynaptic catecholamine re-uptake sites (cf. Trendelenburg).⁸¹ By three weeks and thereafter, however, the eye also develops an enhanced response to noxious stimuli such as neutral formaldehyde¹⁷ or laser irradiation of the iris.⁸² In sympathetically denervated eyes the IOP response to ruby laser light, for example, can reach a level approaching

Table I. Changes in the levels of CGRP-like and substance P-like immunoreactivity in sensorily denervated and normally innervated (control) iris-ciliary bodies

Species	CGRP pmol/g		Substance P pmol/g	
	Denervated	Control	Denervated	Control
tissue				
rat				
iris/cil	1.4 ± 0.1	12 ± 2	—	—
guinea pig				
iris sph.	2.4 ± 1.7	15 ± 2	—	—
dilator	0.4 ± 0.04	6 ± 1	—	—
iris/cil	— ± —	— ± —	7 ± 1	13 ± 1

Data from Unger et al.^{1,10}

Table II. Increase in of IOP, pupil constriction and soluble aqueous protein in the ipsilateral and contralateral rabbit eyes following unilateral infusion of CGRP⁹

Exper. = experimental eye and Contralat = contralateral eye)				
Dose	Response	Time	Exper.	Contralat.
0.6µg	IOP — mmHg	15-20 min	14 ± 3	5 ± 2
0.6µg	pupil —mm	15-45 min	.4 ± .1	.2 ± .2
0.6µg	protein Mg/ml	30 min	13 ± 1	6 ± 1

the mean ocular blood pressure (in excess of 60mmHg) and the pupillary constriction is also prolonged and more intense. The level of PGE-like activity in aqueous humour and the capacity of iris-ciliary tissue to form PGs *in vitro* from endogenous substrate are both greatly raised in the denervated eye. This supersensitivity to injury could not be attributed to raised PG levels since the response in denervated eyes pretreated with indomethacin is still greater than that evoked in normal untreated eyes.⁸² Classically, the enhanced response might be attributed to the withdrawal of uveal vasoconstrictor and iris dilator adrenergic tones which normally would exert functional antagonism to excitation events.

It was surprising, therefore, to find that the concentration of both substance P and CGRP in iris ciliary bodies is increased as much as three to five fold 30 days after sympathetic ganglionectomy (see Table III). Increased substance P is not seen in the first post-operative week^{3,58} but is clearly evident after three weeks³ and remains

elevated at 12 weeks.^{3,83} The hypertrophic changes of the sensory elements do not appear to be associated with the immediate process of sympathetic axon degeneration or to functional supersensitivity of the effector system.^{3,9,75,82-84} Ocular supersensitivity to irritation develops between 21 and 90 days^{82,85} which coincides with increased RIA levels and elevated immuno-histochemical staining of both substance P and CGRP in anterior uveal structures.^{2,3,5,9,72,82} Nerve fibres appeared to be both more abundant and more densely immunostained when compared with normal iris-ciliary bodies. Furthermore, this increase in substance P immunoreactivity appears to occur entirely within sensory fibres since it is depleted by subsequent trigeminal denervation.⁸³ The effect of sympathetomy on sensory activity seems therefore to be a chronic and not an acute or transient effect.

An increase in sensory neuropeptides after sympathetomy is not unique to the eye. Intensification of CGRP immunostaining has also been

Fig. 3. Neurogenic mediation by CGRP and substance P of the immediate ocular response to irritation^{9,13}

Table III. Effect of sympathetic denervation on CGRP-like and substance P-like immunoreactivity levels in extracts of iris ciliary-bodies ^{Data from 3,10}

Species	CGRP pmol/g		Substance P pmol/g	
	Denervated	Contralateral	Denervated	Contralateral
<i>guinea pig</i>				
iris sph.	51.7 ± 7.6	15.7 ± 2.4	—	—
dilator	36.6 ± 8.0	6.5 ± 1.0	—	—
<i>rabbit</i>				
iris sph.	— —	— —	31.3 ± 4.1	15.7 ± 2.7
dilator	— —	— —	17.2 ± 1.5	5.3 ± 1.7

observed in sympathectomised preparations of tongue, palate and epiglottis, all of which receive sensory fibres from the trigeminal system and their sympathetic supply from the superior cervical ganglion.⁸⁶ Similar changes occur in sympathectomised pineal gland,⁸³ cerebral arteries⁸⁷ and male genitalia.⁸⁸ Interestingly, hyperalgesia can develop over a period of time in patients who undergo therapeutic sympathectomy^{89,90} which may be analogous to the enhancement of the ocular response to irritation.^{3,82}

(d) Interaction between sympathetic and sensory nerves

Just as an increase of ocular substance P and CGRP occurs after chronic sympathetic denervation, a reciprocal elevation of specific material associated with sympathetic nerves can be seen in sensorily denervated eyes. Intact adrenergic nerves display, amongst other things, tyrosine hydroxylase (TH) and neuropeptide Y (NPY);⁷ not unexpectedly, immunostaining of both components is abolished in sympathetically denervated uveal tissue.^{2,72} Chronic loss of sensory innervation is accompanied, however, by increases in TH and NPY immunoreactivities.⁷² It would seem, therefore, that these separate nerve types in some way exert a reciprocal modulation of the functional status of the other.

The increase in tissue levels of transmitter substances of either nerve type appears to result from an increase in both the content of individual nerve elements and from the sprouting of new branches and collateral fibres. Fibres in sensorily or sympathetically denervated preparations appeared by micro-

scopic evaluation respectively to be thicker and more densely immunostained but also to be increased in numbers.^{4,72} Such hypertrophic changes might arise from alterations in the availability of endogenous nerve growth factor (NGF) normally formed by somatic tissues.^{91,92} NGF is essential for the growth and function of sensory and sympathetic nerves⁹³⁻⁹⁵ which take up and transport it to their cell bodies,^{93,95-97} where it promotes the synthesis of catecholamine forming enzymes⁹⁸ or neuropeptides.^{84,94,96,99} Nerve fibres within a tissue compete for available NGF thereby placing mutual constraint on the growth and activity of competing nerve elements and the loss of one set of fibres would make more NGF available. Moreover, Heumann and co-workers¹⁰⁰ have shown that synthesis of NGF by innervated tissue is increased after sciatic nerve transection and that Schwann cells elaborate receptors for NGF in the course of regenerative response.

Alternatively, factors produced by a functional nerve fibre might more directly influence the growth and activity of nerve elements within its somatic target area.^{101,102} It has been suggested that neuropeptides, particularly substance P, may promote the neural response to damage¹⁰³ and the outgrowth and development of neurons, including adrenergic neurons.^{98,104,105} CGRP, being present in sensory neurons together with substance P⁶ might exert similar trophic effects.

(e) Species differences in SP responses

Although substance P or a related tachykinin seems the most probable mediator of antidromic miosis in the rabbit eye, its role in the eye of higher mammals is not yet certain. Irrespective

Table IV. Isotonic contraction of isolated iris sphincter muscle preparations from various mammalian species to substance P and bradykinin or to electrical field stimulation. Results are given as molar concentration of drug producing a 50% maximal response EC_{50} responses to electrical field stimulation are either solely cholinergic (atropine sensitive) or display an additional residual non-cholinergic component, both of which are sensitive to tetrodotoxin.^{69,106}

Species	Substance P and Bradykinin				Electrical	TTX sensitive
	X	$10^{-8}M$	X	$10^{-7}M$	field stimulation Atropine	
Rabbit	2.7	(0.7)	4.8	(1.0)	no	yes
Bovine	9.8	(1.7)	3.7	(0.8)	no	yes
Pig	1.7	(0.1)	9.1	(0.3)	nd	nd
Cat	0	(at 500)	0	(at 100)	yes	yes
Dog	0	(at 500)	0	(at 100)	yes	yes
Baboon	0	(at 200)	0	(at 100)	yes	yes
Man	0	(at 100)	0	(at 100)	nd	nd

Values given as mean EC_{50} ($\pm$ SEM); nd = not determined.

of the presence of substance P-like immunoreactive nerves in their iris, isolated sphincter muscle preparations from the cat, dog, monkey and human eye do not contract to authentic substance P⁷⁰ or to any of the other tachykinins.⁷³ Nor are tissues from the same unresponsive species contracted by bradykinin (see Table IV) which, in rabbits iris sphincter preparations, produces contractions through sensory activation.^{28,29} In addition, frequency-dependent contractions to electrical field stimulation of cat, dog and monkey iris sphincter muscle are completely abolished by atropine, which suggests either an absence of a non-cholinergic, spasmogenic neurotransmitter or the absence of appropriate receptors on the muscle or at least of receptors coupled to the myogenic apparatus in these higher mammalian species.¹⁰⁶

The presence of substance P-containing elements within the iris sphincter region, therefore, need not be taken to indicate a direct miotic function. Peptides elaborated in the Gasserian ganglion cell bodies can be translocated along peripheral (as well as central) projections. (cf 23). Since a response to a neurotransmitter occurs by activation of postjunctional effector cells, the elaboration and insertion of tachykinin coupled receptors on the muscle plasma membrane might distinguish those species which respond from those which do not. Two types of tachykinin receptors have been deduced for rabbit iris sphincter muscle from kinetic data;^{6,73} however, both types are activated by all members of the tachykinin family

and conversely none of the peptides contract the iris from the baboon.^{6,73}

In conclusion, there is now substantial evidence for a complex interaction between autonomic and sensory nerves in the anterior uvea.

The importance of neuropeptide mediators in both homeostatic and reactive mechanisms in the mammalian eye is apparent, although much remains to be learned of their precise function and in particular their significance in developmental and pathogenic processes.

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