ARTICLE

5-Hydroxytryptamine$_{1A}$ agonists: potential use in glaucoma. Evidence from animal studies

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Abstract

Various classes of compounds exist to lower intraocular pressure (IOP) in the treatment of glaucoma. None of them is ideal since some patients respond better than others and the side effects vary between individuals. New classes of compounds need to be introduced to allow the clinician greater scope for effective treatment of all patients. It is now generally agreed that the cause of ganglion cell dysfunction in glaucoma is likely to be multifactorial and that concentrating solely on reducing IOP is inadequate. Irrespective of the reason for the dysfunction, the future goal must be to attenuate cell death. This may be achieved with drugs that interact with components of the retina, and is termed 'neuroprotection'. Thus, drugs that can both reduce IOP and act as neuroprotectants would be ideal for the treatment of glaucoma. In this article we summarise studies on animals which show serotonergic 5-HT$_{1A}$ agonists to both reduce IOP when topically applied to the rabbit eye and blunt the damaging effect to the rat retina and ganglion cells induced by glutamate toxicity or ischaemia. Reduction of IOP occurs via stimulation of 5-HT$_{1A}$ receptors associated with the ciliary processes. Neuroprotection of retinal neurones appears to involve the interaction of 5-HT$_{1A}$ agonists with membrane sodium channels and/or 5-HT$_{1A}$ or even possibly 5-HT$_7$ receptors. Various 5-HT$_{1A}$ agonists are used in patients to treat depression, so classes of these drugs have a proven safety profile for use in patients. The animal studies summarised in this article suggest that 5-HT$_{1A}$ agonists need to be considered as a new class of drugs for the treatment of glaucoma.

Key words Glaucoma, 5-Hydroxytryptamine$_{1A}$ agonists, Intraocular pressure, Neuroprotection

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In chronic glaucoma the ganglion cells of the retina appear to die at a slow and variable rate. Anatomical and physiological studies of glaucoma in humans[1] and experimental glaucoma induced in monkeys[2] suggest that retinal ganglion cells with large somata and large-diameter axons, which correspond to the magnocellular visual pathway, may show an increased vulnerability, although this has been questioned.[3] One possible cause of the death of ganglion cells is mechanical damage and/or ischaemic damage to their axons at the level of the lamina cribrosa induced by either elevated intraocular pressure (IOP) or altered blood flow. Another possible cause may be related to the finding that glutamate levels in the vitreous humour of glaucoma patients are elevated.[4] This glutamate could have originated from an injured retina, such as one that had experienced an ischaemic-like insult (hypoxia/anoxia) caused by reduced blood flow or raised IOP. An insult of this nature, which leads to the release of glutamate, would make retinal neurone-types (ganglion cells and a subset of amacrine cells) that contain ionotropic glutamate receptors particularly vulnerable. Anatomical studies on retinas of glaucoma patients support the opinion that both ganglion cells and certain amacrine cells are particularly affected (see review by Osborne *et al.*[5]). In experimental ischaemia, glutamate is released from the retina[6,7] and causes destruction of ganglion and some amacrine cells.[8] The released glutamate may well accumulate in the vitreous humour.

Chronic glaucoma, for which the model is primary open-angle glaucoma (POAG), is perhaps best defined as a heterogeneous group of disorders with a distinctive type of optic nerve damage and characteristic form of field loss. The major causes of glaucoma are likely to be hypoxia/anoxia (affected ocular blood supply to optic nerve head) and/or raised IOP. Therefore, the ideal drug for treatment of glaucoma is a substance which, when topically applied, reduces raised IOP, facilitates ocular blood flow in the optic nerve head and protects the retina from destruction.[9] Experimental studies on animals now give us good reason to believe that certain members of two classes of substances – β-blockers (e.g. betaxolol) and serotonin (5-hydroxytryptamine; 5-HT) 5-HT$_{1A}$ receptor agonists (e.g. 8-hydroxy-2-(di-*N*-propylamino)tetralin or 8-OH-DPAT and flesinoxan) – may fulfil these criteria. Betaxolol

Table 1. *The concentration of biogenic amines in the human aqueous humour*

Amine	Concentration (ng/ml)
Dopamine	0.2
Noradrenaline	1
Serotonin	50

From Martin *et al.*[15]

is already used to treat glaucoma patients and as a consequence our studies on the neuroprotective properties of betaxolol are of particular importance (for details see Osborne *et al.*[10,11]). However, in this article our studies on 5-HT_{1A} agonists will be summarised as they represent a new class of compounds for possible use in glaucoma. 5-HT_{1A} agonists, such as buspirone and ipsapirone, are presently used in the treatment of depression.[12] 8-OH-DPAT and flesinoxan are two of the most potent and selective 5-HT_{1A} receptor agonists and these drugs have proved invaluable in the study of 5-HT_{1A} receptor function.

Serotonin receptors in the anterior uvea

Indirect evidence suggests that some serotonergic nerves may exist in the iris–ciliary body of rabbits.[13] More compelling data show that serotonin is present in the human[14] and rabbit[13] iris–ciliary body as well as the aqueous humour, where the concentration of the amine is greater than that of noradrenaline (Table 1).[15,16] Moreover, binding (Table 2) and secondary messenger studies (Table 3) on iris–ciliary body tissue from the rabbit suggests that 5-HT_{1A} and 5-HT_2 receptors are present.[13,17] We have recently shown that mRNAs encoding the 5-HT_{1A} and 5-HT_7 receptors are associated with the iris–ciliary body complex of the rabbit and that the 5-HT_{1A} receptors are primarily associated with the epithelial cell bilayer of the ciliary processes (Figs. 1, 2).[18] Studies on the isolated human iris–ciliary body complex also support the existence of 5-HT_1-type receptors[19] and our preliminary experiments indicate that the mRNA which encodes the 5-HT_{1A} receptor is present (unpublished data).

Effect of serotonergic drugs on IOP

Topical application of 5-HT has been reported to both elevate[20] and lower[21] IOP in rabbits. In addition, 5-methyl-urapidil (a combined 5-HT_{1A} agonist/α_1-adrenoceptor antagonist) reduces rabbit IOP.[22]

Table 2. *Apparent affinity values (K_i) of various drugs for [^{3}H]5-HT binding to 5-HT_{1A} receptor sites in rabbit iris–ciliary body membranes*

Ligand	K_i value (M)
DP-5-CT	2.5×10^{-10}
MDL 73005EF	3.3×10^{-10}
5-CT	4.9×10^{-10}
5-HT	2.5×10^{-9}
8-OH-DPAT	3.6×10^{-9}
Ketanserin	$> 1.0 \times 10^{-4}$
Zacopride	$> 1.0 \times 10^{-4}$

Data are mean ± SEM value for three or four experiments.

Table 3. *Effects of various antagonists (1 μM) on the 5-HT (1 mM) induced accumulation of inositol phosphates (InsPs) in the rabbit iris–ciliary body*

Agent	% increase in InsPs accumulation relative to the effect of 5-HT
5-HT	100
5-HT + ketanserin	38 ± 8*
5-HT + methysergide	57 + 6*
5-HT + cyproheptadine	90 ± 3
5-HT + mianserin	66 ± 5*
5-HT + MDL 72222	80 ± 9
5-HT + prazosin	80 ± 7

Data are mean ± SEM value for three or four experiments.
*$p < 0.05$ by Student's *t*-test when compared with 5-HT.

8-OH-DPAT was originally thought to be a very selective 5-HT_{1A} agonist[23] but subsequently has been shown to have some affinity for the 5-HT_7 receptor.[24] 8-OH-DPAT when applied topically to the rabbit eye reduces IOP in both the light and dark.[25,26] The full 5-HT_{1A} agonist (+)8-OH-DPAT is a more effective hypotensive agent than the partial agonist (−)8-OH-DPAT (Fig. 3), and this result when taken with the finding that the effect of (±)8-OH-DPAT on IOP was blocked by pretreatment with pindolol, a mixed 5-HT_{1A} antagonist/β-blocker, but not by the specific β-blocker betaxolol, suggests that 8-OH-DPAT lowers IOP via activating 5-HT_{1A} receptors (Fig. 4).[26] Recent studies with a number of 5-HT_{1A} agonists/α_1-adrenoceptor antagonists (e.g. flesinoxan, WB 4101) provide support for the view that 5-HT_{1A} agonists reduce IOP. Flesinoxan is not only highly potent but also a full agonist at 5-HT_{1A} receptors, yet is a relatively weak α_1-blocker.[27] When applied topically to the rabbit, it causes a dramatic reduction in IOP (Fig. 5). Confirmation that the effect involves 5-HT_{1A} receptors was obtained by use of 5-HT_{1A} antagonists, which partially nullify the IOP response of the rabbits to flesinoxan (unpublished observations).

Studies with ketanserin also suggest that antagonists of 5-HT_2 receptors, when applied topically, can lower IOP. For example, topically applied ketanserin lowers IOP in the rabbit[21] and in man.[28,29] It should, however, be borne in mind that ketanserin is known to have an affinity for α_1-adrenoceptors[27] and for this reason the effects of ketanserin on IOP may not be entirely caused by its effect on 5-HT_2 receptors. Additional studies need to be conducted with specific 5-HT_2 ligands to clarify the situation.

Serotonin receptors in the retina

The evidence for serotonin being a neurotransmitter in the mammalian retina (as opposed to the non-mammalian retina) remains a matter of debate because there is a lack of clear data showing the existence of serotonergic neurones.[30,31] However, numerous studies have shown serotonin receptors to be associated with mammalian retinas. Using radiolabelled serotonin or its analogues, saturable 5-HT binding sites were shown to be associated with the retina;[32] moreover, multiple 5-HT receptor subclasses were identified in the rabbit retina, including 5-HT_1-like and 5-HT_2[33] and 5-HT_3.[34]

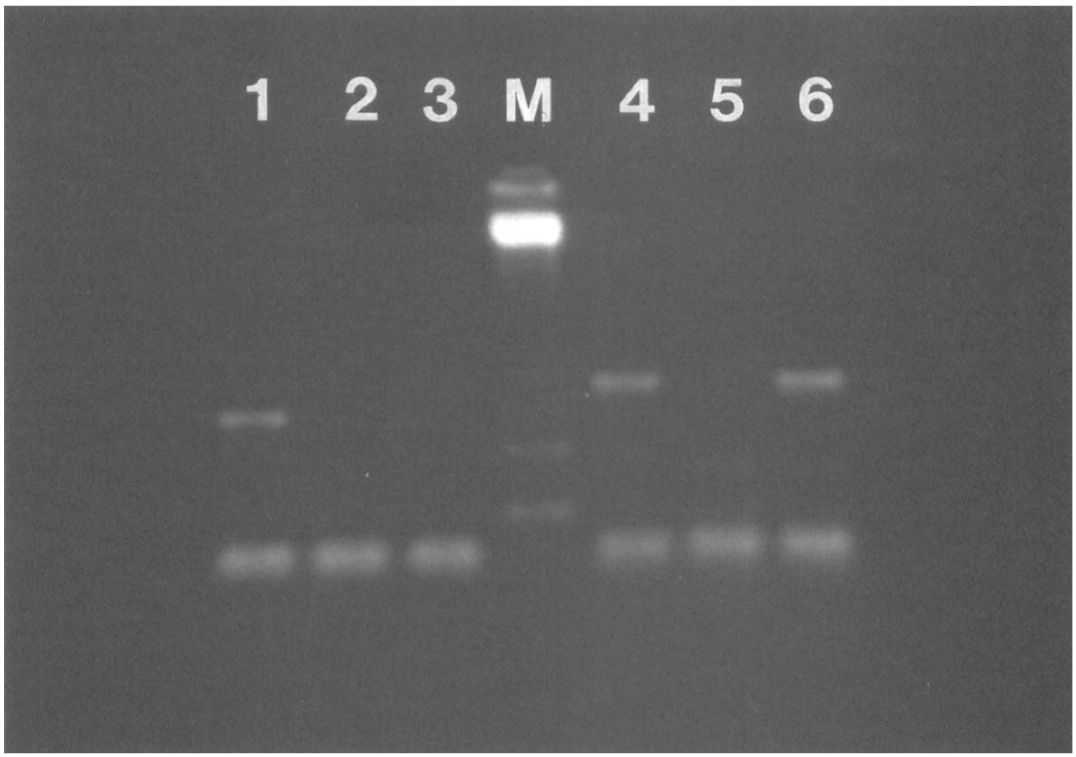

Fig. 1. *Agarose gel electrophoresis of PCR-amplified products of RNA from rabbit ciliary processes (lanes 1 and 4), cornea (lanes 2 and 5) and retina (lanes 3 and 6). The single band located at 357 bp seen in lane 1 corresponds to the expected length of the cDNA product produced by the 5-HT_{1A} receptor primers. The single band located at 464 bp seen in lanes 5 and 7 corresponds to the expected length of the cDNA product produced by the 5-HT_7 receptor primers. No band is detectable in lanes 2 and 3 or lane 6. The 123 bp ladder is shown in between (M).*

Secondary messenger studies also showed that serotonin increased cAMP[35,36] and inositol phosphate production,[37,38] demonstrating that retinal 5-HT receptors are coupled functionally to their known effector systems. At the time it was thought that the serotonergic receptors in the rabbit retina belonged to the 5-HT_{1A} subtype because of the pharmacological profile of the observed response. However, although early studies indicated that 5-HT_{1A} receptors can both stimulate[39] and inhibit[40] adenylate cyclase, it is now widely accepted that 5-HT_{1A}-like stimulations in cAMP production are mediated not through 5-HT_{1A} receptor activation but via the operationally similar 5-HT_7 subtype. Thus the increase in cAMP found in retinal tissue following application of serotonin is most likely due to activation of 5-HT_7 receptors, which are preferentially linked to an increase in cAMP synthesis. Support for this opinion has come from recent work which established the presence of 5-HT_7 mRNA[18] and 5-HT_7-like binding sites in the rabbit retina (Figs. 1, 2). Although 5-HT_{1A} receptors were not detected in rabbit retina it could equally not be concluded that they are absent, and indeed, preliminary RT-PCR data indicate that 5-HT_{1A} mRNA is present in human retina.

Neuropharmacological studies suggest that activation of 5-HT_{1A}, 5-HT_{2A} and 5-HT_3 receptors affect visual processing[41–43] and 5-HT_{2A} receptors are associated with terminals of the photoreceptors and rod bipolar cells in the rabbit retina.[44] However, as far as the other serotonin receptors are concerned (5-HT_{1A}, 5-HT_3 and 5-HT_7 receptors), no information exists on their distribution in the retina. In the context of glaucoma we therefore have no definitive evidence for ganglion cells containing a particular type of serotonin receptor.

Neuroprotective action of 5-HT_{1A} agonists

The direct neuroprotective action of 5-HT_{1A} agonists was demonstrated by use of confluent rat cortical cultures, which were prepared as previously described.[45] The cultures in serum-free medium were exposed to glutamate (100 μM) either alone or together with a 5-HT_{1A} agonist (100 μM flesinoxan or 8-OH-DPAT) for 4 h under normotoxic conditions. In other experiments the cultures were subjected to hypoxic conditions, 95% N_2/5%CO_2, for 5 h at 37 °C with 5-HT_{1A} agonists added before hypoxia where appropriate: reoxygenation was achieved by re-placing the cells in normoxic conditions for 3 h before analyses. Cellular injury (due to L-glutamate or hypoxia/normoxia) was assessed by measurement of lactate dehydrogenase (LDH) release into the cell culture medium.[45] As shown in Table 4 both flesinoxan and 8-OH-DPAT significantly counteracted the effect of L-glutamate or hypoxia/reperfusion. The 5-HT_{1A} antagonist/β-blocker, propranolol (5 μM), did not counteract the effect of flesinoxan or 8-OH-DPAT (Table 4). However, since propranolol had a protective effect on its own (Table 4) (probably because of its calcium channel blocking properties[46]), these experiments could not determine whether the effects of flesinoxan and 8-OH-DPAT are via activation of 5-HT_{1A} receptors.

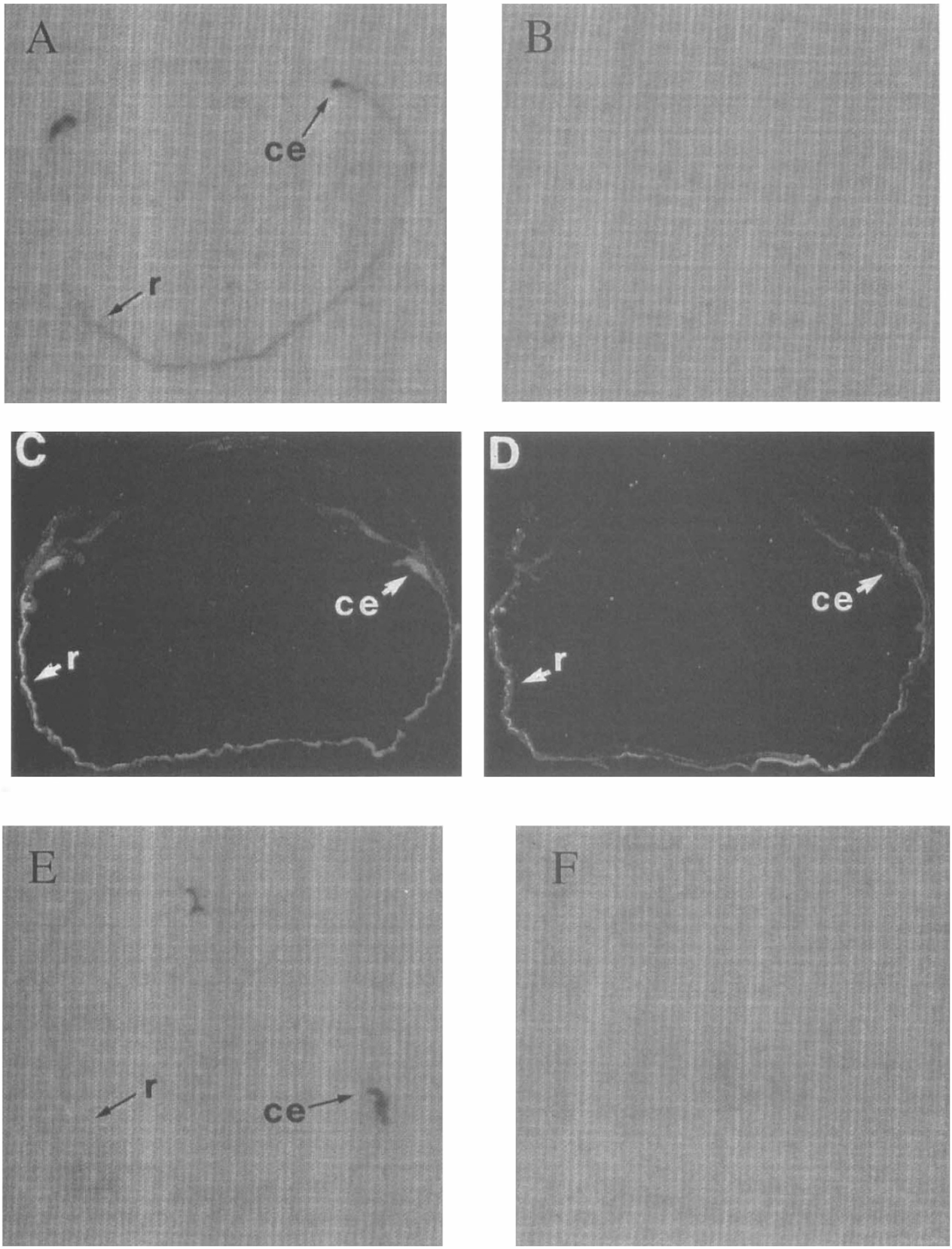

Fig. 2. *Distribution of 5-HT$_{1A/7}$ (A, B) and 5-HT$_{1A}$ (E, F) binding sites and 5-HT$_7$ mRNA (C, D) in sections of the rabbit eye as shown by receptor autoradiography and ISHH. (A) [^{3}H]5-carboxamidotryptamine (2 nM). (B) [^{3}H]5-carboxamidotryptamine (2 nM) + 5-HT (5 μM). (C) ^{35}S-labelled 5-HT$_7$ antisense probe. (D) ^{35}S-labelled 5-HT$_7$ sense probe. (E) [^{3}H]WAY 100–635 (3 nM). (F) [^{3}H]WAY 100–635 (3 nM) + 5-HT (5 μM). Specific 5-HT$_{1A/7}$ binding sites are observed in the retina and ciliary body (A), while 5-HT$_{1A}$ sites are only apparent in the ciliary body (E). Specific 5-HT$_7$ mRNA hybridisation signals appear to be found in the ciliary body, retina and possibly iris (C). ce, ciliary epithelium; r, retina.*

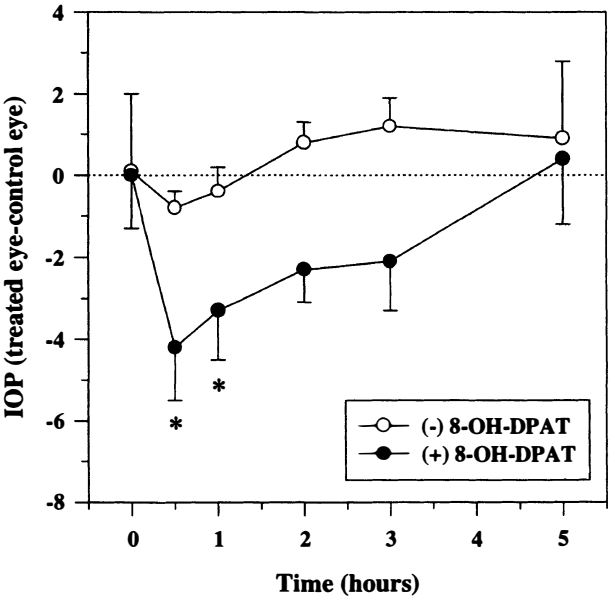

Fig. 3. *Effect of 30 μl of 0.1% (+)8-OH-DPAT and 0.1% (−)8-OH-DPAT on IOP in the dark. Contralateral eyes of rabbits received 30 μl of 0.9% NaCl. Data are represented as mean (± SEM) value calculated by subtracting the IOP of contralateral eyes from the IOP of treated eyes, where n = 5. Baseline IOPs varied from 28 to 30 mm Hg. *p < 0.05 by Student's paired t-test (treated eyes vs contralateral eyes).*

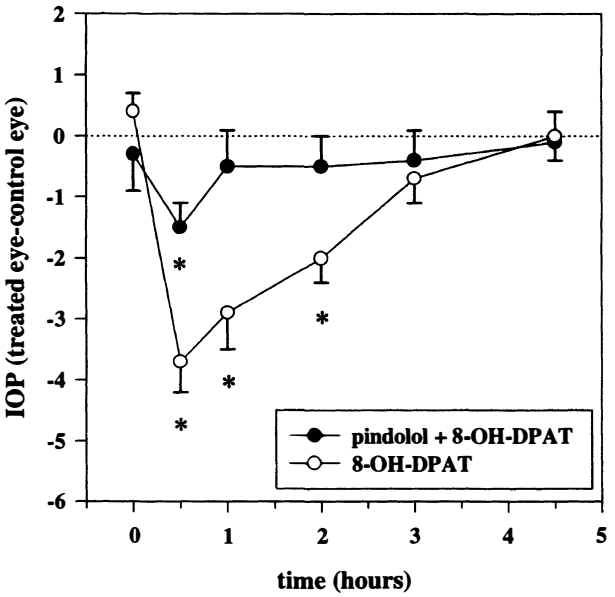

Fig. 4. *Effect of topical administration of 30 μl of 1% pindolol on the IOP response of normotensive NZW rabbits to 30 μl of 0.25% 8-OH-DPAT. Data are represented as mean (± SEM) value calculated by subtracting the IOP of contralateral eyes from the IOP of treated eyes, where n = 9. Contralateral eyes of rabbits received 30 μl of 0.9% NaCl. *p < 0.05 by Student's paired t-test (treated eyes vs contralateral eyes).*

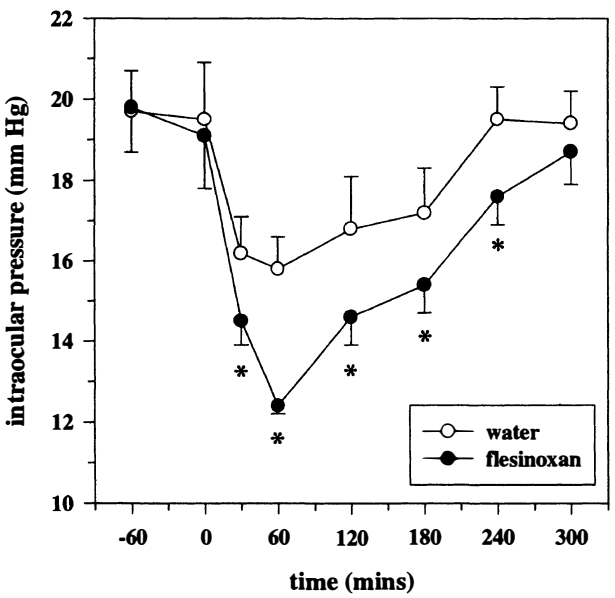

Fig. 5. *Effect of topical administration of 30 μl of 2% flesinoxan on the IOP of normotensive NZW rabbits. Data are represented as mean ± SEM from 6 rabbits. *p < 0.05, by Student's paired t-test (treated vs contralateral).*

To test the general neuroprotective properties of 8-OH-DPAT on the rabbit retina *in vivo*, 8-OH-DPAT was injected directly twice into the vitreous humour (concentration in the vitreous humour on each occasion 100 μM) of one eye, once just before ischaemia and then at the onset of reperfusion. Control animals received injections of vehicle in one eye. Ischaemia was induced in the injected eye by raising the IOP above the systolic blood pressure using a suction-cup procedure[47] for 60 min. Three days after ischaemia (the reperfusion period) the electroretinogram (ERG) was recorded from both eyes and the amplitude of the b-wave determined. The animals were then killed and sections of the retinas 'stained' for the localisation of choline acetyltransferase (ChAT) and parvalbumin immunoreactivities. Ischaemia/reperfusion causes a reduction of the b-wave (Fig. 6) and changes in the ChAT and parvalbumin immunoreactivities (Fig. 7). 8-OH-DPAT significantly counteracted the effects of ischaemia/reperfusion so that the nature of the b-wave and the staining of the ChAT and parvalbumin appeared more normal (Figs. 6, 7).

In 1982 we showed that the antigen Thy-1 is localised to ganglion cells[48] and subsequent studies have deduced that ischaemia/reperfusion to the rat retina causes destruction of the ganglion cells and loss of the mRNA[49] and the antigen[50] for Thy-1. By quantifying the mRNA for Thy-1 in the whole of the retina and relating the content to other retinal mRNAs (cyclophilin, rhodopsin) an assay for ganglion cell survival was constructed.[49] As shown in Fig. 8, intravitreal injection of *N*-methyl-D-aspartate (NMDA; 20 nmol) to the rat retina causes a significant reduction in retinal Thy-1 mRNA level relative to cyclophilin, while this is not the case for rhodopsin mRNA. However, when 8-OH-DPAT (20 nmol) is co-injected into the vitreous humour (to give a concentration of 200 μM in the vitreous humour) at the same time as NMDA the reduction in the retinal Thy-1 mRNA level is significantly attenuated (Fig. 8). We conclude from such experiments that NMDA-induced toxicity to the ganglion cells is attenuated by 8-OH-DPAT.

Our most recent studies show that 8-OH-DPAT can act as a retinal neuroprotectant even when applied topically to the eye. It is assumed that the topically

Table 4. *The effect of 8-OH-DPAT, flesinoxan, propranolol and MK-801 on the glutamate and hypoxia/reperfusion-induced release of lactic dehydrogenase (LDH)*

Treatment				% of LDH released in comparison with total LDH	
Control				10 ± 2	(*n* = 10)
Glutamate			(50 μM)	29 ± 5	(*n* = 10)
Glutamate	+	MK-801	(5 μM)	10 ± 3	(*n* = 4)
Glutamate	+	8-OH-DPAT	(100 μM)	18 ± 4	(*n* = 4)
Glutamate	+	Flesinoxan	(100 μM)	17 ± 2	(*n* = 4)
Glutamate	+	Propranolol	(5 μM)	19 ± 4	(*n* = 4)
Control				12 ± 3	(*n* = 5)
H/R[a]				23 ± 6	(*n* = 5)
H/R[a]	+	MK-801	(5 μM)	12 ± 4	(*n* = 3)
H/R[a]	+	8-OH-DPAT	(100 μM)	14 ± 3	(*n* = 3)
H/R[a]	+	Flesinoxan	(100 μM)	13 ± 5	(*n* = 3)
H/R[a]	+	Propranolol	(5 μM)	15 ± 4	(*n* = 3)

The basal (control) release of LDH into the culture medium was 21 ± 4 mU/ml/mg protein (*n* = 15).
Results are mean ± SEM. Number (*n*) of experiments is shown in parentheses.
All the drugs significantly ($p < 0.05$; using one-way ANOVA followed by Dunnett's test) reduced the effect of glutamate or hypoxia/reperfusion.
Note: MK-801 is an NMDA receptor antagonist.

applied drug reaches the retina through the systemic system, as this appears to be the case in experiments where betaxolol was used.[11] In these experiments, the drug (or vehicle) was topically applied (5 μl of 0.5% solution) 10 min and 5 min before ischaemia, directly after ischaemia and then daily during reperfusion. The b-wave of the ERG was also measured at 2 and 5 days during reperfusion. Fig. 9 shows that the b-wave of the ERG in the 8-OH-DPAT treated animals is significantly less reduced (*n* = 4) than in those animals which received vehicle, thus suggesting a 'protection' against ischaemia/reperfusion. Moreover, the ChAT immunoreactivity (5 days following reperfusion) was completely obliterated in retinas which received ischaemia in all vehicle-treated animals (*n* = 4) but traces of ChAT immunoreactivity were evident in three of the four animals which received 8-OH-DPAT (Fig. 10).

Neuroprotective mode of action of 8-OH-DPAT

8-OH-DPAT is a potent agonist at 5-HT_{1A} and a partial agonist at 5-HT_7 receptors,[24] and both receptor-types exist in the mammalian retina (see above). Thus the neuroprotective effect of 8-OH-DPAT on ischaemia/reperfusion or NMDA-induced effects on the b-wave of the ERG, ChAT and parvalbumin immunoreactivities and ganglion cell Thy-1 mRNA levels may be mediated via an action on 5-HT_{1A} and/or 5-HT_7 receptors. Studies on brain tissues have demonstrated that neuroprotection can be induced by activation of 5-HT_{1A} receptors.[51,52] However, in our studies we could not draw such a conclusion because we did not blunt the neuroprotective effects of 8-OH-DPAT with propranolol. In addition, propranolol is not a very effective 5-HT_{1A} antagonist and more selective and specific antagonists need to be tested.

Laboratory studies show that there are various ways of attenuating neuronal death caused by ischaemia/reperfusion.[5] Substances, for example, which prevent an unusual rise in calcium and/or free radicals in an insulted neurone are likely to act as neuroprotectants.[5] Although we have not tested whether 8-OH-DPAT acts as a free radical scavenger, studies have been conducted to determine whether it can act as a 'calcium channel blocker'. Initial experiments were carried out on rat cortical cultures as described elsewhere.[53] The cortical cultures in a medium containing $^{45}Ca^{2+}$ are exposed to either to NMDA alone or in combination with 8-OH-DPAT. As shown in Table 5, NMDA stimulates an influx of $^{45}Ca^{2+}$ into the neurones and 100 μM 8-OH-DPAT causes a significant attenuation. Similar results have been generated with betaxolol.[11] We next investigated whether 8-OH-DPAT has an affinity for the L-type calcium channel using a radioactive binding procedure described elsewhere.[46] Surprisingly, 8-OH-DPAT had very little affinity for the L-type calcium channel (Fig. 11). Since betaxolol binds to the

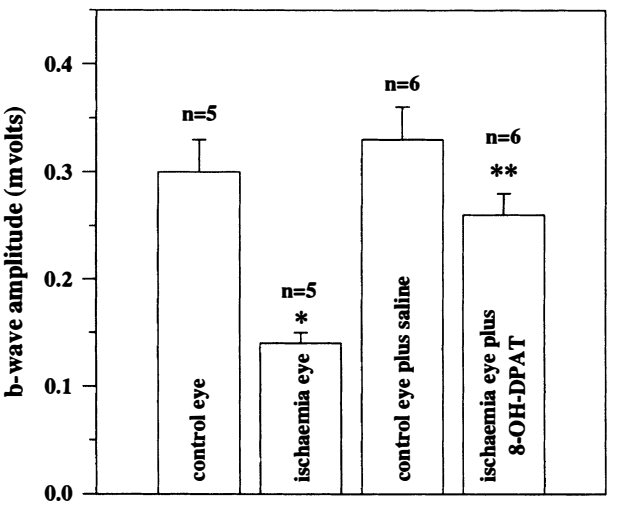

Fig. 6. *Two groups of animals (n = 5) were used. The right eye of one group received 10 μl saline before and after ischaemia; the left eye received 8-OH-DPAT (final concentration in vitreous humour estimated to be 100 μM) at the same times. The left eye of both groups of animals received ischaemia with a suction methodology for 60 min. The amplitudes of the b-wave of the electroretinogram was determined 3 days after ischaemia, i.e. following 3 days of reperfusion. It can be seen that administration of 8-OH-DPAT attenuates the reduction in the b-wave relative to the control. *p < 0.05 compared with control; **p < 0.05 compared with ischaemia.*

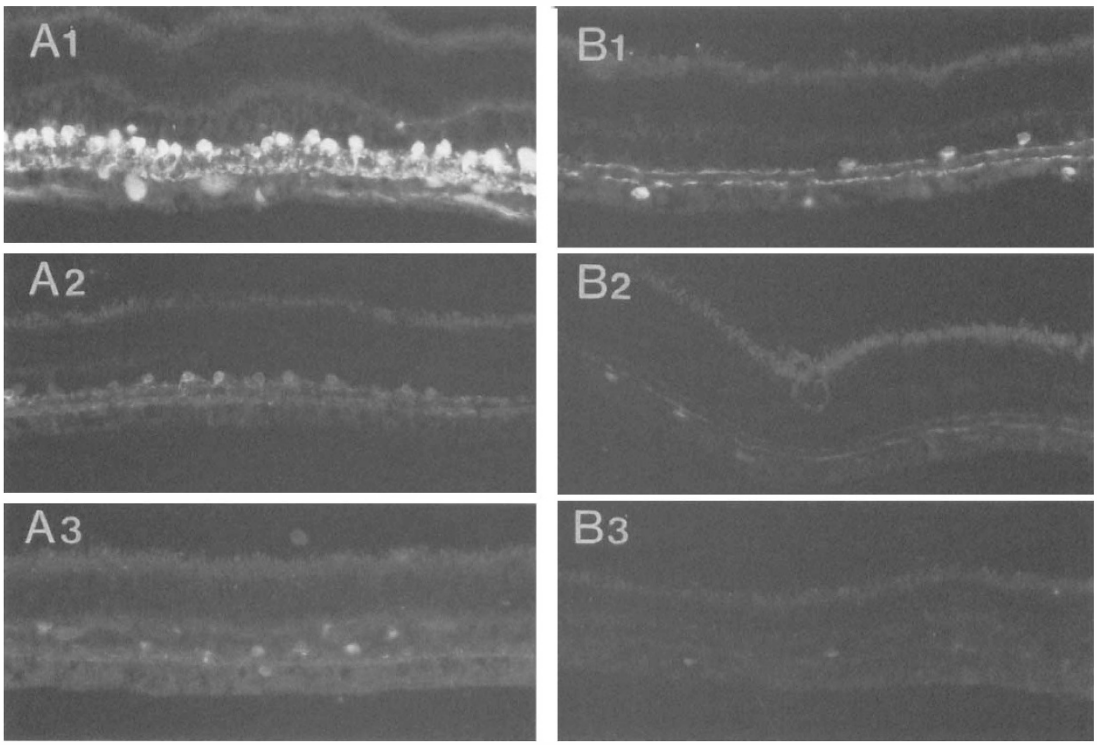

Fig. 7. *(A1) and (B1) The normal distribution of parvalbumin and choline acetyltransferase (ChAT) immunoreactivities in the rabbit retina, respectively. Following ischaemia/reperfusion both the parvalbumin (A3) and ChAT (B3) immunoreactivities are reduced and clearly affected. However, treatment of animals with 8-OH-DPAT clearly blunted the effect of ischaemia/reperfusion, documented by a clear 'staining' for both parvalbumin (A2) and ChAT (B2).*

L-type calcium channel[46] it is concluded that the neuroprotective properties of 8-OH-DPAT and betaxolol are different. The possibility exists that 8-OH-DPAT reduces calcium influx into cortical neurones by acting at other type(s) of calcium channels, such as N-, P/Q- or T-. Another possibility is that it acts directly at 5-HT_{1A} receptors situated on the cortical neurones. However, spiroxatrine (a putative 5-HT_{1A} antagonist) did not blunt the effect of 8-OH-DPAT on the NMDA-induced influx of calcium, which does not support this idea (see Table 5).

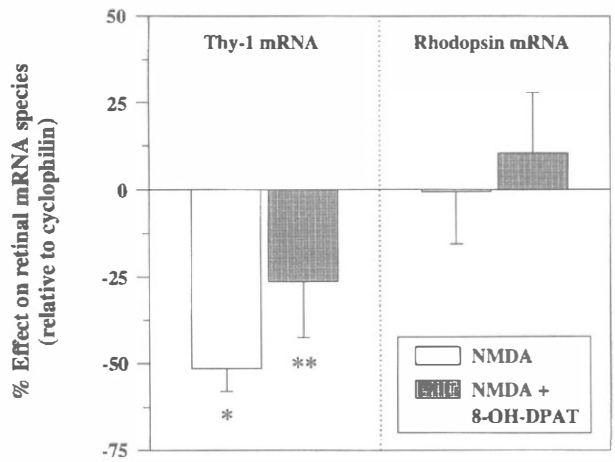

Fig. 8. *The effect of intravitreal injection of* N-*methyl*-D-*aspartate (NMDA; at a final concentration of 200 μM in the vitreous; open bars) or NMDA plus 8-OH-DPAT (both 200 μM; shaded bars) on the overall levels of Thy-1 and rhodopsin mRNA species in the retina (n = 4). Data are derived from densitometric analyses of duplicated RT-PCR experiments. Densitometric readings for Thy-1 and rhodopsin mRNA species were calculated relative to cyclophilin mRNA levels and expressed as percentages of control amounts in each case. Note that rhodopsin mRNA is unaffected by NMDA whereas this compound leads to a significant reduction in Thy-1 mRNA, which is partially ameliorated by 8-OH-DPAT. *p < 0.05, compared with control vehicle injected values using paired Student's t-test (n = 4); **p < 0.05, compared with values obtained for NMDA-treated retinas using paired Student's t-test (n = 4).*

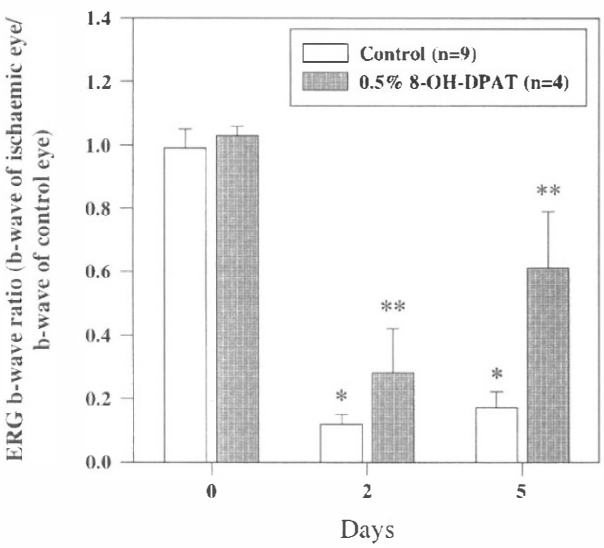

Fig. 9. *Summary of the effect of topically applied 0.5% 8-OH-DPAT on the amplitude of the* b-*wave of the electroretinogram (ERG) following ischaemia. It can be seen that the* b-*wave recovery of untreated animals following ischaemia and reperfusion times of 2 and 5 days is around 15%. In the 8-OH-DPAT treated animals the recovery at 2 days is around 30% and at 5 days greater than 50%. *p < 0.05 compared with t = 0; **p < 0.05 compared with control.*

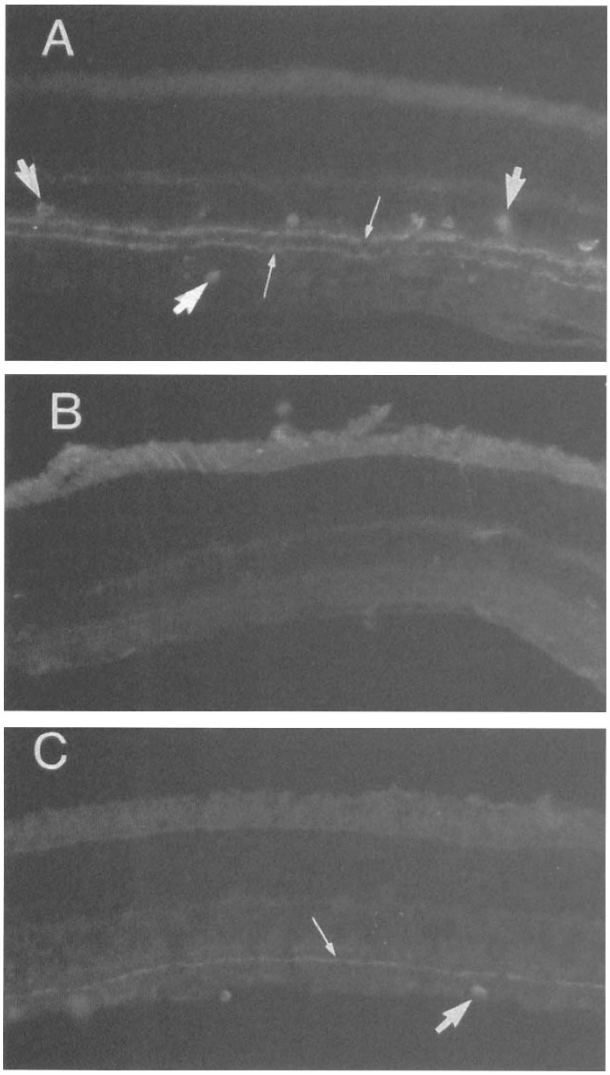

Fig. 10. *(A) Distribution of ChAT immunoreactivity in the rat retina. Immunoreactivity is associated with two bands of fibres in the inner plexiform layer (small arrows) and perikarya on each side (large arrows). Following ischaemia/reperfusion most of the ChAT immunoreactivity is obliterated (B). However, when animals are treated topically with 8-OH-DPAT, the ischaemia/reperfusion-induced effect on the ChAT immunoreactivity is partially blunted with some ChAT 'staining' still apparent (C).*

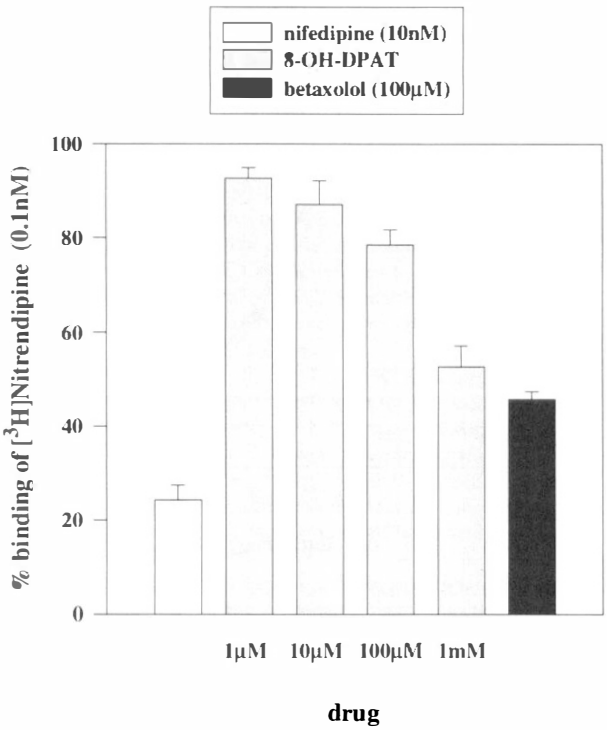

Fig. 11. *Effect of 8-OH-DPAT on the specific binding of 0.1 nM [^{3}H]nitrendipine to rat cortical membranes. Effects of nifedipine and betaxolol are shown for comparison. Each point represents the mean ± SEM of three to five experiments performed in duplicate.*

Recent studies have shown that substances which reduce sodium influx into neurones are also neuroprotective.[54] The neuroprotection may occur by indirectly reducing calcium influx; nevertheless, good evidence exists which demonstrates that activation of voltage-sensitive sodium channels (VSSCs) is involved in the pathophysiology of ischaemic damage. There are three major types of VSSCs in the brain, termed Rat I, Rat II and Rat III, which are responsible for the generation of action potentials in excitable membranes. Several neurotoxins are known to bind with high affinity to specific sites on these VSSCs and alter channel function. Five classes of these neurotoxin binding sites are currently recognised (neurotoxin sites 1–5), the most important of which are sites 1 and 2. Site 1 is located in the vestibule of the channel at the selectivity filter and binding of toxins such as tetrodotoxin and saxitoxin directly blocks the passage of Na^+ ions through the channel. Conversely, site 2 is found in the transmembrane region and is involved in the gating of the channel. Lipid-soluble toxins including veratridine and batrachotoxin bind at site 2 causing a persistent activation of the sodium channel, which can be reversed by tetrodotoxin in a non-competitive fashion. Several local anaesthetics, antiarrhythmics and anticonvulsants inhibit neuronal excitability by interacting with VSSCs at

Table 5. *The effect of 8-OH-DPAT and 8-OH-DPAT plus spiroxatrine on the NMDA-induced accumulation of radioactive calcium by rat cortical cultures*

Treatment						$^{45}Ca^{2+}$ uptake % in comparison with control values	
Control						100	
NMDA			(100 μM)			230 ± 40	(n = 14)
NMDA	+	8-OH-DPAT	(10 μM)			210 ± 38	(n = 5)
NMDA	+	8-OH-DPAT	(100 μM)			148 ± 29	(n = 9)
NMDA	+	8-OH-DPAT	(100 μM)	+	spiroxatrine (10 μM)	152 + 34	(n = 6)
NMDA	+	MK-801	(5 μM)			114 + 11	(n = 4)

Results are mean ± SEM, each carried out in triplicate. Number (n) of experiments is shown in parentheses.
Control cultures accumulated approximately 9000 dpm of $^{45}Ca^{2+}$.
Note: 8-OH-DPAT at 10 μM had no significant effect on the NMDA response.

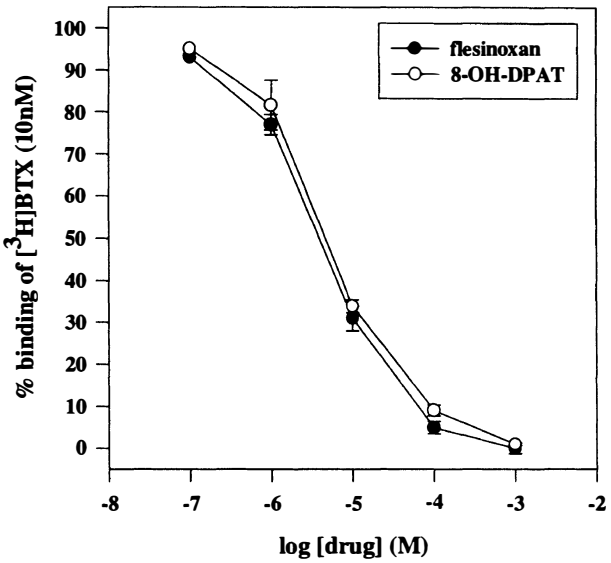

Fig. 12. *Effect of 8-OH-DPAT and flesinoxan on the specific binding of 10 nM [^{3}H]batrachotoxinin (BTX) to rat forebrain membranes. Each point represents the mean ± SEM of three experiments performed in duplicate.*

neurotoxin site 2. More importantly, neuroprotective compounds have also been shown to interact with neurotoxin site 2 in such a way as to inhibit Na^+ influx induced by veratridine or batrachotoxin.[55] The capacity for 5-HT_{1A} agonists to interact with neurotoxin site 2 and potentially to reduce sodium influx was therefore investigated.

In order to ascertain whether 5-HT_{1A} agonists interact with VSSCs, radioligand binding experiments were performed using the neurotoxin site 1 and 2 selective ligands [^{3}H]saxitoxin and [^{3}H]batrachotoxinin, respectively. 8-OH-DPAT and flesinoxan had little effect on the binding of [^{3}H]saxitoxin to rat forebrain membranes (data not shown). However, both compounds potently displaced [^{3}H]batrachotoxinin binding, indicating that the drugs do interact with neurotoxin site 2 (Fig. 12). Our latest preliminary data indicate that this interaction is inhibitory, since the influx of $^{22}Na^+$ into rat cortical synaptosomes induced by veratridine was dose-dependently attenuated by both 8-OH-DPAT and flesinoxan (data not shown).

Conclusions

It is generally agreed that disturbances of ocular blood flow (vascular dysregulation, perfusion pressure changes) and/or IOP is/are involved in the pathogenesis of glaucoma. It is also probable that other factors (age, genes, diet, endocrine abnormalities) may play a part. In any event, it is the death of the ganglion cells that leads to loss of vision. Clearly any drug which reduces the rate of ganglion cell death in glaucoma, irrespective of how death may have occurred, will theoretically benefit a glaucoma patient. If such a drug can additionally reduce the one risk factor that is measurable, i.e. raised IOP, and also possibly facilitate ocular blood flow then the drug in question is potentially important. If the drug can also be tolerated by the patient as well as reaching the retina when applied topically then the criteria for the ideal glaucoma drug are approached.

Many 5-HT_{1A} agonists are used in the treatment of depression so they can be reasonably tolerated by patients. Moreover, 5-HT_{1A} agonists such as urapidil are known to dilate blood vessels, so theoretically could stimulate ocular blood flow. Our studies have shown that 5-HT_{1A} agonists (8-OH-DPAT and flesinoxan) reduce IOP in rabbits. Moreover, we show that 8-OH-DPAT blunts the effects of insults (NMDA toxicity, ischaemia/reperfusion) to the retinas of animals. The neuroprotective effect of 8-OH-DPAT involves reducing the influx of sodium but it may also involve interaction with retinal 5-HT_{1A} and/or 5-HT_7 receptors, although this remains to be demonstrated. Thus it is proposed that 5-HT_{1A} agonists need to be considered as a class of drugs for the treatment of glaucoma.

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