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Serotonin and melatonin in the iris/ciliary processes and their involvement in intraocular pressure

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Abstract. From the evidence available it is clear that melatonin and serotonin receptors exist in the iris/ciliary processes of the rabbit. These receptors may be involved in maintaining the intraocular pressure (IOP). However, the published results on this point are often contradictory, perhaps because of the variation in the species of animals used and the methodology employed. It is also clear that the data obtained from studies on the rabbit cannot be directly applied to man. Nevertheless, present information points to the possibility that drugs influencing specific serotonin and/or melatonin receptors may be used to influence IOP in man and thus have a therapeutic effect.

Key words: intraocular pressure, IOP, melatonin, serotonin, iris/ciliary processes, rabbit

INTRAOCULAR PRESSURE

The major factors determing the level of the eye's intraocular pressure (IOP) are the rate of aqueous humour production and the resistance encountered in the outflow channels. Glaucoma is characterised by an IOP sufficiently elevated to produce ocular tissue damage either transiently or permanently. Primary open-angle glaucoma accounts for about two thirds of all glaucomas (Banks et al. 1968) and is characterised by a raised IOP and loss of retinal ganglion cells. The cause of neuronal loss remains unknown. It has long been assumed that the cause is a deficient blood supply to the optic nerves. Another idea is that the raised IOP distors the lamina criborsa in the optic disc region, damaging neurones as they pass through this tissue. Yet another idea is that the connective tissue of the lamina criborsa is defective, causing distortion and subsequent damage to the retinal neurones. However, increased IOP is considered by many as a major factor in either the direct or indirect cause of glaucoma. The clinical approach to patients presented with an elevated IOP is to concentrate on lowering it.

The level of IOP is related to the rate of aqueous humour production and drainage in the outflow channels. Secretion of aqueous humour is by the ciliary epithelial cells caused by an active transport process. The basis hypothesis (Cole 1977) is that the unpigmented ciliary epithelial cells selectively absorb sodium from the stroma and transport it into the intercellular clefts, which are closed to the stromal side by tight junctions but are open at the aqueous humour side; the development of hyperosmolarity in the clefts leads to osmotic flow of water from the stroma and thus to a continuous flow of fluid along the clefts. The passage of other ions may also be governed by independent active transport processes of e.g. chloride, bicarbonate, potassium ions. The hydrostatic gradient necessary for the process of aqueous humour secretion to take place is influenced by the level of blood pressure in the ciliary arteries.

Outflow of aqueous humour is by two routes generally: Schlemm's canal via the trabecular meshwork and the uveoscleral pathways (Davson 1990). The intricate balance between formation and outflow of the aqueous humour determines the level of IOP. A raised IOP can be due to factors affecting secretion or drainage and various drugs have been developed to lower IOP by having their sites of action on either secretion (e.g. β-adrenergic antagonists) or drainage (e.g. α_2-adrenergic agonists).

INVOLVEMENT OF SEROTONIN IN IOP DYNAMICS

Classification of serotonin receptors

The multiple actions of serotonin or 5-HT (5-hydroxytryptamine) are mediated by the specific interaction of the amine with several receptors. Data from pharmacological and physiological studies identified distinct receptors which were subsequently designated 5-HT_{1A}, 5-HT_{1B}, 5-HT_{1C}, 5-HT_2, 5-HT_3 and 5-HT_4 (Bradley et al. 1986, Osborne and Hamon 1988, Peroutka 1990, Bockaert et al. 1992), although the pharmacology of some studies suggest the existence of additional 5-HT receptor subtypes. Recently, the molecular cloning of thirteen different mammalian receptor subtypes revealed an unexpected heterogeneity within 5-HT receptors (Saudou and Hen 1994). The latest classification of 5-HT receptor subtypes takes into account not only their pharmacological profile and influence on secondary messengers but also their amino acid sequence (Hoyer et al. 1989, 1994, Saudou and Hen 1994).

Except for the 5-HT_3 receptors which are ligand-gated ion channel receptors, all the other 5-HT receptors belong to the large family of receptors interacting with G-proteins. Their amino acid sequence homology and coupling to secondary messengers allow these receptors to be divided into distinct families (see Table I).

The 5-HT_1 family contains receptors that are negatively coupled to adenylate cyclase i.e. the 5-HT_{1A}, 5-$HT_{1B/1D\beta}$ and 5-HT_{1F} (5-$HT_{1F\beta}$) receptors as well as receptors found in *Drosophila* (5-HT_{dro2A}, 5-HT_{dro2B}) (see Saudou and Hen 1994).

TABLE I

5-HT receptor families (from Saudou and Hen 1994)

Superfamily	Family	Transduction system	Subtypes	Comments previous names
G Protein-coupled receptors	5-HT_1	↘ AC	5-HT_{1A} 5-$HT_{1B/D\beta}$ 5-$HT_{1D\alpha}$ 5-HT_{1E} 5-HT_{1F} 5-HT_{dro2A} 5-HT_{dro2B}	The 5-HT_{1B} receptor is the rodent homologue of the 5-$HT_{1D\beta}$ receptor previously called 5-$HT_{1E\beta}$
	5-HT_2	↗ PLC	5-HT_{2A} 5-HT_{2B} 5-HT_{2C}	previously called 5-H_{T2} rat fundus receptor, SLR, 5-HT_{2F} previously called 5-HT_{1C}
	adenylyl cyclase stimulatory 5-HT receptors	↗ AC	5-HT_4 5-HT_6 5-HT_7 5-HT_{dro1}	no molecular data available also called 5-HT_X
	5-HT_5	ion channels?	5-HT_{5A} 5-HT_{5B}	5-HT_5
Ligand-gated ion channels	5-HT_3	Na^+/K^+ channel	5-HT_3	

The 5-HT_2 family includes receptors that stimulate phospholipase C i.e. the 5-HT_{2A} receptor previously known as 5-HT_2 receptor and the 5-HT_{2B} and 5-HT_{2C} receptors which correspond to the previously known 5-HT_{1C} and stomach fundus 5-HT_2-like receptors, respectively.

The adenylate cyclase stimulatory receptors are a heterogenous group including the 5-HT_4 receptor, a non-mammalian (5-HT_{dro1}) serotonin receptor and a further two mammalian receptors termed 5-HT_6 and 5-HT_7 receptors (see Saudou and Hen 1994).

The 5-HT_{5A} and 5-HT_{4B} receptors might constitute a new family of 5-HT receptors as they have little amino acid homology with the 5-HT_1, 5-HT_2, 5-HT_6 and 5-HT_7 receptors. Furthermore, unlike all other G-protein coupled serotonin receptors, the 5-HT_5 receptors do not modulate the activity of adenylate cyclase or phospholipase C. Their effector system remains to be established.

Serotonin in the iris/ciliary body

A transmitter or hormonal role of serotonin in the iris/ciliary body was first suggested by the finding that intravenous injection of the amine to dogs and rabbits lowers their IOP (Chiang 1974), while in the rat a strong constriction of the retinal blood vessels was reported (Tammisto 1965). Studies by Moro et al. (1981) then showed that following an intravenous injection of 5,7-dihydroxytryptamine to destroy serotonergic terminals, the rabbits' pupil diameter was decreased but the IOP remained unaltered.

Studies on the rabbit iris/ciliary body provide only weak evidence for the presence of a serotoner-

TABLE II

Effects of various antagonist (1μM) on the 5-HT (1mM) induced accumalation of InsPs in ICB of rabbit

Agent	% Increase in InsPs accumulation relative to the effect of serotonin
5-HT	100%
5-HT plus ketanserin	38± 8*
5-HT plus methysergide	57± 6*
5-HT plus cyproheptidine	90±3
5-HT plus mianserin	66±5*
5-HT plus MDL 72222	80±9
5-HT plus prozosin	80±7
5-HT plus atropine	112±9

Results are mean values ±SEM where n = 3-4 ;* Significant inhibition as evaluated by Student's t-test $P<0.05$; ICB = iris/ciliary processes.

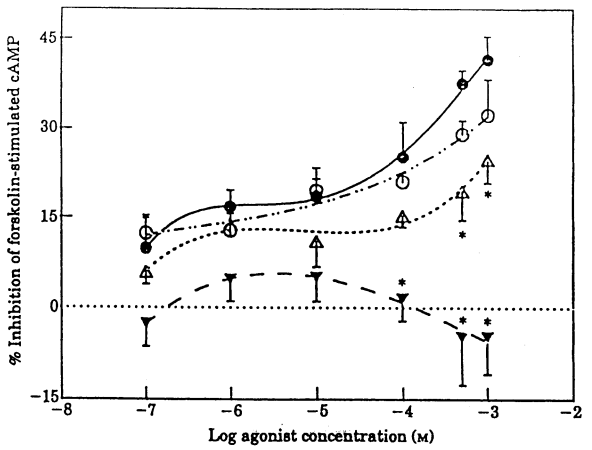

Fig. 1. Dose-response curve for the effects of serotonin (○) and 5-CT (●) on forskolin-stimulated cAMP levels in rabbit iris-ciliary body homogenates. It can be seen that the antagonists propranolol (1μM, Δ) and spiperone (1mM,▼) end to counteract the inhibition of the forskolin-elevated cAMP caused by 5-CT. Values are means SEM where n = 3-5. *$P<0.05$ (from Barnett and Osborne 1993).

gic innervation (Osborne and Tobin 1987, Tobin et al. 1988). While HPLC analysis of the iris/ciliary body tissue demonstrated the existence of low amounts of serotonin, immunohistochemical attempts to localise the amine have proved negative. However, interestingly, when the iris/ciliary body is exposed to exogenous serotonin, the presence of 5-HT immunoreactive fibres can be extensively demonstrated (Osborne and Tobin 1988). The relatively high density of 5-HT accumulating fibres raises the possibility that the amine has been taken up into noradrenergic fibres. This is supported by the fact that the distribution of tyrosine-hydroxylase (enzyme involved in the synthesis of noradrenaline) immunoreactive fibres in the iris/ciliary body closely parallels the localisation of serotonin-accumulating fibres (Tobin et al. 1988). As serotonin has been found to also exist in an aqueous humour (Martin et al. 1988) it is possible that the low amounts of amine associated with the iris/ciliary body as found by HPLC (Tobin et al. 1988) represent amine taken up by the tissues and/or contamination.

Regardless of whether serotonin nerves actually exist in the iris/ciliary body the presence of 5-HT receptors is good. Specific binding sites for the amine have been reported and characterised as being 5-HT_1-like in nature (Mallorga and Sugrue 1987). Serotonin also reduces the forskolin-induced elevation of cAMP content in rabbit (Fig. 1) and human iris/ciliary body tissues (Tobin and Osborne 1989, Barnett and Osborne 1993) thus supporting the idea for the existence of 5-HT_1 receptors. As spiperone and propranolol are particularly effective in counteracting the reduction of elevated cAMP caused by the 5-HT_1 agonist, 5-carboxamidotryptamine (Fig. 1), the presence of 5-HT_1-type receptors is suggested. Molecular biology studies are now required to confirm these pharmacological observations.

As well as a 5-HT_{1A} type receptor negatively coupled to adenylate cyclase, another type of serotonin receptor associated with the activation of phospholipase C exists in the iris/ciliary body. As shown in Fig. 2 serotonin stimulates inositol phosphates accumulation in a dose dependent manner in the iris/ciliary body and this effect is partially counteracted by ketanserin, methysergide and mianserin (Table II). Such studies argue for the occurence of 5-$HT_{2A/2C}$-type receptors (Tobin et al. 1988). Clearly molecular biological studies are re-

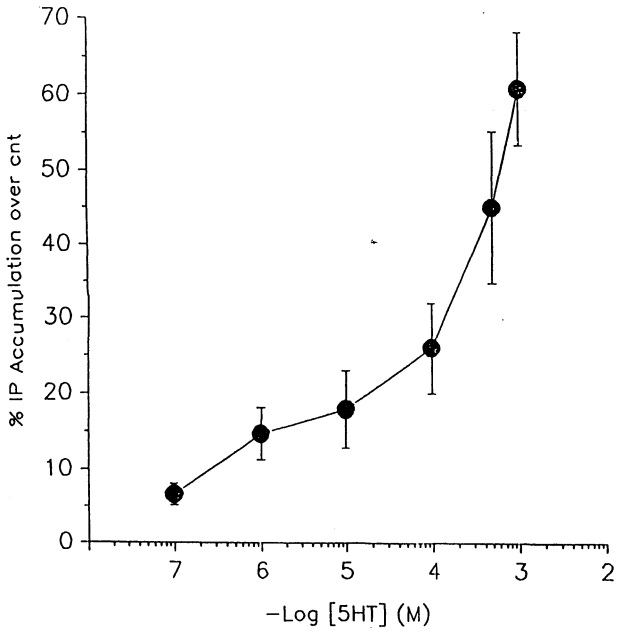

Fig. 2. Dose-response curve for serotonin stimulated accumulation of inositol phosphates (IP) in the iris-ciliary body (n = 5-7 ±SEM).

quired to clarify more percisely the receptor-type(s) present.

Serotonin and IOP

Topical application of serotonin to the rabbit eye has been described to decrease (Krootila et al. 1987a) or increase (Meyer-Bothling et al. 1993) IOP. The 5-HT_1 agonist, 5-CT, when applied topically to the rabbit eye has also been reported to increase the IOP (Fig. 3)(Meyer-Bothling et al. 1993). In addition, intracameral injection of serotonin into the rabbit eye has been reported either to have no effect (Chiou et al. 1985) or raise the IOP (Krootila et al. 1987b). A rationale for such apparently contradictory results is difficult to provide but may reflect time of serotonin administration, handling of the animals as well as method for recording IOP. Diurnal changes in the rabbits IOP have been shown to occur (Chiou et al. 1985) and differences do exist between pigmented and non-pigmented rabbits.

The effect of i.v. injections of methysergide (affinity for 5-$HT_{2C/2A}$ and to a lesser extend 5-HT_{1A}

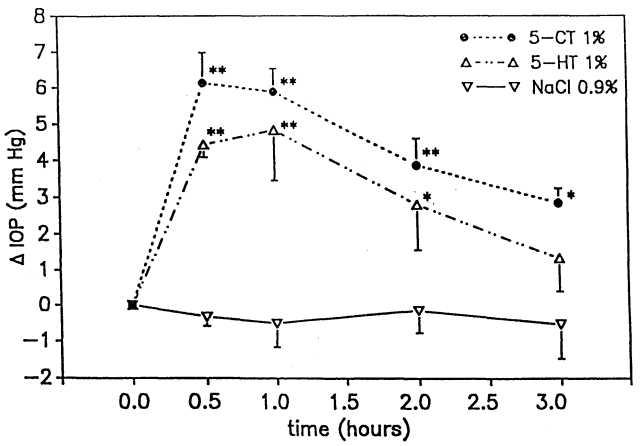

Fig. 3. IOP in rabbits after topical application of 1% 5-HT, 1% 5-CT or saline (control). Data are given as changes in IOP (in mmHg) as means ±SEM (n = 12 in each case). * P<0.05, ** P<0.001 compared to IOP at time 0.00 h (from Meyer-Bothling et al.).

receptors) or ketanserin (affinity for 5-$HT_{2C/2A}$ receptors) on the acute irritative response in the rabbit eye caused by topically applied formaldehyde (1%) has also been investigated (Krootila et al. 1987b). Methysergide lowered the IOP response while ketanserin produced no effect. Such data argue for the involvement of 5-HT_{1A} receptors in the control of IOP supporting the findings of Meyer-Bothling et al. (1993) as shown in Fig. 3.

The involvement of 5-$HT_{2C/2A}$ receptors in maintaining IOP is implicated by further studies involving the use of ketanserin. Topical application of ketanserin to rabbit, cat and monkey eyes has been reported to lower IOP (Chang et al. 1985, Krootila et al. 1987a, Chiou and Li 1992). This effect was more prominent in eyes with intact sympathetic innervation than in sympathectomised eyes. According to Chang et al. (1985) ketanserin probably suppresses aqueous humour formation by antagonising the action of noradrenaline on α_1-adrenoceptors. The effect of oral ketanserin on IOP in glaucomatous human patients was investigated by Costagliola et al. (1991). One hour after oral ketanserin administration both the systolic arterial pressure and IOP was decreased. These reductions were more evident after 3 h. Costagliola et al. (1991) be-

lieve that ketanserin's action on 5-HT_{2A} receptors resulting in an increase in outflow of aqueous fluid.

INVOLVEMENT OF MELATONIN IN IOP DYNAMICS

Melatonin receptors

Within the last few years a number of reports have appeared describing the binding characteristics of radioactive melatonin or the selective agonist iodomelatonin to a variety of tissues (Bittman 1993, Morgan et al. 1994). The results show a great deal of variation with a more than a 100-fold differencies in K_D values suggesting more than one-type of melatonin binding site (receptor). This, however, has not been established because of the paucity of pharmacological tools available. It is also possible that the variation in binding parameters reported by different authors reflects the differencies in methodology employed.

In many studies melatonin binding sites have been shown to be regulated by guanine nucleotides (Carlson et al. 1989, Morgan et al. 1994). Moreover, melatonin and melatonin analogues such as iodomelatonin have been shown to inhibit forskolin-stimulated cAMP accumulation in various tissues (Carlson et al. 1989, Wiechmann and Wirsig-Wiechmann 1993, Morgan et al. 1994) revealing the existence of at least a subset of melatonin receptors which are negatively coupled to cAMP production.

Autoradiography has recently revealed the presence of specific ^{125}I-iodomelatonin binding sites associated with the rabbit/iris ciliary process (Osborne and Chidlow 1994). The binding of iodomelatonin to iris/cilary process homogenates was found to be saturable with a single population of binding sites. Interestingly and importantly, serotonin has little affinity for the iodomelatonin binding sites (Fig. 4) compared with a variety of melatonin analogues (Osborne and Chidlow 1994). In addition, iodomelatonin, like serotonin, decreased the forskolin elevated cAMP levels but, unlike serotonin, was not antagonised by propranolol.

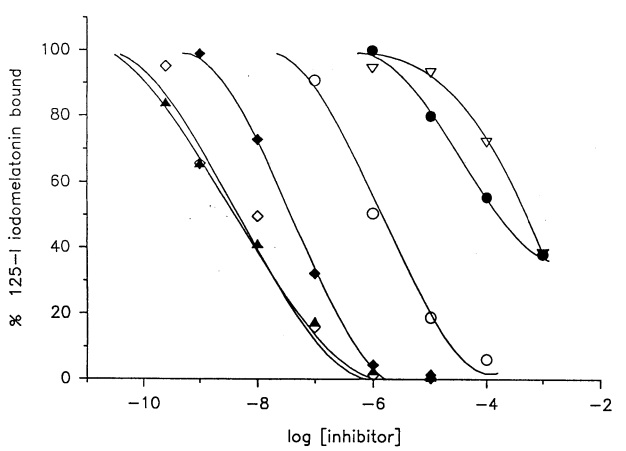

Fig. 4. The effect of melanonin and a number of analogues on the specific binding of ^{125}I-iodomelatonin to membrane homogenates of the ICB. Data are mean values for three separate experiments each carried out in triplicate. ▲ = 6-chloromelatonin, □ = melatonin, ◆ = 6-OH-melatonin, ○ = N-acetyloserotonin, ● = 5-metoxytryptamine, ▽ = serotonin (from Osborne and Chidlow 1994).

Melatonin and IOP

A diurnal change in the IOP is a well-known phenomenon observed in animals and man (Hendkind et al. 1973, Rowland et al. 1981). The mechanisms by which these changes occur remain elusive, although melatonin is implicated. Rabbits have daily rhythms of melatonin levels in the aqueous humour (Chiou et al. 1985, Yu et al. 1990, Liu and Dacus 1991) and iris/ciliary body (Aimoto et al. 1985), melatonin being higher during the dark than the light period. IOP and aqueous flow also peak during the dark in rabbits (Liu and Dacus 1991, Yoshitomi and Gregory 1991), and it has been suggested that melatonin is partially responsible for the increase of IOP which occurs during the dark phase of the circadian cycle (Aimoto et al. 1985, Chiou et al. 1985).

Humans also have diurnal rhythms of IOP (Kitazawa and Horie 1975, Samples et al. 1988) and aqueous flow (Oshika et al. 1988, Samples et al. 1988) which are generally higher during the daytime than night-time i.e. opposite of what has been observed for the rabbit. It has been reported that exogenous melatonin decreases IOP in humans

(Samples et al. 1988) although it appears not to have an effect on aqueous flow rate (Heinrich et al. 1990). Thus it appears as if melatonin may influence IOP in opposite directions in rabbits and humans.

The influence of melatonin on IOP in animals, however, has led to contradictory data. Intracameral injections of melatonin to rabbits (Chiou et al. 1985) and cats (Rhode et al. 1985) significantly raise the IOP in a dose-dependent manner by decreasing aqueous outflow capacity. Yet topical application of melatonin to animals had no influence on IOP (Chiou et al. 1985, Rhode et al. 1985, Kiuchi et al. 1993). Melatonin injected into the vortex vein of rabbits has also been reported to increase the IOP (Rhode et al. 1993) while Kiuchi et al. (1993) delivered melatonin to the rabbit by four routes: topical application, intravenous injection, intra-arterial infusion and intravitreal injection to record no obvious alteration in IOP.

An interesting observation regarding melatonin administration to the rabbit eye has been reported by Matusowa and Aganov (1989). In their experiments rabbit eyes were treated with single daily doses of melatonin for three weeks. This caused an increase in IOP and histological examination of the eyes revealed all the typical signs normally associated with glaucoma in man. These results raise the possibility that one cause of glaucoma in man could be altered melatonin levels in the eye. It is clearly necessary to substantiate the findings reported by Matusova and Aganov (1989).

GENERAL CONCLUSIONS

From the evidence available it is clear that melatonin and serotonin receptors exist in the iris/ciliary process of the rabbit. Some evidence exists to suggest that these receptors are involved in maintaining the IOP. However, the published results are often contradictory, perhaps because of the variation in the species of animals used and the methodology employed. It is also clear that data obtained from studies on the rabbit cannot be directly applied to man. Nevertheless, present information points to the possibility that drugs influencing specific serotonin and/or melatonin receptors may be used to influence IOP in man and thus have a therapeutic effect.

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