Characteristics of $[^3H]5$-Hydroxytryptamine Binding to Iris–Ciliary Body Tissue of the Rabbit

G. Chidlow, L. M. De Santis,† N. A. Sharif,† and N. N. Osborne

Purpose. This study sought to identify, characterize, and localize subtypes of 5-hydroxytryptamine (HT) receptors in rabbit iris–ciliary body.

Methods. Radioligand binding assays were performed with $[^3H]5$-hydroxytryptamine on membranes prepared from rabbit iris–ciliary bodies and on tissue sections subsequently developed by autoradiography.

Results. $[^3H]5$-HT appeared to bind to a single population of receptors in membrane preparations of rabbit iris–ciliary body. The apparent affinity of the ligand ($K_v$) was 2.19 nM, and the density of binding sites was 58.3 fmol/mg protein. Binding of $[^3H]5$-HT exhibited guanosine-5-triphosphate sensitivity. Competitive inhibition experiments were performed to differentiate between 5-HT receptor subtypes. A relative potency order of 5-CT $>$ 5-HT $>$ 8-OH-DPAT $>$ ipsapirone $>$ RU24969 $>$ sumatriptan $>$ ritanserin $>$ ketanserin was demonstrated. The apparent inhibitory constants for the ligands tested fit with the profile expected of binding to 5-HT$_{1A}$ receptors. Inhibition studies with $[^3H]5$-HT plus 100 nM 8-OH-DPAT (which inhibits binding to 5-HT$_{1A}$ receptors only) representing total binding, indicated that no further displacement occurred when ligands preferentially selective for 5-HT$_{1B}$, 5-HT$_{1D}$, or 5-HT$_{1C}$ were tested. Total binding of $[^3H]5$-HT in tissue sections developed by autoradiography was displaced completely by 100 nM 8-OH-DPAT. Melatonin showed little affinity for the $[^3H]5$-HT binding sites.

Conclusions. A population of 5-HT$_{1A}$ receptors is present in rabbit ciliary processes. There is no evidence to suggest the presence of 5-HT$_{1B}$, 5-HT$_{1D}$, or 5-HT$_{2C}$ receptors in the iris–ciliary body.

Serotonin (5-HT) is known to have a wide-ranging transmitter function in both central and peripheral nervous tissues. The multiple actions of 5-HT are mediated through the specific interaction with receptors. According to the latest classification,$^1$ there are at least three and probably seven classes or families of serotonin receptor. With the exception of the 5-HT$_3$ receptors, which are ligand-gated cation channels, all the other 5-HT receptors are G-protein coupled. The families of receptors are defined according to their amino acid sequence homology and their coupling to secondary messengers. The 5-HT$_1$ class of receptors are negatively coupled to adenylate cyclase, whereas the 5-HT$_3$ family of receptors, when activated, stimulate the enzyme phospholipase C. 5-HT$_{1A}$, 5-HT$_{1D}$, and 5-HT$_7$ receptors are all thought to be positively coupled to adenylate cyclase, but these receptors have not yet been properly characterized. Little is known about the 5-HT$_1$ family of receptors.

Evidence has been gradually accumulating that suggests a role for 5-HT in the iris–ciliary body. Serotonin has been shown biochemically to be present in the iris–ciliary body of a number of species, including rabbit,$^2,3$ and human.$^4$ In addition, 5-HT levels have been measured in the aqueous humor.$^5$ Studies on the iris–ciliary body of various species provide no direct evidence for the presence of serotonergic nerves,$^3,7$ yet when rats, guinea pigs,$^6$ and rabbits$^2$ are preloaded with L-tryptophan and a monoamine oxidase inhibitor, subsequent immunohistochemical staining of the iris and ciliary body reveals 5-HT positive fibers. Moreover, when the rabbit iris–ciliary body is exposed to...
exogenous serotonin, a larger population of indoleamine-accumulating cells is demonstrated. However, some of these cells may be noradrenergic in nature. Specific binding sites for \( ^3H \)5-HT in the rabbit iris–ciliary body were reported by Mallorga and Sugrue in 1987 and were characterized as belonging to the 5-HT\(_2\) family of receptors. In support of these data were secondary messenger studies showing that serotonin attenuates the forskolin-induced elevation of adenine 3',5'-cyclic monophosphate (cAMP) content in rabbit iris–ciliary body. Some of these cells may be noradrenergic in nature.

The presence within the rabbit iris–ciliary body of a further serotonin receptor, belonging to 5-HT\(_2\) class, was inferred from the observation that, in tissue pieces, serotonin stimulates inositol phosphate accumulation.

The presence of 5-HT, 5-HT-accumulating cells, and 5-HT receptors in the rabbit iris–ciliary body raises the question whether serotonin has a physiological role in the tissue. A number of studies have looked at the effect that serotonin and some of its analogues have on intraocular pressure (IOP). Topical application of 5-HT has been shown to reduce increase IOP, whereas intracameral injection of the amine has been demonstrated to elevate IOP. Topical application of the 5-HT\(_1\) agonist 5-carboxamidotryptamine (5-CT) raised IOP, but similar treatment with the 5-HT\(_2\) antagonist ketanserin leads to the opposite effect. Furthermore, when an acute irritation of the rabbit eye was induced by topical formaldehyde, intravenous injection of methysergide, a 5-HT\(_1\)A and 5-HT\(_2\) antagonist, decreased the raised IOP.

In the current study, we sought to identify, characterize, and localize serotonergic receptors in rabbit iris–ciliary body by radioligand binding using a new, high-specific activity \( ^3H \) 5-HT salt. Moreover, we assessed the affinity of a range of serotonergic agonists and antagonists and melatonin for the serotonin binding sites.

**METHODS**

**Materials**

5-hydroxy\( ^3H \)tryptamine (\( ^3H \) 5-HT) TFA salt (80 to 100 G/mmol) was obtained from Amersham (Amer- sham, UK). All drugs and reagents were purchased from Merck (Lutterworth, UK) or Sigma (Poole, UK) except 5-Methoxy-dimethyltryptamine (5-Meo-DMT), (±)-8-OH-DPAT, N,N-dipropyl-5-carboxamido tryptamine (DP-5-CT), RBl (St. Albans, UK); 5-Carboxamidotryptamine, Sumatriptan, Glaxo (Greenford, UK); RU 24969, Roussel-UCLAF (Paris, France); Ipsapirone, Tropenwerke (Koln, Germany); Buspirone, Bristol–Meyers Squibb (Wallingford, CT); Mesulergine, Metergoline, Methysergide, Sandoz (Basel, Switzerland); Spiperone, Spiroxatrine, Ketanserin, Ritan- erin, Janssen (Geer, Belgium); MDL 73005EF, Marion Merrell Dow (Cincinnati, OH); SC-53116, G.D. Searle & Co. (Skokie, IL); SL81-0385-03, SL86-0094-10, Synthelabo (Paris, France); YM-8054, Yamanouchi (Osaka, Japan).

**Iris–Ciliary Body Membrane Preparation**

Freshly enucleated albino rabbit eyes were obtained from a local slaughterhouse. Iris–ciliary bodies were carefully dissected, rinsed in Kreb's physiological solution, and stored at −80°C until used. Iris–ciliary bodies were thawed, weighed, minced finely with a pair of scissors, and homogenized by a motor-driven teflon homogenizer in 0.32 M sucrose. The homogenate was centrifuged at 300 g for 10 minutes at 4°C, and the supernatant was collected. The pellet was homogenized and centrifuged again as above. The supernatants were combined, diluted \( 1/2 \) with cold Tris–HCl (50 mM, pH 7.7, at 25°C) and centrifuged at 48,000 g for 30 minutes at 4°C. The pellet was resuspended in Tris–HCl, and the suspension was incubated at 37°C for 15 minutes to remove any endogenous 5-HT and then recentrifuged as above. The final pellet was suspended in binding buffer (Tris–HCl, 50 mM, pH 7.7, at 25°C; CaCl\(_2\), 4 mM; pargyline, 10 \( \mu \)M; and ascorbic acid, 0.1%) and used immediately in the binding assay. Protein concentrations were determined by the method of Bradford using bovine serum albumin as a standard.

**Radioligand Binding Assay**

To each assay tube was added 50 \( \mu l \) \( ^3H \) 5-HT, 50 \( \mu l \) binding buffer, 50 \( \mu l \) of drug to be tested or binding buffer, and, to initiate the assay, 350 \( \mu l \) of membrane suspension. Incubation occurred at room temperature in a shaking water bath for a period of 40 minutes. Binding was terminated by the addition of 3.5 ml of ice-cold buffer (Tris–HCl, 50 mM, pH 7.7, at 25°C), rapidly vacuum filtration through Whatman GF/B glass fiber filters and 2 × 3.5 ml washes with the same buffer. The filters were placed in scintillation vials, covered by 5 ml Insta-gel (Packard, Pangbourne, UK) and counted in a scintillation spectrometer (LKB Wallac, Turku, Finland).

Specific binding was defined as the difference between total binding and binding obtained in the presence of 100 \( \mu M \) 5-methoxytryptamine (5-MeO-T; non-specific binding). For saturation experiments, seven concentrations of \( ^3H \) 5-HT (0.5 nM to 10 nm) were used. In competition studies, the ability of unlabeled analogs to displace 2 nM \( ^3H \) 5-HT was assessed at concentrations ranging from 10 pM to 1 mM.

**Data Analysis**

Binding data from saturation experiments were analyzed by nonlinear computer-assisted analysis for the
binding constants \( K_d \) and \( B_{max} \). The apparent inhibition constant \( K_i \) for each drug was calculated using the nonlinear curve fitting program "LIGAND" developed by Munson and Rodbard. The curves were analyzed according to a one-site model.

**Autoradiography**

Iris–ciliary bodies were obtained as detailed above and frozen on to microtone chucks. Frozen sections (10 μm) through the ciliary body were cut, thaw mounted onto slides, and stored at -20°C for 24 hours. The slides containing the sections were incubated in buffer (Tris-HCl 50 mM, pH 7.7, at 25°C) for 30 minutes at room temperature. The sections were then incubated for 40 minutes at room temperature in incubation buffer (Tris-HCl, 50 mM, pH 7.7, at 25°C; CaCl₂, 4 mM; pargyline, 10 μM and ascorbic acid, 0.1%) containing 4 nM [³H] 5-HT to give total binding. Nonspecific binding was determined by displacing the radioligand with 100 μM 5-MeO-T. 5-HT₁A binding sites were identified by comparing the number of [³H] 5-HT binding sites observed in the presence of 100 nM 8-OH-DPAT with total binding. Specific binding to 5-HT₁B, 5-HT₁D, or 5-HT₁C sites is revealed by incubating the sections with 4 nM [³H] 5-HT + 100 nM 8-OH-DPAT and including in the incubation medium 20 nM RU 24969, 100 nM sumatriptan, or 100 nM mesulergine, respectively. Slides are compared with sections incubated with 4 nM [³H] 5-HT + 100 nM 8-OH-DPAT alone. After incubation, the slides were washed twice for 10 seconds in cold buffer, dipped in cold distilled water, and then air-dried. The sections were exposed to Amersham [³H]-hyperfilm, stored in the dark for 60 days at 4°C, and developed with Kodak (Hemel Hempstead, UK) Dektal developer.

**RESULTS**

**Autoradiographic Analysis of [³H] 5-HT Binding to Ciliary Body Sections**

As shown in Figure 1A, the binding of 4 nM [³H] 5-HT to the ciliary body is restricted to the ciliary processes. The methodology used does not allow for the discrimination of binding sites between stroma, pigmented ciliary epithelium, and nonpigmented ciliary epithelium. No binding is associated with the ciliary musculature. The [³H] 5-HT binding is displaced almost completely in the presence of 100 nM 8-OH-DPAT, an appropriate concentration of ligand to inhibit the high affinity 5-HT₁A receptor (Fig. 1B). When 100 nM sumatriptan, a ligand used to displace from 5-HT₁B receptors (not shown), 100 nM mesulergine, a ligand used to displace from 5-HT₁C receptors (Fig. 1C), or RU 24969, a ligand used to displace from 5-HT₁B receptors (not shown) is included with [³H] 5-HT + 100 nM 8-OH-DPAT in the incubation, no further displacement is observed.

**Binding to Membrane Preparations of Iris–Ciliary Body**

Figure 2 shows a time course of association of [³H] 5-HT binding to iris–ciliary body membranes. Binding is rapid over the first 10 minutes, with saturation occurring at 40 minutes. Therefore, 40 minutes was chosen as the optimum incubation time for all subsequent experiments.
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TABLE 1. Apparent Affinity Values ($K_i$) of Various Drugs for $[^3H]$ 5-HT Binding in ICB

<table>
<thead>
<tr>
<th>Ligand</th>
<th>$K_i$ value (M)</th>
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<tr>
<td>DP-5-CT</td>
<td>$2.514 \times 10^{-10}$</td>
</tr>
<tr>
<td>MDL 73005EF</td>
<td>$3.382 \times 10^{-10}$</td>
</tr>
<tr>
<td>5-CT</td>
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<tr>
<td>5-HT</td>
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</tr>
<tr>
<td>8-OH-DPAT</td>
<td>$3.644 \times 10^{-9}$</td>
</tr>
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<td>5-MT</td>
<td>$5.932 \times 10^{-9}$</td>
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<tr>
<td>Ipasiprone</td>
<td>$7.663 \times 10^{-9}$</td>
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<tr>
<td>SL 86-0094-10</td>
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<td>Spiroxatrine</td>
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<tr>
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<td>5-MeO-DMT</td>
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<tr>
<td>RU 24969</td>
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<tr>
<td>Methysergide</td>
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<tr>
<td>Busapronne</td>
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<tr>
<td>5,6 DHT</td>
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<tr>
<td>Sumatriptan</td>
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<tr>
<td>5,7 DHT</td>
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<td>Spiperone</td>
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<tr>
<td>Ritanserin</td>
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<tr>
<td>Melatonin</td>
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<td>SL81-0385-03</td>
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Saturation Analysis

A representative saturation curve for $[^3H]$ 5-HT binding in membrane preparations from iris–ciliary body is shown in Figure 3. Binding is saturable (0.5 nM to 10 nM) and specific binding, defined as the difference between total binding and binding obtained in the presence of 100 µM 5-MeO-T, ranged from approximately 60% to 85% of total binding at the $K_i$ concentration of the ligand. Scatchard transformation of the saturation data produced a linear plot indicating the likelihood of one population of labeled $[^3H]$ 5-HT binding sites. $K_i$ and $B_{max}$ values of 2.19 nM and 58.3 fmol/mg protein, respectively, were obtained.

Pharmacologic Properties

To explore the pharmacology of the $[^3H]$ 5-HT binding sites more thoroughly, we performed competition curves with a range of drugs including drugs selective for 5-HT$_{1A}$ receptors, drugs selective for 5-HT receptors other than the 5-HT$_{1A}$ site, and melatonin. The data from competition experiments are summarized in Table 1, and representative competition curves of $[^3H]$ 5-HT binding in the presence of various serotonergic agonists and antagonists are depicted in Figures 4 and 5, respectively.

The data show that the indoles and indole derivatives are the most potent displacers of $[^3H]$ 5-HT binding, with DP-5-CT and 5-CT each having a subnanomolar $K_i$ value and 5-HT, 5-MeO-T, 5-MeO-DMT, and RU 24969 $K_i$ values in the low nanomolar range. All these compounds are known to have high potency at brain 5-HT$_{1A}$ receptors. 8-OH-DPAT is highly selective for...
5-HT\textsubscript{1A} receptors in brain membranes\cite{36}, and the K\textsubscript{i} value in this study of 3.6 nM is consistent with the presence of 5-HT\textsubscript{1A} binding sites in rabbit iris–ciliary body. The apparent K\textsubscript{i} values of the known 5-HT\textsubscript{1A} partial agonists spiroxatrine (9.2 nM), metergoline (24 nM), and methysergide (50 nM) are consistent with values expected for displacement from 5-HT\textsubscript{1A} receptors. The partial agonists ipsapirone and buspirone are potent at and selective for brain 5-HT\textsubscript{1A} binding sites with reported K\textsubscript{i} values of <10 nM and ~25 nM. In this study, ipsapirone and buspirone displayed K\textsubscript{i} values of 7.6 nM and 149 nM. 5-HT\textsubscript{1A} receptors are characterized by an insensitivity to ketanserin, and we report a K\textsubscript{i} value of >100 \mu M for this compound.

Figure 4 shows that melatonin (K\textsubscript{i} value 7.5 \mu M) has only low affinity for the [\textsuperscript{3}H] 5-HT binding sites. Figure 6 shows that in the iris–ciliary body membrane preparation guanosine-5-triphosphate (GTP) inhibited [\textsuperscript{3}H] 5-HT binding in a dose-dependent manner. A concentration of 10\textsuperscript{-3} M GTP caused a 26.2% reduction in specific binding.

Although the apparent K\textsubscript{i} values of virtually all the drugs tested and the Hill coefficients of a number of compounds—including 5-MT, sumatriptan, buspirone, 5,6 DHT and melatonin—indicate the presence of a single population of 5-HT\textsubscript{1A} receptors, some of the other Hill slopes tended to be more erratic, varying in range from 0.5 to 0.8. Thus, we considered it necessary to inhibit the binding of [\textsuperscript{3}H] 5-HT to 5-HT\textsubscript{1A} receptors by the use of 100 nM 8-OH-DPAT and then ascertain which drugs compete with the residual binding. 8-OH-DPAT (100 nM) displaces approximately 80% of specific [\textsuperscript{3}H] 5-HT binding (Fig. 6). When RU 24969 (20 nM), mesulergine (100 nM), or sumatriptan (100 nM) is included with [\textsuperscript{3}H] 5-HT and 8-OH-DPAT in the incubation medium, little or no further displacement is seen (Fig. 7).

**DISCUSSION**

The rapid advancements in serotonin receptor classification over the past few years, coupled with the devel-
The heterogeneity of serotonin receptors, including the 5-HT subtype, has been extensively studied. The 5-HT1A receptor, known for its high affinity for all 5-HT receptor subtypes, has been the focus of numerous studies due to its role in various physiological processes. The 5-HT1A receptor is involved in the regulation of neurotransmitter release, synaptic plasticity, and neuronal excitability.


dvelopment of better ligands and the recent availability of a new, high-specific activity [3H] 5-HT salt, have given us the opportunity to characterize more fully the serotonin receptor subtypes present in the rabbit iris–ciliary body. The heterogeneity of serotonin receptors was revealed by radioligand binding studies using [3H] 5-HT.20-25 [3H] 5-HT has been the most widely used label for the characterization of serotonin receptors because of its high affinity for all the 5-HT1 receptors and the 5-HT2C and 5-HT2B receptors. Peroutka and Snyder26 showed that 5-HT3 (now termed 5-HT3A) binding sites cannot be labeled with [3H] 5-HT.

The current study demonstrates clearly that [3H] 5-HT TFA labels a population of 5-HT1A receptors in the rabbit iris–ciliary body and more specifically in the ciliary processes. The study also finds no evidence for the presence of any other subtypes to which [3H] 5-HT is known to bind. The results confirm the findings of Mallorga and Sugrue8 and Tormay et al24 that 5-HT1 binding sites exist in the tissue. There are several observations supporting this conclusion. First, inhibition of [3H] 5-HT binding by a range of drugs of differing selectivities yielded the expected pharmacologic characteristics of a 5-HT1A subtype. Thus, not only was the pharmacologic profile of the [3H] 5-HT binding sites consistent with the expected profile of binding to a 5-HT1A subtype, the apparent K values of those compounds tested are close to the reported K values in tissues containing the 5-HT1A subtype.25 Second, computer modeling indicated the existence of a single class of binding sites for 5-HT with a K value similar to that expected for 5-HT1A receptors.

Third, inhibition studies with [3H] 5-HT plus 100 nM 8-OH-DPAT (which inhibits binding to 5-HT1A receptors only), representing total binding, indicated that no further displacement occurred when ligands preferentially selective for 5-HT1B, 5-HT1D, 5-HT1D, or 5-HT2C were tested. Fourth, total binding of [3H] 5-HT in tissue sections developed by autoradiography was displaced completely by 100 nM 8-OH-DPAT.

The inevitable variability between the purity of individual membrane preparations and the rapid catastrophe of the unstable, high-affinity, tritiated 5-HT are likely to be the causes of the differences between Hill coefficients of compounds tested. It is also possible that some of the drugs tested do not possess favorable binding properties, i.e., they may be binding to acceptor sites, glass, or filters. The erratic nature of the Hill plots also could be caused by negative cooperative interactions between certain ligands and the [3H]5-HT, such as when an excess of unlabeled ligand accelerated the dissociation process of the radioligand. Finally, it cannot be excluded that [3H]5-HT binds to multiple receptor sites and that the Hill coefficients of some of the compounds reflect differences in affinities for the bound receptors.

The highly selective 5-HT1A agonist 8-OH-DPAT, an aminotetralin,20 remains unique in its combination of high efficacy and striking potency and has proved to be a useful tool in allowing the discovery of multiple [3H] 5-HT binding sites in the same tissue.21-25 The specificity of 8-OH-DPAT however, has been called into question recently because it does not discriminate readily between 5-HT1A and 5-HT1D receptors.20 5-HT7 receptors are positively coupled to adenylate cyclase when the receptors are expressed in cell lines,27,28 and, in the rabbit iris–ciliary body, two studies have demonstrated that serotonin does not stimulate cAMP production but reduces forskolin-elevated cAMP levels.9,10

Since the study of Mallorga and Sugrue,8 cloning techniques have shown that 5-HT1B receptors exist only in rodents; however, two subtypes of 5-HT1D receptors exist in nonrodent mammals—5-HT1D and 5-HT1D—possessing very similar pharmacologies. The 5-HT1D subtype is the nonrodent homologue of the rat 5-HT1B subtype.29 Therefore, 5-HT1B receptors would not be found in the rabbit, but 5-HT1D receptors would be present in rabbit brain and possibly in the iris–ciliary body. The reported affinity of sumatriptan for the 5-HT1D subtype (Ki < 10 nM) is significantly lower than its affinity for the 5-HT1A subtype (Ki > 100 nM). In our study, the affinity of sumatriptan (Ki = 290 nM) and its Hill slope (0.82) is evidence for the lack of either subtype of 5-HT1D subtype in rabbit iris–ciliary body.

There is a conflict in the literature between whether 5-HT1D receptors exist in the rabbit iris–ciliary...
body. A secondary messenger study by Tobin et al\(^2\) showed clearly that serotonin stimulates an increase in inositol phosphate accumulation in a dose-dependent manner. Furthermore, it was demonstrated that the 5-HT\(_2\) antagonist methysergide and the mixed 5-HT\(_1\)–\(\alpha\)\(_1\)-adrenergic antagonist ketanserin can affect IOP.\(^3\) Yet, neither [\(^3\)H] ketanserin,\(^4\) a label for 5-HT\(_2A\) receptors, nor in this study [\(^3\)H] 5-HT, a label for 5-HT\(_2B/C\) receptors, has been shown to bind specifically to 5-HT\(_3\) receptors. It is possible that the density of 5-HT\(_3\) receptors within the iris–ciliary body is low and that consequently radioligand binding techniques are not sensitive enough to reveal the receptors. This hypothesis is supported by the fact that high concentrations of serotonin were needed in the secondary messenger study to stimulate inositol phosphate accumulation to any significant degree above basal levels.\(^2\)

Zacopride, a 5-HT\(_3\) antagonist and a 5-HT\(_4\) agonist, and SC-53116, a 5-HT\(_4\) agonist, were both weak inhibitors of the specific [\(^3\)H] 5-HT binding, indicating the lack of potency of these ligands at 5-HT\(_1\)A receptors. The affinity of 5-HT for 5-HT\(_3\) and 5-HT\(_4\) receptors is fairly low (EC\(_{50}\) values of 100 nM to 1000 nM).\(^3\) Because the concentration of radioactive 5-HT used in the displacement studies was 2 nM, binding to potential 5-HT\(_3\) or 5-HT\(_4\) receptors would not occur to a significant enough degree to reveal whether these receptors are located in the rabbit iris–ciliary body.

The presence of high-affinity melatonin receptors negatively coupled to adenylate cyclase within the rabbit iris–ciliary body recently has been demonstrated.\(^3\) Serotonin receptors in the rabbit iris–ciliary body are also negatively coupled to adenylate cyclase,\(^5,6\) and the two indoleamines are structurally similar. Thus, we considered it pertinent to assess the affinity of melatonin for the [\(^3\)H] 5-HT binding sites. Melatonin was demonstrated to have approximately 1000-fold lower potency at the serotonin receptors than at the melatonin receptors, and, hence, the receptors can be considered distinct entities.

In radioligand binding studies, the presence of GTP and other guanine nucleotides decreases the affinity of agonists for 5-HT\(_{1\alpha}\)\(^7\) and 5-HT\(_3\)\(^8\) binding sites. The effect of GTP on 5-HT\(_1\)A sites labeled with [\(^3\)H]8-OH-DPAT\(^4\) is approximately an order of magnitude more potent than detected for heterogeneous 5-HT\(_3\) sites labeled with [\(^3\)H]5-HT.\(^9\) In this study, GTP reduces the binding of [\(^3\)H]5-HT to iris–ciliary body membranes in a similar manner to that found for heterogeneous 5-HT\(_3\) sites.\(^9\) Thus, a discrepancy exists between this result and our other findings. Whether this discrepancy reflects a difference between [\(^3\)H]8-OH-DPAT and [\(^3\)H]5-HT or between 5-HT\(_1\)A receptors in rabbit iris–ciliary body and rat brain remains to be established.

Although the autoradiographic localization of [\(^3\)H]5-HT binding showed the presence of 8-OH-DPAT-sensitive binding sites in the ciliary processes, unfortunately the “emulsion film” technique does not have a high enough resolution to distinguish between the cell layers present in the tissue. Nevertheless, functional 5-HT\(_1\)A receptors are preferentially associated with the ciliary processes, and, therefore, a functional role in aqueous humor secretion must be considered a possibility, especially in light of the known effects of serotonin and some of its analogs on IOP.

**Key Words**

autoradiography, 5-HT\(_1\)A receptors, [\(^3\)H]5-hydroxytryptamine, rabbit iris–ciliary body, radioligand binding assay

**Acknowledgment**

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**References**

10. Barnett NL, Osborne NN. The presence of serotonin (5-HT\(_3\)) receptors negatively coupled to adenylate cy-


