Pharmacologic Evidence for 5-HT1A Receptors Associated With Human Retinal Pigment Epithelial Cells in Culture

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Purpose. The authors investigate the possible presence of 5-hydroxytryptamine (5-HT1) type serotonin receptors negatively coupled to adenylate cyclase activity in cultured human retinal pigment epithelial (RPE) cells.

Methods. Adenylate cyclase activity was assessed by the determination of cellular adenosine 3':5' cyclic monophosphate (cAMP) levels and the effects of serotonin on both basal and forskolin-stimulated cAMP levels studied.

Results. Serotonin at 100 µM had no effect on the basal levels of cAMP in cultured human RPE cells, but attenuated by 43.6% the stimulation in cAMP production induced by forskolin (5 µM). This effect was dose dependent for serotonin with half-maximal inhibition (EC50) occurring at approximately 1.5 × 10^{-9} M. The 5-HT1 receptor agonists 8-hydroxy [2-di-n-propylamino] tetralin (8-OH DPAT), buspirone, 5-carboxamidotryptamine, and RU24969 mimicked the inhibitory effect of serotonin in a dose-dependent manner. The actions of serotonin and 8-OH DPAT (10 µM) were dose-dependently attenuated by the serotonergic antagonists spiroxatrine, propranolol, and spiperone. Pretreatment of RPE cell cultures with pertussis toxin abolished the serotonin-induced reduction of forskolin-elevated cAMP levels. Stimulation of cAMP production by the β-adrenoceptor agonist isoproterenol at 0.1 µM, but not at 10 µM or 100 µM, was also attenuated by serotonin (100 µM), whereas cAMP production induced by the adenosine receptor agonist 5'-[N-ethyl]-carboxamidoadenosine (NECA) at 1 µM, 10 µM, and 100 µM was unaffected. Serotonin and 8-OH DPAT dose-dependently inhibited isoproterenol-stimulated (0.1 µM) cAMP production with EC50 values of approximately 10 µM, and pertussis toxin pretreatment partially blocked these effects.

Conclusions. Cultured human RPE cells possess 5-HT1A receptors negatively coupled to cAMP production through a pertussis toxin-sensitive G protein. These receptors show differential effects on forskolin-, isoproterenol-, and NECA-stimulated cAMP production, which may reflect a unique spatial distribution of receptor proteins or the phenotypic heterogeneity of RPE cells that is the result of or that is preserved in culture. Invest Ophthalmol Vis Sci. 1997;38:510-519.

Retinal pigment epithelial (RPE) cells perform several functions that are crucial to the functional integrity of the photoreceptors, including phagocytosis of shed outer segment discs, maintenance of the ion and fluid homeostasis of the subretinal space, and uptake and processing of retinoids for use in the visual cycle. Many of these important functions are modulated by mediators of the adenylate cyclase (AC) and phospholipase C (PLC) signal transduction systems. For example, increased adenosine 3':5' cyclic monophosphate (cAMP) levels and protein kinase C activity inhibit the ingestion of rod outer segments by cultured RPE cells, subretinal fluid absorption is decreased by cAMP, and ion transport across the RPE cells is modified by factors coupled to AC and PLC activity. The identification of cell surface receptors linked to these second messenger systems may thus provide clues as to how RPE cell function is controlled in vivo.

Cultured human RPE possess numerous G-pro-
tein-coupled receptors that modulate AC and PLC activity. Beta2 adrenoceptor,17 A2 adenosine receptor,18 and vasopressin17,18 and /32 bradykinin18 receptors are coupled to AC and stimulate cAMP production, whereas M3 muscarinic acetylcholine,14-16 H1 histamine,16 V1 vasopressin,17,18 and /32 bradykinin18 receptors are coupled to PLC activity and stimulate production of inositol phosphates and intracellular calcium mobilization. Cultured human RPE cells also possess melatonin receptors negatively coupled to AC through an inhibitory G protein (G1) that attenuate the stimulation of cAMP production by forskolin, a direct activator of AC.19 Working on cultured rat RPE cells, we recently identified serotonin (5-HT2A) receptors positively coupled to inositol phosphate turnover and intracellular Ca2+ mobilization.20 However, serotonin (5-hydroxytryptamine; 5-HT) fails to influence PLC activity in cultured human RPE,15,16 suggesting that important species differences may occur in the types of G-protein-coupled receptors associated with RPE cells. We have therefore undertaken an investigation into the possible presence of alternative subtypes of 5-HT receptor and report that cultured human RPE possess 5-HT1A receptors negatively coupled to cAMP production.

MATERIALS AND METHODS

Materials

Postmortem human eyes were obtained from Bristol Eye Bank (Bristol, UK) after removal of the cornea for transplant surgery. [2, 8-3H]-adenosine 3':5' cyclic monophosphate (33 Ci/mmol) was purchased from Amersham International (Amersham, United Kingdom). Fetal bovine serum (European Community approved), Hams-F10, fungizone (amphotericin B), glutamine, 0.25% trypsin solution, and 24-multiwell plates (NUNC) were from Gibco (Paisley, United Kingdom), and 25 cm² and 75 cm² tissue culture flasks were from Falcon (Oxford, United Kingdom). The 8-hydroxy[2-di-n-propylamino] tetralin (±S-OH DPAT) and mianserin were from Research Biochemicals International (St. Albans, United Kingdom); 5-carboxymidotryptamine (5-CT), and sumatriptan from Glaxo (Greenford, United Kingdom); RU24969 from Roussel-UCLAF (Paris, France); buspirone from Bristol-Meyers Squibb (Wallingford, CT); methysergide, metergoline, and SDZ 21009 from Sandoz (Basel, Switzerland); MDL 72222 from Marion Merrel Dow (Cincinnati, OH); and ketanserin, spiperone, and spiroxatrine from Janssen Pharmaceuticals (Geel, Belgium). All other standard chemicals and biochemicals were obtained from Sigma (Poole, United Kingdom) or Merck (Lutterworth, United Kingdom).

Methods

Primary cultures of human RPE cells were established as described previously19 and grown to confluence in 25-cm² flasks containing Hams-F10 culture medium (Hams-F10 [with L-glutamine], 10% fetal bovine serum, 0.4% glucose, 2-mM glutamine, 2.5 μg/ml amphotericin B, and 100 μg/ml gentamycin). Cultures were passaged with a ratio of 1:3 in 75-cm² flasks, and cells between passages 2 through 4 used for experimentation. Cells were immunostained routinely with the monoclonal antibody K 8.13 (Sigma) to test for the presence of cytokeratins and to confirm the purity and nature of the cells present because potential contaminating cells do not express cytokeratins.21

The effects of drug treatment on cellular cAMP levels were determined as described by Nash and Osborne.19 Briefly, culture medium was removed from cells in 24-multiwell plates, replaced with serum-free Hams-F10, and cells were incubated at 37°C/5% carbon dioxide for 2 to 4 hours. Cells then were incubated in 200 μl of experimental buffer (Hams-F10 with 20-mM Hepes, pH 7.4) for 5 minutes at 37°C after which 10 μl of drug was added. An experimental layout of 5 minutes preincubation with antagonist, a further 5-minute treatment with serotoninergic agonist, and then incubation with forskolin, isoproterenol, or 5'-[N-ethyl]-carboxamidoadenosine (NECA) for 5 minutes was used throughout. The reaction then was terminated by boiling for 3 minutes. Fifty microliter aliquots were removed and cAMP levels determined using the method of Brown et al,22,23 which uses a specific cAMP-binding protein isolated from bovine adrenal cortex to detect cellular cAMP by competition with a standard amount of tritiated cAMP. Details of the methodology can be found in the study by Nash and Osborne.19

Statistical Analysis

Statistical significance was determined using Student's t test for paired data, and a P < 0.05 was considered significant. Half-maximal (EC50) values were calculated by determination of the concentration of agonist required to achieve half the maximum response observed at saturating drug concentrations.

RESULTS

The biochemical studies were performed on cultured RPE cells derived from 13 different human donors aged between 14 and 65 years old. Some variations were observed in the responsiveness to test drugs between particular cell cultures. To minimize any complications in interpretation of results arising from such variation, results were compiled, as far as possible,
TABLE 1. Effect of Serotonin on Basal and Forskolin (5 μM)-stimulated Levels of cAMP in Cultured Human RPE Cells

<table>
<thead>
<tr>
<th>Drugs Added</th>
<th>cAMP (pmol/well/5 min)</th>
</tr>
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<tbody>
<tr>
<td>Basal</td>
<td>3.95 ± 0.97</td>
</tr>
<tr>
<td>Serotonin (100 μM)</td>
<td>3.65 ± 0.84</td>
</tr>
<tr>
<td>Forskolin (5 μM)</td>
<td>61.91 ± 3.44</td>
</tr>
<tr>
<td>+ Serotonin (100 μM)</td>
<td>34.90 ± 3.82*</td>
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</table>

cAMP = cyclic adenosine monophosphate; RPE = retinal pigment epithelial.
Results are expressed as mean ± SEM values from seven separate experiments carried out in triplicate.
* P < 0.001 versus forskolin addition alone using Student’s paired t-test.

from experimental data obtained from two or more cell lines to give an average response for the observed effects.

The effect of 5-HT on cAMP metabolism in cultured human RPE cells is listed in Table 1. Exposure of human RPE cells to 100-μM 5-HT for 10 minutes had no effect on the basal levels of cAMP. Forskolin at 5 μM, however, elevated cellular cAMP levels by 1,467.3% after 5 minutes. In contrast to its effects on basal levels, a 5-minute preincubation with 100-μM 5-HT attenuated the ability of forskolin to increase cAMP production by 43.6%. This effect of 5-HT was mimicked by 5-CT, RU24969, and sumatriptan (general 5-HT1 receptor agonists) and 8-OH DPAT and buspirone (specific 5-HT1A receptor agonists) (Fig. 1) with an order of efficacy of 8-OH DPAT (52.0%) > 5-CT (47.8%) > 5-HT (44.3%) > buspirone (41.3%) > RU24969 (38.4%) > sumatriptan (34.4%). The reduction in forskolin-stimulated cAMP production was dose dependent for 5-HT, 8-OH DPAT, and buspirone (Fig. 2A), and 5-CT and RU24969 (Fig. 2B) with EC50 values of 1.5 × 10^-9 M, 1.9 × 10^-9 M, 1.6 × 10^-8 M, and 4.0 × 10^-8 M for 8-OH DPAT action, respectively.

The effect of 5-HT on forskolin action was found to be pertussis toxin-sensitive (Fig. 5). An 18-hour incubation with pertussis toxin (100 ng/ml) failed to influence basal levels of cAMP and those induced by forskolin. However, the treatment potentiated the

![Figure 1](https://lovos.arvojournals.org/)

**Figure 1.** Effect of various serotonin (5-HT1) receptor agonists on the stimulation of adenosine 3'5'-cyclic monophosphate production by forskolin (5 μM) in cultured human retinal pigment epithelial cells. The results are mean ± standard error of the mean value from three separate experiments performed in triplicate. The effect of drug compared with forskolin stimulation alone was statistically significant (P < 0.05) in all cases by Student’s t-test for paired data.
FIGURE 2. (A) Dose response curves are shown for the effect of 5-HT (O) and the 5-HT1A receptor agonists, 8-OH DPAT (●) and buspirone (□) on the stimulation of cAMP production by forskolin (5 μM) in cultured human retinal pigment epithelial cells. (B) Dose response curves are shown for the effect of the general 5-HT, receptor agonists, 5-CT (O), and RU24969 (●). The data are presented as mean ± standard error of the mean value from between 4 and 10 separate experiments performed in triplicate. cAMP = adenosine 3':5' cyclic monophosphate.

stimulation of cAMP production by the β-adrenoceptor agonist isoproterenol by approximately 32.4% and completely attenuated the 5-HT (100 μM)-induced reduction of forskolin-stimulated cAMP production.

Cultured human RPE cells have been previously shown to possess β2-adrenoceptor11 and A9 adenosine12 receptors. Thus, the influence of 5-HT on the ability of isoproterenol and NECA to stimulate cAMP production was determined (Fig. 6). Isoproterenol stimulates cAMP production in cultured human RPE cells with an EC50 value of approximately 0.2 μM and a saturating concentration of 10 μM, whereas NECA has an EC50 value of approximately 1 μM and saturation at approximately 10 μM (data not shown). Interestingly, serotonin had little or no effect on maximally stimulating concentrations of isoproterenol (100 and 10 μM) but inhibited by 64.8% the stimulation of cAMP production with 0.1-μM isoproterenol. In contrast, 5-HT had no effect on the stimulation of cAMP synthesis through the adenosine receptor, whether activated by maximal or submaximal concentrations of NECA. Elevation of cAMP levels by 0.1-μM isoproterenol was dose-dependently inhibited by both serotonin and 8-OH DPAT, although, unlike their effect on forskolin, significant inhibition was witnessed only at concentrations greater than micromolar. However, at 1 mM, both drugs achieved near-complete attenuation of isoproterenol-stimulated cAMP levels (Fig. 7). The stimulation of cAMP production by isoproterenol at 100 μM was unaffected by preincubation with a range of concentrations of 5-HT and, in addition, no effect of 5-HT or 8-OH DPAT was observed on NECA (1 μM)-stimulated cAMP production across a range of agonist concentrations (data not shown).

As for the 5-HT-induced inhibition of forskolin-

FIGURE 3. Effect of various antagonists (0.1 μM) on the 5-HT-(10 μM) induced reduction of forskolin- (5 μM) stimulated cAMP levels in cultured human retinal pigment epithelial cells. The results are mean ± standard error of the mean value from between three and eight separate experiments conducted in triplicate. *P < 0.05 when compared with 5-HT effect alone using Student’s t-test for paired data. cAMP = adenosine 3':5' cyclic monophosphate.
FIGURE 4. Dose-dependent antagonism by spiroxatrine (A), spiperone (B), and propranolol (C) of the 10-μM induced effect of 5-HT (●) and 8-OH DPAT (○) on forskolin (5 μM) stimulation of cAMP production in cultured human retinal pigment epithelial cells. Data are expressed as mean ± standard error of the mean values from between four and seven experiments performed in triplicate. cAMP = adenosine 3':5' cyclic monophosphate.

FIGURE 5. Effect of pertussis toxin on the serotonin-induced reduction of forskolin-stimulated cAMP levels and the stimulation of cAMP production by isoproterenol in cultured human retinal pigment epithelial cells. Data are mean ± standard error of the mean value from three separate experiments carried out in triplicate. *P < 0.01 when compared with isoproterenol stimulation in the absence of pertussis toxin by Student’s t test for paired data. **P < 0.05 when compared with forskolin stimulation of cAMP production alone by Student’s t test for paired data. cAMP = adenosine 3':5' cyclic monophosphate.

DISCUSSION

Cultured rat RPE cells have been shown recently to possess 5-HT2A receptors that, when activated, increase inositol phosphate turnover and mobilize intracellular Ca2+ stores.20 However, no serotonin effect on the PLC signal transduction system is observed in human RPE cell cultures.15,16 The data presented here show that, in contrast to their effect on rat RPE cells, serotonin receptors negatively couple to cAMP production in cultured human RPE cells. Fourteen different serotonin receptors currently have been characterized, and these have been classified into 7 different families.24-26 The 5-HT1 receptor family is negatively coupled to AC activity, whereas 5-HT4, 5-HT6, and 5-HT7 receptors are positively coupled to the enzyme. The 5-HT2 receptors are positively coupled to PLC activity, and the 5-HT3 receptor is a ligand-gated cation channel. No second messenger system coupled to the 5-HT3 receptors has yet been identified. Cultured human RPE cells thus possess a receptor attenuating the agonist-induced inhibition of isoproterenol-stimulated cAMP production.

elevated cAMP levels, the effect of 5-HT (100 μM) on isoproterenol (0.1 μM) action was pertussis toxin-sensitive, as was the effect of 8-OH DPAT (100 μM) (Table 2). However, in these experiments, the pertussis toxin pretreatment was only partially effective in
5-HT₁A Receptors Associated With Human RPE

FIGURE 6. Effect of 5-HT on the stimulation of cAMP production by various concentrations of isoproterenol and NECA. Data are expressed as mean ± standard error of the mean value from three separate experiments conducted in triplicate. *P < 0.01 when compared with stimulation of cAMP production by isoproterenol alone. cAMP = adenosine 3'5' cyclic monophosphate.

The maximal reduction of forskolin-stimulated cAMP production induced by 5-HT is similar to values obtained from human iris ciliary processes, guinea pig hippocampus, and selected cell lines expressing human 5-HT₁A receptors, although significantly lower than the maximal-induced effect in transfected NIH-3T3 cells. The 5-HT-induced effect was mimicked by 8-OH DPAT, buspirone, 5-CT, RU24969, and sumatriptan (Fig. 1), and these drugs act similarly on the stimulated AC in hippocampus from a number of species and NIH-3T3 cells transfected with human 5-HT₁A receptors.

TABLE 2. Effect of Pertussis Toxin (100 ng/ml) on the 5-HT- and 8-OH DPAT-induced Reductions of Isoproterenol-stimulated cAMP Levels in Cultured Human RPE Cells

<table>
<thead>
<tr>
<th>Drugs Added</th>
<th>% Stimulation of cAMP Production Relative to Basal Levels</th>
</tr>
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<tbody>
<tr>
<td>Control</td>
<td>17.6 ± 3.5</td>
</tr>
<tr>
<td>Isoproterenol (0.1 μM)</td>
<td>1046.0 ± 60.3</td>
</tr>
<tr>
<td>+ 5-HT (100 μM)</td>
<td>150.9 ± 8.1*</td>
</tr>
<tr>
<td>+ 8-OH DPAT (100 μM)</td>
<td>649.0 ± 65.6*</td>
</tr>
</tbody>
</table>

cAMP = cyclic adenosine monophosphate; RPE = retinal pigment epithelial.
Cells were pretreated with pertussis toxin for 18 hours prior to experimentation. Results are mean ± SEM values from three separate experiments conducted in triplicate.
* P < 0.02 versus isoproterenol stimulation of cAMP production by Student's paired ttest.
† P < 0.05 versus drug effect on isoproterenol-induced stimulation in the absence of PTX.
The ability of 8-OH DPAT to mimic 5-HT action has been considered diagnostic of 5-HT₁₅ receptors, although the recent discovery that the newly cloned 5-HT₇ receptor shows high affinity for 8-OH DPAT has led to a reappraisal of its diagnostic role. However, 5-HT₁₅ receptors are positively coupled to AC and thus unlikely to mediate the effect of 8-OH DPAT on cAMP metabolism in human RPE cells. The observed inhibition of forskolin-stimulated cAMP production by 8-OH DPAT in cultured human RPE is thus strong evidence for the presence of 5-HT₁₅ receptors in these cells. Buspirone binds with high affinity and selectivity for 5-HT₁₅ receptors and reduces forskolin-stimulated cAMP production in hippocampus and NIH-3T3 cells expressing human 5-HT₁₅ receptors. In contrast, buspirone has no effect on stimulated cAMP levels in Chinese hamster ovary cells transfected with human 5-HT₁₅ receptors and is an antagonist at 5-HT₁₅ receptors in primary cultures of cortical neurons. In human RPE cells, buspirone reduced forskolin-stimulated cAMP levels by 41.3% (Fig. 1) and thus the human RPE 5-HT₁₅ receptors show greater similarity to those in mouse hippocampus than those in cortical neurons. Sumatriptan has been reported to have high affinity and selectivity for 5-HT₁₅ receptors but has also been shown to interact with calf hippocampus 5-HT₁₅ receptors to reduce forskolin-stimulated AC. A similar effect is evident in human RPE cells (Fig. 1).

The potency order obtained for the action of the agonists on forskolin-stimulated cAMP production (Fig. 2) is in fair agreement with that obtained from mouse and calf hippocampus and NIH-3T3 cells expressing human 5-HT₁₅ receptors. However, important differences are evident in the response of these 5-HT₁₅ receptors to the agonists. Most notable is the high potency (low EC₅₀ value) of 5-HT for its receptor, which is comparable to that of 8-OH DPAT, in cultured human RPE. In NIH-3T3 cells transfected with human 5-HT₁₅ receptors, the potency of 5-HT was 10 times less than that for 8-OH DPAT than it was in hippocampus. Furthermore, the half-maximal effect of 5-CT was observed to be 100-fold lower in cultured human RPE cells than that witnessed in other systems, and the potencies of buspirone and RU24969 were at least 10-fold that observed previously (Fig. 2).

The action of 5-HT on forskolin-stimulated cAMP production was antagonized by spiroxatrine, spiperone, propranolol, methysergide, and metergoline (Fig. 3). Spiperone classically has been used to antagonize 5-HT₁₅ receptor-mediated responses and potently attenuates inhibition of forskolin-stimulated AC activity in hippocampus, and HeLa and CHO cells transfected with the human 5-HT₁₅ receptor. Beta adrenoceptor receptor antagonists also are known to have high affinity for 5-HT₁₅ receptors, reflecting the similarity of these two receptor types, and propranolol inhibits 5-HT and 8-OH DPAT action on 5-HT₁₅ receptors in rat hippocampus. Spiroxatrine has high affinity and selectivity for 5-HT₁₅ receptors but has been described variously as an antagonist and a partial agonist. In human RPE cells, spiroxatrine appears to act as a full antagonist and is more potent than are propranolol and spiperone at inhibiting both 5-HT and 8-OH DPAT action (Fig. 4). The partial agonists methysergide and metergoline have been described as agonists at 5-HT₁₅ receptors in hippocampal neurons, but in human RPE cell cultures act as antagonists as found in mouse cortical and striatal neurons.

The 5-HT-induced inhibition of forskolin-stimulated cAMP production was observed to be pertussis toxin-sensitive (Fig. 5) as expected for receptors negatively coupled to Gₛ proteins. Basal levels of cAMP were unaffected by pertussis toxin, whereas iso- proterenol-stimulated levels were found to be potentiated. Pertussis toxin preincubation attenuated 5-HT action by near 100%. To prove conclusively that pertussis toxin is inhibiting Gₛ protein action, however, adenosine diphosphate-ribosylation of the α₁ subunit must be shown.

Studies on the action of 5-HT on isoproterenol- and NECA-stimulated cAMP production provided some unexpected results. No effect of 5-HT on NECA action could be observed with either maximal or submaximal concentrations of NECA (Fig. 6) or different concentrations of 5-HT (data not shown). A similar differential effect on second-messenger production was observed by us for the effect of epidermal growth factor on the stimulation of cAMP production in human RPE cells where epidermal growth factor was found to potentiate the effects of forskolin and isoproterenol but not of NECA. Two explanations for these effects are possible. The failure of 5-HT and 8-OH DPAT to modulate NECA-stimulated cAMP production might result from their respective receptors being sequestered from each other in the human RPE cell membrane. The RPE are highly polarized and are known to show polarized and localized expression of membrane proteins (e.g., the Na⁺/K⁺ ATPase). Thus, the restriction of movement of membrane receptors and effector systems to specific domains is not improbable. If the A₂ adenosine receptor signal transduction system is sequestered from the 5-HT₁₅ receptors, then this also may explain why the inhibitory effect 5-HT on forskolin action fails to reach 100% because a certain level of AC will sequester with the adenosine receptors.
The recent description of different phenotypes of human RPE cells in culture might offer an alternative explanation. Two distinct subpopulations of cultured RPE cells have been identified, one showing an epithelial phenotype and the other fusiform, and these subsequently were found to show heterogeneity in the presence of phosphoproteins in the cell periphery. Using bovine RPE explants, Burke et al. also identified similar phenotypic variations in RPE cells in situ. It is possible, therefore, that subpopulations of human RPE cells in culture might express a different complement of cell surface receptors. Thus, if one subpopulation expresses adenosine receptors but not 5-HT<sub>1A</sub> receptors, no effect of 5-HT on NECA-stimulated cAMP production would be observed. The presence of one subpopulation might explain the inability of 5-HT to inhibit fully the forskolin-induced response because if only one phenotype expresses 5-HT<sub>1A</sub> receptors, then 100% inhibition of cAMP production could not be achieved. The ability to separate these two phenotypes of human RPE cell based on differential adhesion offers an attractive method to assess possible heterogeneity in the possession of cell surface receptors in the future.

The action of 5-HT on isoproterenol-stimulated cAMP levels is harder to understand because at high concentrations, both 5-HT and 8-OH DPAT inhibit the action of low concentrations of isoproterenol (Fig. 7). One possible explanation for these effects is that like adenosine receptors, the β-adrenoceptors are sequestered from the 5-HT<sub>1A</sub> receptors due to either of the possibilities discussed above and that high concentrations of 5-HT and 8-OH DPAT are able to directly inhibit isoproterenol binding to the β-adrenoceptor, thus reducing cAMP production. This, however, would not explain the partial attenuation of the 5-HT-induced response by pertussis toxin. Another explanation might include the coupling of a low density of 5-HT<sub>1A</sub> receptors to the β<sub>2</sub>-adrenoceptors or the interaction of the receptors with different subtypes of G-protein subunit or ACs. However, currently, there is no evidence for either of these possibilities. The actual explanation of this interesting phenomenon must therefore await future research.

In conclusion, the results presented show that cultured human RPE cells possess functional 5-HT<sub>1A</sub> receptors that mediate inhibition of forskolin-stimulated cAMP production. This is in contrast to cultured rat RPE cells, which express 5-HT<sub>2A</sub> receptors coupled to inositol phosphate production and [Ca<sup>2+</sup>]<sub>i</sub> mobilization. The role of the 5-HT<sub>1A</sub> receptors associated with human RPE cells is unknown, although the wide range of effects mediated by cAMP in vitro suggests they may be involved in the control of RPE cell function. Although it is unknown how serotonin may reach the RPE cells to trigger responses, several possibilities exist. One source may be directly from the serotonin-accumulating amacrine cells situated in the inner retina, whereas another could be from the choroidal blood supply. An alternative source is from the centrifugal serotonergic nerve fibers, which originate in the raphe nuclei or the suprachiasmatic nucleus. These fibers terminate in the outer plexiform layer in the rat retina, from where serotonin could reach the RPE cells more easily than from the inner plexiform layer or the choroid. The presence of this retinopetal pathway might suggest some degree of central control of the circadian rhythms of the outer retina.

Key Words: adenylyate cyclase, cAMP (adenosine 3′:5′ cyclic monophosphate), pertussis toxin, retinal pigment epithelial cells, 5-HT<sub>1A</sub> serotonin receptors

References


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