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Serotonin receptor subtype mRNA expression in human ocular tissues, determined by RT-PCR

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Purpose: To determine the relative density and tissue localization of mRNAs for serotonin (5-hydroxytryptamine; 5HT) receptor subtypes in human ocular tissues and correlate with their possible functions in the eye.
Methods: Total RNA was extracted from human ocular tissues samples from multiple donors and transcribed into cDNA. An optimized reverse transcriptase polymerase chain reaction (RT-PCR) procedure was then used to amplify the signals using primers designed against human 5HT receptor cDNAs. The PCR products were analyzed by gel electrophoresis and confirmed by endonuclease digestion.
Results: Variable amounts of total RNA were extracted from different tissues with the least amount from ciliary epithelium and most amount from retina. $5HT_2$ receptor subtype mRNAs were the most abundant with $5HT_{2A}$ and $5HT_{2B}$ being the most predominant in the retina, ciliary body, ciliary epithelium, choroid, conjunctiva, and iris. Ciliary body, choroid, and conjunctiva were most enriched in $5HT_3$ receptor mRNA, with relatively lower levels in the iris. $5HT_4$ receptor mRNA was most enriched in the retina, ciliary body, choroid, conjunctiva, and somewhat detectable in the iris. $5HT_5$ receptor mRNA was abundant in the retina, ciliary body, and iris. $5HT_6$ receptor mRNA was the least abundant of all subtypes studied and could only be detected in the iris. $5HT_7$ receptor mRNA was enriched in the ciliary body, choroid, conjunctiva, and iris, with much lower levels in the retina and ciliary epithelium. Optic nerve tissue of 1-2 donors exhibited the presence of $5HT_{2B}$, $5HT_5$, and $5HT_7$ receptor mRNAs. Data for human trabecular meshwork cells indicated a high density of mRNAs for $5HT_{2A}$ and $5HT_{2B}$, with much lower levels of $5HT_{2C}$, $5HT_5$, and $5HT_7$ receptor mRNAs.
Conclusions: Human ocular tissues differentially expressed mRNAs for the various 5HT receptor subtypes. These studies suggest a diverse range of possible physiological and pharmacological functions of 5HT receptors in these human ocular tissues.

The indolamine, serotonin (5-hydroxytryptamine; 5HT), is a major endogenous neurotransmitter in the mammalian body [1,2]. In the eye, 5HT is found in the aqueous humor [3,4], and ciliary body [5] and serotonergic innervation of many ocular tissues has been described based on many different types of studies [6-13]. Various radioligand binding [7-9], biochemical and pharmacological [10-13], and other tissue-based functional studies [14] have indicated the presence of a variety of 5HT receptors and their subtypes in ocular tissues of different species. Recent reverse transcriptase polymerase chain reaction (RT-PCR)-based investigations have revealed the presence of certain 5HT receptor subtypes in porcine conjunctiva [15], rabbit ocular tissues [16], and in human ciliary body [17].

Seven major guanine nucleotide coupled receptors, and the subtypes within each of these families, are known to mediate the different functions of 5HT [1,2]. Consequently, it is important to know the distribution of these receptors and their mRNAs and the down-stream coupling mechanisms associated with these receptors. There is a relative paucity of information on these latter aspects relative to the eye. Thus, in an attempt to address the latter aspects we and others have begun to perform a range of studies using a variety of different techniques [6-13] and different tissues and cells derived from eyes of various species (see above). Presently, we have mapped the relative distribution and density of 5HT receptor mRNAs in human ocular tissues obtained from numerous donors to shed further light on possible functions of 5HT in human ocular physiology and pharmacology. A preliminary report of some of these data was previously presented in abstract form [18,19].

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METHODS

Total RNA isolation: Total RNA from tissues possessing a high content of collagen and/or adipose (e.g., optic nerve) was extracted using the Tri-Pure isolation Reagent kit (Roche Molecular Biochemicals, Laval, Quebec, Canada). RNA extraction from all other tissues utilized the RNeasy Midi Kit (QIAGEN Inc., Mississauga, Ontario, Canada). Manufacturer's instructions were followed for all procedures [20]. Methods optimized for dealing with pigmented tissues were adopted to insure isolation of high quality RNA [20,21]. The concentration and purity of total RNA was determined by UV light absorption using a GeneQuant *pro* RNA/DNA calculator (Biochrom Ltd.; Cambridge, UK). Preparations were discarded if they had a ratio of optical densities at 260 nm/280 nm lower than 1.6 [21]. Furthermore, the presence of intact RNA was determined by loading it on 1% agarose-formalde-

hyde gels and subjecting them to electrophoresis. After ethidium bromide staining, RNA isolates were deemed intact if the UV fluorescence of the 28S rRNA band was twice the intensity of the 18S rRNA band and if no UV fluorescence was detected below the 18 rRNA band [21].

RT-PCR for mRNA detection: Sense and antisense-specific primers were synthesized at the Central Facility of the Institute of Molecular Biology and Biotechnology at McMaster University [21]. All primers were designed to span intron-exon boundaries to distinguish between amplification of mRNA and genomic DNA and were based on published cDNA sequences [15,17,22]. All primers were designed following BLAST retrieval of human cDNA sequences from GenBank searches. Reverse transcription polymerase chain reaction (RT-PCR) procedures were utilized to detect the mRNAs for various 5HT receptors in samples obtained from numerous donor eyes as previously described [15,17,21,22] (Table 1). Total RNA (25 to 97 μg) was isolated from the tissue samples and then converted into cDNA in a 10 μl reverse transcription reaction containing 0.5 mg of total RNA of each sample; 1X first strand buffer (75 mM KCl; 50 mM Tris-HCl, pH 8.3; 3.0 mM $MgCl_2$); 1.7 mM $MgCl_2$; 1 mM each dNTP; 10 mM dTT; 2.5 mM oligo (dT)18 and 5 U/μl of SuperScript™ II reverse transcriptase. Reactants were incubated at 42 °C for 60 min, heated at 95 °C for 5 min then cooled at 4 °C for a minimum of 5 min and a maximum of 30 min. This was followed by PCR performed on 5 μl of cDNA preparation (obtained from 0.5 μg of total RNA), to which was added 44 μl of a PCR master mix containing 1X PCR buffer (55 mM KCl; 13 mM Tris-HCl, pH 8.3); 1 mM $MgCl_2$; 10% dimethylsulphoxide (DMSO); 1.25 U/50 μl AmpilTaq Gold® with GeneAmp® DNA polymerase and 0.2 μM each sense and anti-sense primer in a total volume of 50 μl. A "hot start" PCR method was performed in a GeneAmp® PCR System 2400 thermocycler (Perkin Elmer, Norwalk, CA) using the following parameters: an initial denaturing step of 10 min at 95 °C; denaturing at 94 °C for 30 s; annealing at the optimal temperature for 30 s; extending at 72 °C for one min. The final polymerization step was extended an additional 7 min. Unless otherwise specified, 40 cycles of PCR was performed. Precautions were taken to avoid product contamination. PCR set-up, amplification, and product processing were performed using dedicated equipment in separate rooms. In addition, several control reactions were routinely run in parallel during RT-PCR analysis including RT reactions run in the absence of the reverse transcriptase enzyme to confirm the absence of genomic DNA and/or cDNA contamination and RT reactions without RNA to check for reagent contamination. As well, PCR amplification of 1.5 ng of human genomic DNA served as a negative control. Positive control RT-PCR reactions were performed using purchased total human lung or brain RNA. PCR amplification reactions were evaluated through electrophoresis of 12 μl of PCR product on a 1.5% agarose gels containing 1 μg/ml ethidium bromide and visualized by UV transillumination on a GeneGenius Imager (Synoptics Ltd., Cambridge, UK). Initial product identification was made by comparison to the positive control and the molecular weight ladder. Endonuclease digestion was used to confirm product identity. Briefly, digestion of each 5HT receptor mRNA amplification product was performed using the appropriate restriction endonuclease enzyme in a final reaction volume of 25 μl. Following digestion, products were resolved by 2.5 h of electrophoresis at 90 V on a 2.0% agarose-TBE gel stained with 1 μg/ml ethidium bromide. Gels were visualized and photographed by GeneGenius and GeneSnap software. Confirmation of appropriate splice products was made by comparison to the molecular weight ladder and to the positive control tissues (human lung or total human brain). The other internal control probed was mRNA for the housekeeping enzyme glycerol-3-phosphate dehydrogenase

Table 1.

Donor number	Age	Gender	Cause of death
1	98	Female	Cardiac arrest
2	89	Female	Respiratory failure
3	80	Female	Respiratory failure; chronic obstructive pulmonary disorder
4	91	Female	Colon cancer; hypertension
5	79	Female	Congestive heart failure; myocardiac infarction
6	88	Male	Congestive heart failure
7	84	Female	Chronic obstructive pulmonary disorder; pneumonia
8	82	Male	Peritonitis; pneumonia
9	76	Male	Cancer; liver failure
10	84	Female	Pneumonia

Human donor information relative to the use of the ocular tissues for RT-PCR studies.All subjects were Caucasian.

Table 2.

Tissue	Total yield (μg)	Number of donors
Ciliary Epithelium	4.3± 0.5	4
Iris	27.5± 6.8	7
Optic Nerve	27.6±16.8	8
Ciliary Body	43.6± 8.6	8
Conjunctiva	48.5±15.0	4
Choroid	97.1±13.8	7
Retina	196.8±27.4	8

Total RNA yields (mean±SEM) from various human ocular tissues. RNA isolated from 4-8 individual donor eye tissues is shown. Each sample was handled separately and kept separate for the subsequent RT-PCR experiments.

Table 3.

5HT receptor	Positive control tissue	PCR product length (bp)	Endonuclease enzyme used for PCR product verification	Endonuclease digestion products (bp)
2A	Brain	277	Bcl1	193, 85
2B	Lung	432	HaeIII	348, 84
2C	Brain	288	HaeIII	38, 150, 100
3	Brain	393	Aci1	126, 267
4	Brain	398	Alu1	310, 88
5	Brain	486	Alu1	38, 60, 388
6	Brain	198	Pst1	73, 125
7	Brain	772	Bcl1	312, 171, 289

Amplification and confirmation of human 5HT receptor mRNAs. The data shown were used to confirm the identity of the 5HT receptor mRNA being detected in human ocular tissues relative to the positive control tissues (human brain and lung).

(G3PDH). Other aspects of these procedures have been previously described [15,17,22,23].

RESULTS

In order to perform the RT-PCR studies, total RNA had to be extracted from each sample and the corresponding cDNA constructed. Tissues from various donors yielded different amounts of total RNA, as shown in Table 2. Endonuclease digestion products helped confirm the 5HT receptor subtype mRNAs being detected by the current RT-PCR procedures (Table 3).

Examples of mRNA signals detected for $5HT_{2A}$ (Figure 1), $5HT_{2B}$ (Figure 2), and $5HT_3$ (Figure 3) receptors in numerous tissues, along with positive controls (5HT receptor mRNAs from human brain and lung) and internal control enzyme (G3PDH) mRNA expression (Figure 4) are shown. The relative intensity of the mRNA signals determined visually, thus qualitatively, revealed differential density and distribution of the various 5HT receptor subtype mRNAs studied (Table 4, Table 5).

DISCUSSION

Despite attempts to perform careful dissections and uniform processing of postmortem human donor eye tissues, we found a certain amount of variability in the amount of total RNA isolated from these tissues. However, the mRNA signals detected by RT-PCR were normalized by the use of uniform amounts of RNA and cDNA preparations, and typically the same number of PCR cycles. In addition, endonuclease digestion products helped confirm the identity of the receptor mRNA under study. Positive controls such 5HT receptor mRNAs from human brain and lung, and the mRNA for the internal housekeeping enzyme glycerol-3-phosphate dehydrogenase (G3PDH) also helped validate the RT-PCR procedures employed in the current studies.

With respect to the relative density and distribution of the mRNAs for the various 5HT receptor subtypes examined, human conjunctival tissue, in the anterior segment of the eye, expressed a high density of mRNAs for $5HT_{2A}$, $5HT_{2B}$, $5HT_7$, $5HT_4$, and $5HT_3$ receptors with $5HT_{2C}$ and $5HT_6$ receptor mRNAs being undetectable and $5HT_5$ receptor mRNA levels being quite low (Table 4, Table 5). Turner et al. [15] recently reported the presence of $5HT_{1D}$ and $5HT_{1F}$ receptor mRNAs in human conjunctiva in addition to $5HT_7$ receptor. Interestingly, the porcine and human conjunctiva had a different profile to the rabbit conjunctiva in terms of the 5HT receptor mRNA expression [15] thus pointing to some species differences. Along with the detection of mRNAs and functional receptor proteins for $5HT_7$ receptors coupled to cAMP production in rabbit and human corneal epithelium/cells [11,24-28] and conjunctiva [15] it appears that the $5HT_7$ receptor may be involved in modulation of fluid and chloride secretion [11,24-28] in these ocular surface tissues. The linkage to functions of the receptor mRNAs for $5HT_{2-4}$ receptors we detected in human conjunctiva remains to be determined.

Within the anterior uveal tissues, we found mRNAs for the majority of the 5HT receptor subtypes in human ciliary body/ciliary epithelium with greatest apparent abundance of $5HT_{2A-C}$, $5HT_7$, $5HT_5$, and $5HT_4$ receptors. Our data confirmed the recent report by Chidlow et al. [17] who also found $5HT_{1A}$ receptor mRNA in the ciliary body, but we extended the observations to $5HT_{3-5}$ receptor mRNAs and the finding that $5HT_6$ receptor mRNAs could not be found in the human ciliary body. Functional correlates for such observations include the pharmacological characterization of $5HT_{1A}$ receptors in rabbit cili-

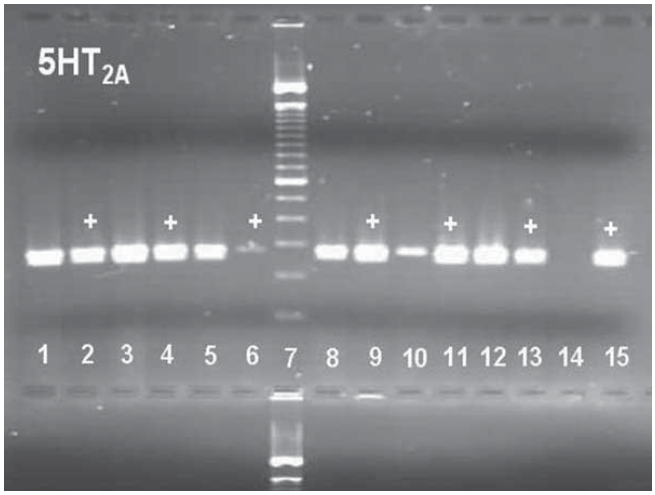

Figure 1. Representative RT-PCR mRNA signals for $5HT_{2A}$ receptor in human ocular tissues. The legend for the different lanes are as follows: 1. Retina; 2. positive control (brain); 3. Ciliary body; 4. positive control (brain); 5. Ciliary epithelium; 6. positive control (brain); 7. Molecular weight markers; 8. Choroid; 9. positive control (brain); 10. Conjunctiva; 11. positive control (brain); 12. Iris; 13. positive control (brain); 14. Optic nerve; 15. positive control (brain). The positive controls are also identified in the figure with a plus symbol.

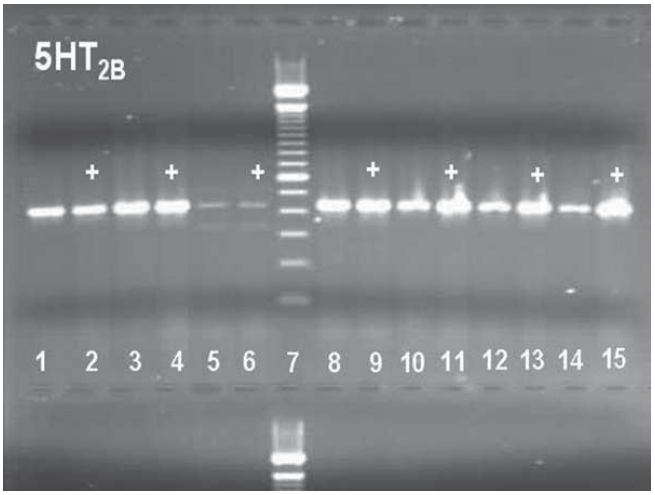

Figure 2. Representative RT-PCR mRNA signals for $5HT_{2B}$ receptor in human ocular tissues. The legends for the different lanes are as follows: 1. Retina; 2. positive control (lung); 3. Ciliary body; 4. positive control (lung); 5. Ciliary epithelium; 6. positive control (lung); 7. Molecular weight markers; 8. Choroid; 9. positive control (lung); 10. Conjunctiva; 11. positive control (lung); 12. Iris; 13. positive control (lung); 14. Optic nerve; 15. positive control (lung). The positive controls are also identified in the figure with a plus symbol.

ary-body [7,8], negative coupling to adenylate cyclase [29,30], antagonism of muscarinic functions via the $5HT_{1A}$ receptors [31]. Functional coupling of $5HT_2$ receptors in human ciliary muscle cells to phospholipase C [32], and thus generation of inositol phosphates and mobilization of intracellular Ca^{2+} in bovine ciliary epithelium [10] and ciliary muscle contraction/relaxation via $5HT_{2/3}$ receptors [14] can be related to the RT-PCR data in the present studies and those of others as mentioned above. These collective data for the presence of $5HT_2$ receptors in the ciliary muscle/epithelium and human trabecular meshwork [33] strongly support the efficacious intraocular pressure (IOP) lowering effects in the cynomolgus monkey of $5HT_2$ agonists of different structural classes [34-37], primarily via the uveoscleral outflow pathway [38] even though the contributions from the conventional outflow pathway cannot be ruled out [33]. However, there appear to be major species differences in the ocular hypotensive effects of 5HT agonists, since $5HT_2$ agonists do not lower IOP in the rabbit [34] but $5HT_{1A}$ agonists are ocular hypotensive agents in this species [39-42]. However, the physiological relevance of the $5HT_4$, $5HT_5$, and $5HT_7$ receptor mRNAs we found in human ciliary body remains to be defined.

The human iris appears highly enriched in $5HT_{2A}$, $5HT_{2B}$, $5HT_7$, and $5HT_5$ receptor mRNAs (Table 4). Our observations pertaining to $5HT_2$ and $5HT_7$ receptor mRNAs in the iris confirm observations of Chidlow et al. [17]. Unfortunately, there are major species differences in the density and functions of iridial 5HT receptors and thus gross generalizations cannot be made as to the functions of these receptors. Thus, while 5HT receptors coupled to phosphoinositide turnover [43] and inhibition of adenylate cyclase [29,30] and stimulation of cAMP production coupled to iridial relaxation [43] have been reported in rabbit iris, direct changes of papillary diameter [44] cannot be ascribed to any specific 5HT receptor subtype even though $5HT_2$, $5HT_{1A}$, and $5HT_7$ receptors appear to be involved based on the second messenger data. However, pharmacological characterization of iridial 5HT receptor subtypes represents a fertile area of research in the future.

In terms of the posterior chamber of the eye, we found the human retina and choroid to be well endowed in mRNAs for numerous 5HT receptor subtypes. These observation complement the previous detection of serotonergic innervation of the retina [45] and finding of retinal cell's ability to accumulate exogenous 5HT [46-48]. Due to the complexity of the different cell types of the retina, it is difficult to define which receptors may be present on which cell types from our data. Our detection of $5HT_{2A-C}$ receptor mRNAs in the human retina can be correlated with reports of $5HT_2$ receptor coupled phospholipase C mechanisms in rabbit retina [49] and in rat retinal pigment epithelial cells [50,51] and pre-synaptic localization of retinal $5HT_{2A}$ receptors [52]. Even though $5HT_3$ binding sites have been detected in rat [9] and rabbit retina [53], and electrophysiological studies using measurement of channel activity associated with these receptors [54-56] have lent credence to their existence, we were unable to detect mRNA for $5HT_3$ receptor in human retinal tissue, hence suggesting additional species differences. However, there appears to be a better correlation between species for the 5HT receptors positively coupled to adenylate cyclase. Thus, we found strong mRNA signals for $5HT_4$ and $5HT_5$ receptors and weaker but detectable levels of $5HT_7$ receptor mRNA in human retina and others have reported on 5HT receptor-mediated elevation of

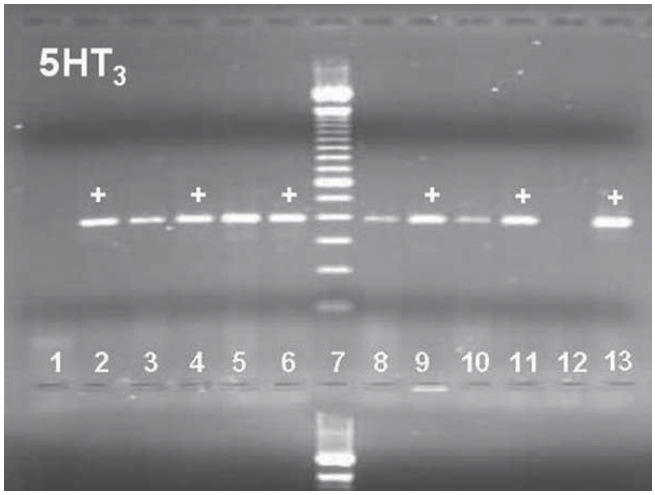

Figure 3. Representative RT-PCR mRNA signals for $5HT_3$ receptor in human ocular tissues. The legends for the different lanes are as follows: 1. Retina; 2. positive control (brain); 3. Ciliary body; 4. positive control (brain); 5. Choroid; 6. positive control (brain); 7. Molecular weight markers; 8. Conjunctiva; 9. positive control (brain); 10. Iris; 11. positive control (brain); 12. Optic nerve; 13. positive control (brain). The positive controls are also identified in the figure with a plus symbol.

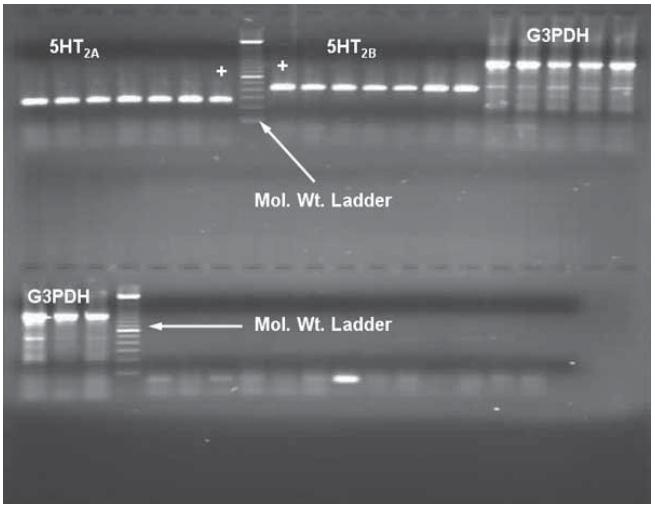

Figure 4. RT-PCR mRNA signals for $5HT_{2A}$ and $5HT_{2B}$ mRNA signals in human ciliary body samples. The top left side of the figure shows the $5HT_{2A}$ mRNA signals (along with a positive control, brain, shown with a plus sign) from individual donor samples followed by the molecular weight ladder, and then the $5HT_{2B}$ mRNA signals and the respective positive control. The top right side (and bottom left side) of the figure shows the mRNA signals for the internal control house-keeping enzyme, glycerol-3-phosphate dehydrogenase (G3PDH).

TABLE 4.

5HT receptor subtype mRNA signals in human conjunctiva								
Donor #	$5HT_{2A}$	$5HT_{2B}$	$5HT_{2C}$	$5HT_3$	$5HT_4$	$5HT_5$	$5HT_6$	$5HT_7$
1	+++	+++	-	+	++	-	-	-
2	-	-	-	-	-	+	-	+++
3	+++	+++	-	++	+++	+	-	+++
4	++	+++	-	++	+++	-	-	+++

5HT receptor subtype mRNA signals in human ciliary body								
Donor #	$5HT_{2A}$	$5HT_{2B}$	$5HT_{2C}$	$5HT_3$	$5HT_4$	$5HT_5$	$5HT_6$	$5HT_7$
1	+++	+++	++	+++	+++	+++	-	+++
2	+++	+++	++	++	++	++	-	+++
3	+++	+++	-	-	++	++	-	+++
4	+++	+++	+	+++	++	++	-	+
5	+++	+++	+	+	++	-	-	+++
6	+++	+++	+++	-	+++	++	-	+++

5HT receptor subtype mRNA Signals in human ciliary epithelium								
Donor #	$5HT_{2A}$	$5HT_{2B}$	$5HT_{2C}$	$5HT_3$	$5HT_4$	$5HT_5$	$5HT_6$	$5HT_7$
1	+++	-	x	x	x	x	x	X
2	+++	+	+	x	x	x	x	-
3	+++	-	+	x	x	x	x	+
4	+++	+	+	x	x	x	x	+

5HT receptor subtype mRNA signals in human TM cells								
Donor	$5HT_{2A}$	$5HT_{2B}$	$5HT_{2C}$	$5HT_3$	$5HT_4$	$5HT_5$	$5HT_6$	$5HT_7$
Data from 8 donors	+++	+++	+	-	-	+	-	+

5HT receptor subtype mRNA signals in human iris								
Donor #	$5HT_{2A}$	$5HT_{2B}$	$5HT_{2C}$	$5HT_3$	$5HT_4$	$5HT_5$	$5HT_6$	$5HT_7$
1	+++	+++	-	+	+	++	+	+++
2	+++	-	-	-	+	+	+	++
3	+++	+++	-	x	x	+	+	-
4	+++	+++	+	++	++	++	-	+++

RT-PCR-based detection of 5HT receptor mRNA signal expression in tissues of the ocular surface and of anterior chamber of the human eye. In the table, the single plus sign (+) indicates an extremely faint but present band, the double plus sign (++) indicates an easily visible band, the triple plus sign (+++) denotes a very strong band, the minus sign (-) shows negative for the presence of a band, and an "x" indicates that the experiment was not completed (due to lack of available tissue or total RNA).

cAMP in rabbit retinae [57-59] with properties primarily reflecting $5HT_7$ receptor pharmacology.

Human choroidal tissue appeared to express the same 5HT receptor mRNA profile as the retina with the exception that $5HT_3$ receptor mRNA signal was quite robust in the choroid (Table 4). This raises the question whether the studies where retinal $5HT_3$ binding sites and electrophysiological observations were made [45,53,54] were contaminated with choroidal tissue. At any rate, we have also recently detected a high density of [^{3}H]-5HT and [^{3}H]-ketanserin-labeled binding sites on human choroidal tissue using quantitative autoradiographic techniques [32] suggesting that this tissue is dependent on the serotonergic system for some of its functions.

Even though we have limited data for mRNAs for 5HT receptors in the human optic nerve, it was interesting that relatively strong mRNA signals were observed for $5HT_{2B}$, $5HT_5$, and $5HT_7$ receptor in some of the donor tissue (Table 4). We are unable to ascribe any function to these observations but perhaps these reflect mRNAs undergoing axonal transport to the retinal ganglion cells where we have indeed detected very high levels of $5HT_5$, $5HT_{2B}$, and, to a lesser extent, $5HT_7$ receptor mRNAs. However, much more work is needed to confirm and extend these observations.

In conclusion, RT-PCR detection of 5HT receptor subtype mRNAs in numerous human ocular tissues has been successfully performed. While it is difficult to associate these observations with functional evidence for the existence of the various 5HT receptors in these tissues in every case, our data provide a foundation for future research to discover the physiological and pharmacological relevance of the 5HT receptors in the human ocular tissues.

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TABLE 5.

5HT receptor subtype mRNA signals in human retina								
Donor #	$5HT_{2A}$	$5HT_{2B}$	$5HT_{2C}$	$5HT_3$	$5HT_4$	$5HT_5$	$5HT_6$	$5HT_7$
1	++	++	+	-	+++	+++	-	-
2	+	++	-	-	+++	++	-	-
3	-	+	+	-	++	+	-	+
4	+++	++	++	-	+++	+++	-	+
5	+++	++	++	-	+++	+++	-	+
6	+++	++	++	-	+++	+++	-	+
7	+++	x	x	-	+++	+++	-	+
8	x	++	++	x	x	x	x	x
5HT receptor subtype mRNA signals in human choroid								
Donor #	$5HT_{2A}$	$5HT_{2B}$	$5HT_{2C}$	$5HT_3$	$5HT_4$	$5HT_5$	$5HT_6$	$5HT_7$
1	+	++	+	+++	++	+	+	-
2	+++	+++	++	+++	++	+	-	+++
3	+++	+++	++	+++	+++	+	-	+++
4	+++	+++	++	+++	++	-	-	++
5HT receptor subtype mRNA signals in human optic nerve								
Donor #	$5HT_{2A}$	$5HT_{2B}$	$5HT_{2C}$	$5HT_3$	$5HT_4$	$5HT_5$	$5HT_6$	$5HT_7$
1	-	+	-	-	-	-	-	-
2	+	-	-	x	x	++	+	++
3	-	+++	-	-	+	-	-	-

RT-PCR-based detection of 5HT receptor mRNA signal expression in tissues of the posterior chamber of the human eye. In the table, the single plus sign (+) indicates an extremely faint but present band, the double plus sign (++) indicates an easily visible band, the triple plus sign (+++) denotes a very strong band, the minus sign (-) shows negative for the presence of a band, and an "x" indicates that the experiment was not completed (due to lack of available tissue or total RNA).

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