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5-Hydroxytryptamine$_{1C}$ Receptor Density and mRNA Levels in Choroid Plexus Epithelial Cells after Treatment with Mianserin and (−)-1-(4-Bromo-2,5-dimethoxyphenyl)-2-aminopropane

ERIC L. BARKER[1] and ELAINE SANDERS-BUSH

Department of Pharmacology, School of Medicine, Vanderbilt University, Nashville, Tennessee 37232-6600

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SUMMARY

5-Hydroxytryptamine (5HT)$_{1C}$ and 5HT$_2$ receptors display paradoxical down-regulation when exposed to receptor antagonists *in vivo*, a property that is unique to these two subtypes of serotonin (5HT) receptors. Because of the absence of cell culture model systems, the mechanisms involved in this paradoxical down-regulation have been difficult to explore. The present study focuses on the regulation of 5HT$_{1C}$ receptors in primary cultures of rat choroid plexus epithelial cells. Exposure of the epithelial cell cultures to 100 nM mianserin, a receptor antagonist, or (−)-1-(4-bromo-2,5-dimethoxyphenyl)-2-aminopropane, an agonist, for 72 hr caused a loss of 5HT$_{1C}$ receptor binding sites, as determined by [^{3}H]mesulergine binding to crude membrane preparations. No significant changes in K_d values were observed. Neither the agonist nor antagonist caused a significant change in binding sites after 24 hr. A solution hybridization assay was used to determine whether the down-regulation by mianserin or (−)-1-(4-bromo-2,5-dimethoxyphenyl)-2-aminopropane was accompanied by a decrease in the steady state level of 5HT$_{1C}$ receptor mRNA. These studies showed that neither treatment caused an alteration in the levels of 5HT$_{1C}$ receptor mRNA. Thus, it is possible to reproduce the *in vivo* regulatory effects of drugs on 5HT$_{1C}$ receptors in choroid plexus epithelial cells in culture, including the atypical down-regulation by receptor antagonists. Using this cell culture model system, indirect transynaptic effects and decreases in receptor mRNA levels have been ruled out as mechanisms accounting for the down-regulation.

Adaptive regulation of neurotransmitter receptors occurs as the central nervous system attempts to maintain homeostasis in response to drugs and other external stimuli. Exposure of a receptor to an agonist initiates a complex cascade of events. First, a desensitization or decreased ability of the agonist to elicit a further response occurs. This reflects a functional uncoupling of the receptor from its effector system. Next, the receptors are sequestered and internalized away from the cell surface and, finally, upon prolonged agonist exposure, down-regulation or actual loss of receptor protein is observed. Conversely, disuse of receptors caused by either chemical denervation or chronic pharmacological antagonism leads to supersensitivity and up-regulation, manifested as an increase in receptor density and responsiveness. These general regulatory phenomena have been demonstrated for many hormone, growth factor, and neurotransmitter receptors.

The 5HT$_{1C}$ and 5HT$_2$ receptors, which are members of a large family of 5HT receptors, typically undergo down-regulation in response to agonists (1-3) but deviate from receptor regulation dogma by exhibiting a paradoxical down-regulation after chronic antagonist treatment (4, 5). In addition to this atypical regulation, the 5HT$_{1C}$ and 5HT$_2$ receptors have many other properties in common. Both receptors are positively linked to the phosphoinositide hydrolysis signaling cascade, their pharmacological profiles are similar, and their primary amino acid structures indicate a high degree of sequence homology (6). The present study focuses on the regulation of 5HT$_{1C}$ receptors, which are highly localized to the epithelial cells of the choroid plexus (7, 8). *In vivo* studies have shown that the administration of quipazine, a serotonin receptor agonist, causes down-regulation of 5HT$_{1C}$ receptors in rat choroid plexus (3), whereas the administration of the receptor antagonist mianserin also causes down-regulation of the receptors (5). This atypical regulation of the 5HT$_{1C}$ receptor has been recently confirmed in rat spinal cord after treatment with a number of other agonists and antagonists (9).

The perplexing question has been how agents with seemingly opposite pharmacological properties can elicit the common adaptive response of down-regulation. Experiments designed to address the mechanisms involved in agonist- and antagonist-induced down-regulation have been hindered by the lack of a

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[1] Current address: Department of Anatomy and Cell Biology, Emory University School of Medicine, Atlanta, GA 30322.

ABBREVIATIONS: 5HT, 5-hydroxytryptamine; (−)-DOB, (−)-1-(4-bromo-2,5-dimethoxyphenyl)-2-aminopropane; bp, base pair(s).

cell culture model with a homogeneous population of cells, where experimental conditions can be strictly controlled. The use of cell culture models is critical to eliminating transynaptic effects potentially involved in the atypical regulation observed *in vivo*. A transynaptic mechanism has been associated with the chronic administration of the $5HT_{1C/2}$ receptor antagonist ritanserin, which attenuates the presynaptic $5HT_{1A}$ receptor-mediated inhibitory feedback regulation of 5HT release (10). A blunting of this autoregulatory response may increase synaptic 5HT levels, which in turn could lead to a down-regulation of postsynaptic receptors. To avoid transynaptic mechanisms, we have utilized primary cell cultures enriched in choroid plexus epithelial cells to determine whether $5HT_{1C}$ receptor down-regulation occurs *in vitro*. In these cultures, $5HT_{1C}$ receptors exhibited a delayed down-regulation after both agonist and antagonist treatment, consistent with previous results obtained *in vivo*.

A growing body of evidence suggests that down-regulation of various receptors is accompanied by alterations in gene transcription or mRNA stability (for review, see Ref. 11). For example, β_2-adrenergic receptor density and mRNA levels are both decreased upon prolonged exposure to receptor agonists (12, 13). The decrease in mRNA levels is caused by a destabilization of receptor mRNA (14). To determine whether transcriptional or post-transcriptional regulation of mRNA plays a role in $5HT_{1C}$ receptor down-regulation, the levels of $5HT_{1C}$ receptor mRNA were examined after treatments that caused a loss of receptor binding sites. These studies showed that neither agonist nor antagonist treatment led to an alteration in steady state levels of $5HT_{1C}$ receptor mRNA.

Experimental Procedures

Materials. Twenty-day-old male Sprague-Dawley rats were obtained from Sasco, Inc. (Omaha, NE). Pronase was purchased from Boehringer Mannheim (Indianapolis, IN). Cell culture dishes were obtained from Falcon/Becton Dickinson and Co. (Lincoln Park, NJ). Cell medium, dialyzed calf serum, gentamicin, and S1 nuclease were purchased from GIBCO/BRL Life Technologies Inc. (Grand Island, NY). [^{3}H]Mesulergine (78 Ci/mmol) was obtained from Amersham Corp. (Arlington Heights, IL) and (±)-[5,7-^{3}H]CGP-12177 (51.9 Ci/mmol) and [α-^{32}P]CTP (800 Ci/mmol) from New England Nuclear Corp. (Boston, MA). Restriction endonucleases were purchased from New England Biolabs, Inc. (Beverly, MA), T7 RNA polymerase and Riboprobe Gemini II Core System from Promega Corp. (Madison, WI), and pBKS II(−) from Stratagene Cloning Systems (La Jolla, CA). Mianserin HCl was obtained from Research Biochemicals Inc. (Natick, MA) and (−)-DOB from the National Institute on Drug Abuse (Rockville, MD). All other chemicals were obtained from Sigma Chemical Co. (St. Louis, MO) or Fisher Scientific Co. (Fair Lawn, NJ).

Primary cultures of choroid plexus epithelial cells. Cultures were prepared as described previously (15). Epithelial cells were liberated by incubation at 37° in Hanks' buffer containing Pronase and type I DNase. The pooled cells were plated in 100-mm-diameter culture dishes and incubated at 37° in minimum essential medium with D-valine, 15% dialyzed calf serum, and 10 μg/ml gentamicin, in a humidified incubator with 5% CO_2. After 3 days of culture, medium was changed to Ham's F-12 without serum. Treatments began at least 24 hr and not longer than 48 hr later, with fresh drug added to cultures each day of treatment. The cultures were enriched with populations of cells displaying typical epithelial morphology, with few contaminating cells morphologically similar to fibroblasts.

$5HT_{1C}$ receptor binding. Crude membranes were prepared for binding assays by a modification of the method of Ivins and Molinoff (16). Cells were washed with phosphate-buffered saline, incubated in ice-cold hypotonic buffer for 10 min, detached with a cell scraper, and centrifuged for 1 hr at 27,000 × *g*. The resulting pellet was resuspended by trituration and centrifuged for another 1 hr at 27,000 × *g*. The pellet was suspended in 50 mM Tris·HCl buffer, pH 7.4, homogenized with a Brinkmann Polytron (5 sec, setting 7), and used in binding assays. Saturation binding of [^{3}H]mesulergine was performed using 10 μM 5HT to define nonspecific binding. Assay tubes were incubated for 30 min at 37° and filtered using a Brandel harvester with Whatman GF/C filters that were previously soaked in 3% polyethylenimine, pH 9.5. Protein concentration was determined using the method of Bradford (17), with bovine serum albumin as a standard.

β-Adrenergic receptor binding. C6 glioma cells were maintained at 37° in Ham's F-10 medium supplemented with 0.5% glutamine, 2.5% fetal calf serum, and 15% horse serum, in a humidified atmosphere containing 5% CO_2. For studies of receptor density, cells were plated on 100-mm culture dishes, with treatments beginning when the cells reached confluency. β-Adrenergic receptor binding in crude membrane preparations was determined using six concentrations of [^{3}H]CGP-12177 ranging from 0.1 to 2.5 nM. Nonspecific binding was defined by the addition of 10 μM *dl*-propranolol. Assays were incubated at 25° for 30 min and processed as described above.

Determination of $5HT_{1C}$ receptor mRNA by solution hybridization. $5HT_{1C}$ receptor mRNA standards were prepared from cells transfected with $5HT_{1C}$ receptor cDNA. Rat $5HT_{1C}$ receptor cDNA (18) was subcloned into the *Eco*RI site of the expression vector pCMV2 (19). NIH3T3 fibroblasts were transfected with the $5HT_{1C}$ receptor cDNA/pCMV2 construct and the selection marker pRSVneo by the high efficiency calcium phosphate method (20). Transfected cells were maintained at 37° in Dulbecco's modified Eagle medium with 10% bovine calf serum, in a humidified incubator in an atmosphere of 5% CO_2. Neomycin-resistant colonies were isolated and screened for 5HT-mediated phosphoinositide hydrolysis. A clonal line expressing approximately 10,000 fmol/mg of protein of $5HT_{1C}$ receptors (referred to as B3–6 cells) was used to prepare $5HT_{1C}$ receptor mRNA for control experiments.

Total RNA from B3–6 cells or choroid plexus epithelial cells was isolated over a cesium chloride gradient by a modification of a previously described method (21). Medium was removed and buffer (4 M guanidinium isothiocyanate, 25 mM sodium citrate, 0.5% sarcosyl, 0.1 M β-mercaptoethanol) was added. Cell suspensions were sheared several times through a 22-gauge needle, layered on a cushion of 5.7 M cesium chloride, and centrifuged at 260,000 × *g* for 4 hr at 22°. The resulting pellet was resuspended, precipitated with ethanol, and quantitated by measuring absorbance at 260 nm. Fifteen to 20 μg of total RNA from choroid plexus epithelial cells were used in each determination.

A solution hybridization S1 nuclease protection assay was developed based on the original procedure introduced by Sharp *et al.* (22), except that a ^{32}P-labeled riboprobe was used instead of a ^{32}P-labeled DNA probe. A ^{32}P-labeled single-stranded RNA probe for the $5HT_{1C}$ receptor was prepared as follows: the 1.6-kilobase *Hind*III fragment of the rat $5HT_{1C}$ receptor cDNA was cloned and oriented into pBKS II(−) such that, when the plasmid is linearized with *Age*I, a 570-bp antisense riboprobe is synthesized using T7 RNA polymerase. This probe spans nucleotides 1143–1617, coding for the amino acids from the beginning of the second cytoplasmic loop through the third cytoplasmic loop of the $5HT_{1C}$ receptor. The predicted length of the protected fragment in a protection assay is 480 bp. Total RNA from B3–6 cells yielded the predicted single fragment at 480 bp, whereas RNA isolated from the choroid plexus epithelial cells yielded two protected fragments, the predicted 480-bp fragment and an unidentified fragment of 400 bp. A rat β-actin ^{32}P-labeled riboprobe was used as an internal control. A 199-bp fragment from the rat genomic β-actin DNA (nucleotides 2452–2651; exon 4 and 57 nucleotides of intron 4) was generated by the polymerase chain reaction and cloned into pBKS II(−) (23). When the plasmid is linearized with *Eco*RI and transcribed with T7 RNA polymerase, a 265-bp riboprobe is produced, which yields a fragment of 143 bp in a protection assay. Total RNA was hybridized overnight at 45°

with 200,000 cpm of each labeled probe in 30 μl of hybridization buffer [40 M piperazine-*N*,*N*′-bis-(2-ethanesulfonic acid), pH 6.4, 1 mM EDTA, pH 8, 400 mM NaCl, 80% formamide]. The samples were then digested with S1 nuclease for 30 min at 37° and precipitated with isopropanol, and the protected fragments were isolated by electrophoresis on a 4% acrylamide, 7 M urea gel. A representative S1 nuclease assay of total RNA isolated from B3–6 cells is shown in Fig. 1.

Quantitative measurements of $5HT_{1C}$ receptor and β-actin mRNA levels were obtained by using a PhosphorImager (Molecular Dynamics, Sunnyvale, CA) or by densitometric analysis of X-ray films using Image 1.38 software (Wayne Rasburn, National Institutes of Health, Bethesda, MD). Standard curves, generated using increasing amounts of B3–6 cell RNA, were linear for both $5HT_{1C}$ receptor mRNA and β-actin mRNA. To determine whether β-actin mRNA is a valid internal standard, the actual density units for the β-actin mRNA bands were compared over the time course of the treatments. These analyses showed that β-actin mRNA does not change during the course of the experiments and is not altered by the treatments. This validates the use of β-actin as an internal control to normalize the $5HT_{1C}$ receptor mRNA values. In each experimental sample, $5HT_{1C}$ receptor mRNA abundance was normalized by dividing the $5HT_{1C}$ receptor mRNA density value by the corresponding β-actin value.

Results

Down-regulation of $5HT_{1C}$ receptors in choroid plexus epithelial cells by mianserin and (−)-DOB. Exposure of primary cultures of choroid plexus epithelial cells to 100 nM (−)-DOB, a $5HT_{1C}$ receptor agonist, for 72 hr led to a 45% loss of $5HT_{1C}$ receptor binding sites (Fig. 2; Table 1). Additionally, 72-hr treatment with 100 nM mianserin, an antagonist, induced a 33% decrease in binding sites (Fig. 3; Table 1). No significant shifts in the K_d values for [^{3}H]mesulergine were observed. Neither (−)-DOB nor mianserin altered receptor density in the choroid plexus epithelial cells after 24 hr of treatment (Table 1).

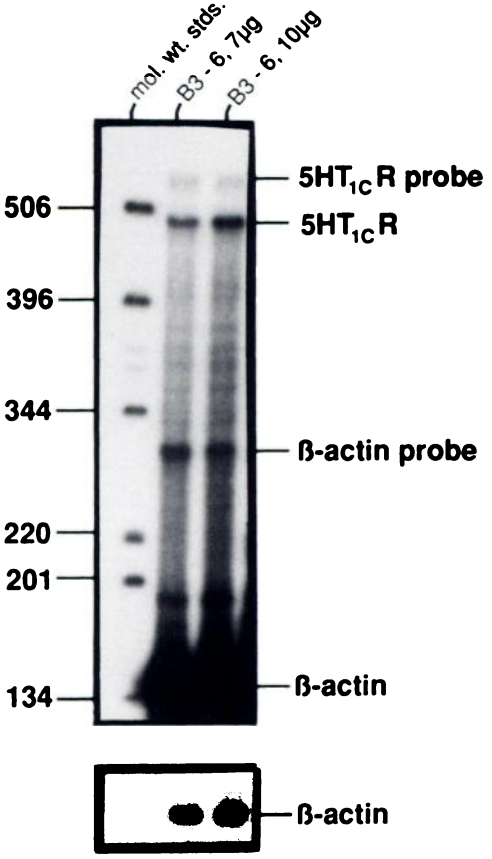

Fig. 1. S1 nuclease protection assay of $5HT_{1C}$ receptor (*$5HT_{1C}R$*) mRNA. Total RNA was isolated from fibroblasts expressing the $5HT_{1C}$ receptor cDNA (B3–6 cells) and was assayed as described in Experimental Procedures. *First lane*, molecular weight markers; *second lane*, 7 μg of total RNA; *third lane*, 10 μg. *Lower*, a second film exposed for a shorter time. For quantitations, the gels were analyzed using a PhosphorImager, which eliminates problems associated with the unequal signals for the $5HT_{1C}$ receptor and β-actin.

Effects of mianserin on β-adrenergic receptors in C6 glioma cells. The question still remains whether the effect of the $5HT_{1C}$ receptor antagonist mianserin is a specific effect on $5HT_{1C}$ receptors or is a generalized phenomenon that would cause a decrease in other cell surface proteins. Classically, receptor specificity would be addressed by attempting to block the effect of a drug with a receptor-selective antagonist. However, because both agonists and antagonists elicit down-regulation of $5HT_{1C}$ receptors, such studies would be uninterpreta-

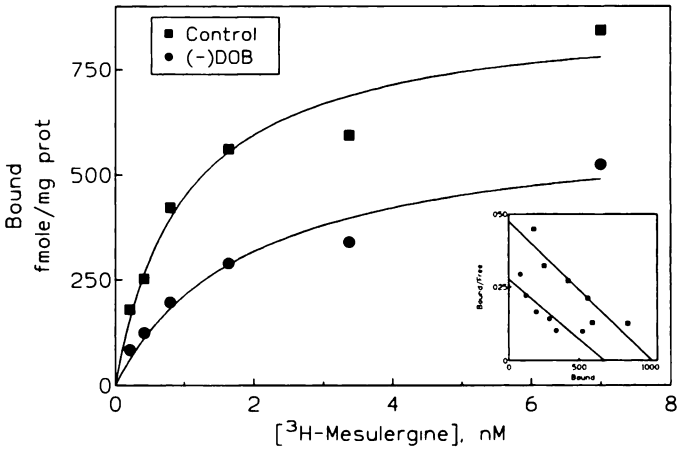

Fig. 2. Down-regulation of $5HT_{1C}$ receptors in choroid plexus epithelial cells by (−)-DOB. Cells were treated with 100 nM (−)-DOB for 72 hr, with fresh drug added each day of treatment. Radioligand binding was then performed as described in Experimental Procedures. *Data points* are means of duplicate determinations and are representative of three separate experiments. The *curve* was plotted using the equation for a rectangular hyperbola (GraphPad InPlot). *Inset*, the saturation binding curve was transformed to a Scatchard plot. See Table 1 for a summary of all of the data.

TABLE 1

Induction of down-regulation of $5HT_{1C}$ receptors by 3-day treatment with mianserin or (−)-DOB

Primary cultures of choroid plexus epithelial cells were treated with 100 nM mianserin or (−)-DOB for 72 hr (groups I and II) or 24 hr (group III). The values are means ± standard errors, with the number of experiments shown in parentheses. Differences between mean values in groups I and II were determined by paired *t* tests using GraphPad InStat (Intuitive Software for Science, La Jolla, CA). The treatments in group III were compared using one-way analysis of variance, followed by Dunnett's multiple-comparisons test.

	B_{max}	K_d
	fmol/mg of protein	*nM*
Group I (72 hr)		
Control (6)	1100 ± 315	1.0 ± 0.1
Mianserin (6)	740 ± 270*	1.4 ± 0.3
Group II (72 hr)		
Control (3)	650 ± 120	0.9 ± 0.2
(−)-DOB (3)	360 ± 130*	1.1 ± 0.2
Group III (24 hr)		
Control (3)	715 ± 84	0.8 ± 0.2
Mianserin (3)	829 ± 169	1.4 ± 0.2
(−)-DOB (3)	667 ± 91	0.9 ± 0.1

* $p < 0.05$, compared with control.

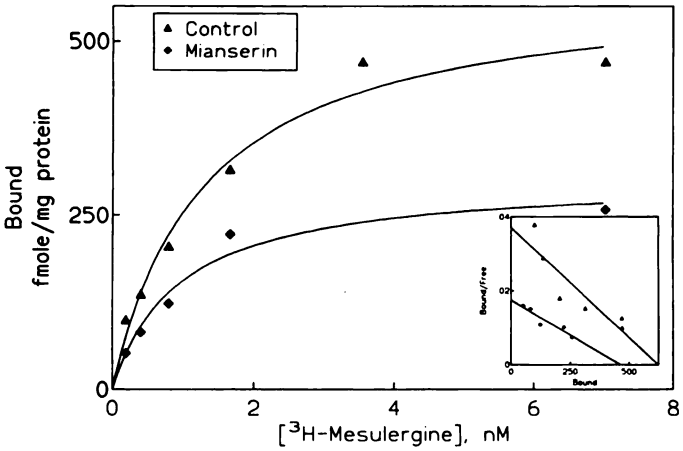

Fig. 3. Down-regulation of $5HT_{1C}$ receptors in choroid plexus epithelial cells by mianserin. Cells were treated with 100 nM mianserin for 72 hr, with fresh drug added each day of treatment. Radioligand binding was then performed as described in Experimental Procedures. *Data points* are means of duplicate determinations. The *graph* is representative of six experiments. The *curve* was plotted using the equation for a rectangular hyperbola. *Inset*, binding data were transformed to a Scatchard plot. See Table 1 for a summary of all of the data.

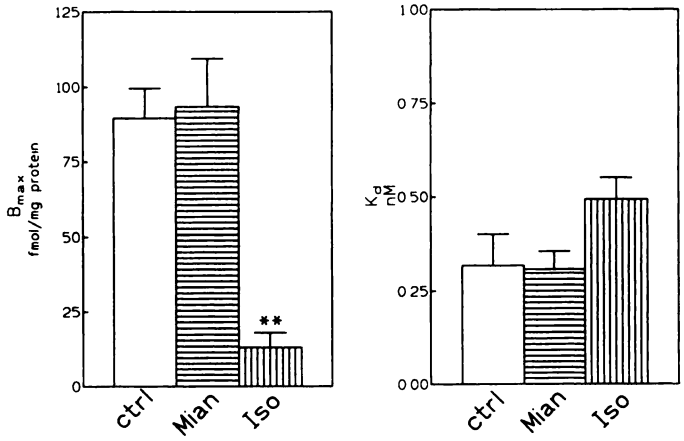

Fig. 4. Effect of 72-hr treatment with mianserin on β-adrenergic receptors. C6 glioma cells were exposed to either 100 nM mianserin (*Mian*) or 1 μM isoproterenol (*Iso*) for 72 hr, with fresh drug added each day of treatment. [3H]CGP-12177 saturation binding experiments were performed as described in Experimental Procedures. *ctrl*, control. Each *bar* represents the mean ± standard error of three separate experiments. Data were analyzed by one-way analysis of variance, followed by *post hoc* tests using Dunnett's multiple-comparisons test. **, $p < 0.01$.

ble. The question of receptor specificity was addressed in an indirect fashion by looking at the effect of mianserin on another type of cell surface receptor, β-adrenergic receptors. In C6 glioma cells, the β-adrenergic receptor agonist isoproterenol induced down-regulation of the β-adrenergic receptors, as would be expected, but 72-hr treatment with mianserin did not decrease β-adrenergic receptor density (Fig. 4). These results strengthen the argument that mianserin has a specific effect on $5HT_{1C}$ receptors. It was not possible to confirm this in the choroid plexus epithelial cells, because the density of β-adrenergic receptors was too low for quantitative measurement.

Effects of mianserin and (−)-DOB on $5HT_{1C}$ receptor mRNA levels. As with the many *in vivo* studies of regulation of $5HT_2$ and $5HT_{1C}$ receptors, the experiments detailed above simply describe the existence of atypical regulation by antagonists and do not explore the mechanisms that may account for this effect. Reports indicate that receptor regulation may also include regulation at the level of transcription (11). Therefore, it was of interest to determine whether down-regulation of $5HT_{1C}$ receptors by antagonist or agonist is accompanied by an alteration in the steady state levels of $5HT_{1C}$ receptor mRNA. Representative autoradiographs of S1 nuclease protection assays of RNA isolated from cells treated with mianserin or (−)-DOB are shown in Figs. 5 and 6, respectively. Neither mianserin nor (−)-DOB induced an alteration in steady state levels of $5HT_{1C}$ receptor mRNA during the treatment period (Table 2). It was important to determine whether the S1 nuclease protection assay had adequate sensitivity to detect changes in $5HT_{1C}$ receptor mRNA levels equivalent to those found in the radioligand binding studies. To do this, two samples of B3–6 RNA (7 and 10 μg or 9 and 12 μg) were included in all protection assays (for example, see Fig. 5). Analyses of these data showed a significant difference between

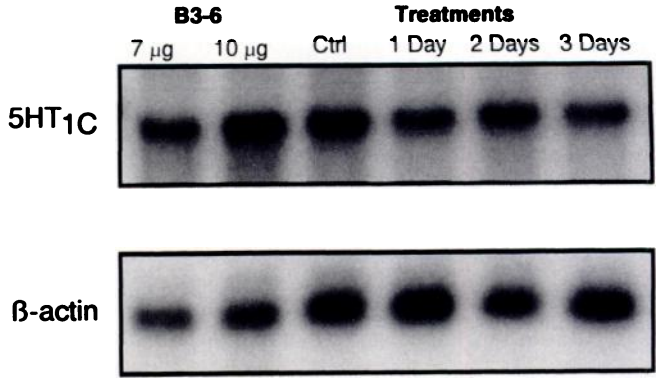

Fig. 5. S1 nuclease protection assay of $5HT_{1C}$ receptor mRNA after treatment with mianserin. Choroid plexus epithelial cells were treated with 100 nM mianserin for 3, 2, or 1 day(s), with fresh drug added each day. Total cellular RNA was isolated and assayed as described in Experimental Procedures. Two concentrations (7 μg and 10 μg) of RNA from NIH3T3 fibroblasts transfected with $5HT_{1C}$ receptor cDNA (B3–6 cells) were included as internal controls. *Ctrl*, control. *Upper*, $5HT_{1C}$ receptor mRNA; *lower*, β-actin mRNA. This autoradiograph is representative of four separate assays with two determinations for each RNA sample. See Table 2 for summary of the data.

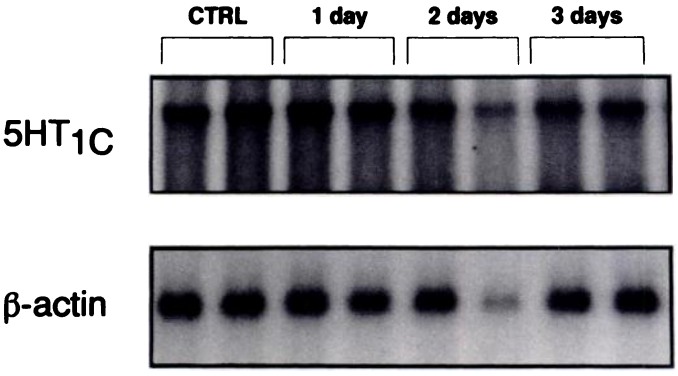

Fig. 6. S1 nuclease protection assay of $5HT_{1C}$ receptor mRNA after treatment with (−)-DOB. Choroid plexus epithelial cells were treated with 100 nM (−)-DOB for 3, 2, or 1 day(s), with fresh drug added each day. Total cellular RNA was isolated and assayed as described in Experimental Procedures. *CTRL*, control. *Upper*, $5HT_{1C}$ receptor mRNA; *lower*, β-actin mRNA. This autoradiograph shows two determinations for each treatment group and is representative of four separate assays. See Table 2 for summary of the data.

TABLE 2

Effect of mianserin and (−)-DOB treatments on $5HT_{1C}$ receptor mRNA in choroid plexus epithelial cells

Cells were treated as described for Figs. 5 and 6. Abundance of $5HT_{1C}$ receptor mRNA was calculated by normalizing the 480-bp $5HT_{1C}$ receptor mRNA band to the corresponding β-actin band. Band intensities were calculated using a PhosphorImager or Image 1.38 software. Values represent means ± standard errors (four separate assays with two determinations per RNA sample). Data were analyzed by one-way analysis of variance, followed by *post hoc* tests using Dunnett's multiple-comparisons test. No significant differences were found.

Treatment	$5HT_{1C}$ receptor mRNA levels	
	Mianserin	(−)-DOB
	% of control	
Control	100 ± 10.8	100 ± 3.6
1 day	98 ± 6.7	117 ± 10.5
2 days	109 ± 11.1	101 ± 13.8
3 days	90 ± 8.2	92 ± 15.6

the two control samples, verifying that at least a 25–30% decrease in $5HT_{1C}$ receptor mRNA levels was detectable.

Discussion

Pharmacological experiments have suggested a role for $5HT_{1C}$ receptors in the actions of hallucinogens (24–26), antidepressants (5), and antipsychotic agents (27–29). (−)-DOB, while being a $5HT_{1C}$ receptor agonist, is also a hallucinogenic amphetamine derivative. Studies aimed at identifying the mechanisms by which (−)-DOB, lysergic acid diethylamide, and other hallucinogens cause desensitization and down-regulation of $5HT_{1C}$ receptors will provide insight into the adaptive responses mediated by these substances of abuse. Additionally, the 5HT antagonist mianserin is a therapeutically effective antidepressant. Identification of the mechanism(s) of $5HT_{1C}$ receptor down-regulation by this drug may reveal novel actions involved in its antidepressant action. $5HT_{1C}$ receptors are also important in the function of the choroid plexus (15, 30), the tissue that regulates the secretion and composition of cerebrospinal fluid. Therefore, regulation of $5HT_{1C}$ receptors in the choroid plexus could play an important role in the homeostasis of cerebrospinal fluid content.

The present results show that exposure to both the receptor agonist (−)-DOB and the antagonist mianserin caused a down-regulation of $5HT_{1C}$ receptors in choroid plexus epithelial cell cultures. By comparing the agonist- and antagonist-induced regulation of $5HT_{1C}$ receptors, similarities in mechanisms of action might be elucidated, thus providing clues about how two opposing agents elicit a common adaptive response. Because previous studies were carried out in whole animals, indirect effects associated with transynaptic or interneuronal mechanisms may have been involved. For example, the administration of ritanserin, a $5HT_{1C/2}$ antagonist, attenuates $5HT_{1A}$ receptor-mediated inhibition of 5HT release (10). This in turn may increase synaptic levels of 5HT, with subsequent down-regulation of postsynaptic receptors. Such transynaptic mechanisms are eliminated in cell cultures, where the classical presynaptic and postsynaptic components do not exist and the microenvironment around the receptor can be strictly controlled. The present data showing down-regulation in cultured choroid plexus epithelial cells indicate that transynaptic or interneuronal mechanisms are not required for drug-induced regulation of the $5HT_{1C}$ receptor.

Many mechanisms have been suggested to explain the atypical down-regulation of $5HT_{1C}$ and $5HT_2$ receptors by antagonists such as mianserin. One obvious possibility is that the drug is actually not an antagonist but is, rather, a partial agonist at the receptor. This suggestion has been extensively investigated, looking at the phosphoinositide hydrolysis response, and these studies have uniformly failed to find any agonist effects of mianserin at $5HT_{1C}$ or $5HT_2$ receptors (5, 31, 32). Mianserin is an extremely lipophilic molecule and is difficult to wash out of membrane preparations. The apparent down-regulation could be simply due to residual drug that is tenaciously bound to the receptor, thereby preventing the radioligand from binding to the receptor and being manifest as a decrease in receptor binding sites. The failure of 24-hr treatment with mianserin to decrease receptor density in the cultured cells suggests that residual drug is not the mechanism responsible for the paradoxical down-regulation of $5HT_{1C}$ receptors. The effects of mianserin on $5HT_{1C}$ receptors in the cultured cells could be due to a nonspecific decrease in protein synthesis or turnover. However, mianserin treatment does not decrease the levels of the iron-carrier protein transferrin (15) or β-actin mRNA (present results), indicating that mianserin does not have generalized effects on RNA or protein synthesis. The issue of heterologous or nonspecific decreases in cell surface receptors was more difficult to address, because there were no readily detectable receptors in choroid plexus epithelial cell cultures other than the $5HT_{1C}$ receptor. The potential effects of mianserin on β-adrenergic receptors were therefore examined in C6 glioma cells. It is known that two other antidepressants, desipramine and oxaprotiline, induce down-regulation of β-adrenergic receptors in C6 glioma cells (33, 34). Our finding that mianserin did not reduce β-adrenergic receptor density in the C6 glioma cells implies a specific action of mianserin on 5HT receptors and rules out a universal down-regulation of β-adrenergic receptors by clinically effective antidepressant drugs. However, because we did not have a positive control showing that the atypical down-regulation of $5HT_{1C}$ receptors occurred in C6 glioma cells, these results do not entirely rule out a nonspecific or heterologous mechanism of down-regulation.

Although both $5HT_{1C}$ and $5HT_2$ receptors display atypical regulation, temporal studies of receptor density suggest possible differences in the mechanisms. For example, previous studies of $5HT_2$ receptors in P11 cell cultures have shown a rapid down-regulation by agonists that is apparent within 24 hr (2, 35). Also, *in vivo*, a single dose of mianserin results in a decrease of $5HT_2$ receptors in rat cerebral cortex (36). This is not the case for the $5HT_{1C}$ receptor. *In vivo* studies of $5HT_{1C}$ receptor regulation by various agonists and antagonists failed to find a decrease in $5HT_{1C}$ receptors 24 hr after a single dose of the drugs (3, 5, 9). Additionally, our data indicate that $5HT_{1C}$ receptors were not decreased after 24-hr treatment with either agonist or antagonist in choroid plexus epithelial cells in culture, consistent with *in vivo* studies.

The delay in the observed loss of $5HT_{1C}$ receptors suggests the possibility of regulation at the level of gene transcription. Regulation of receptors at the transcriptional level has been suggested by many reports of down-regulation accompanied by a decrease in receptor mRNA levels (for review, see Ref. 11). However, a recent preliminary report indicates that 5HT-induced down-regulation of $5HT_2$ receptors in cultured cells is not accompanied by an alteration in $5HT_2$ receptor mRNA levels (35). Furthermore, Roth and Ciaranello (37) have shown that, in rat brain, chronic mianserin treatment decreases $5HT_2$

receptor binding without altering $5HT_2$ receptor mRNA levels. The present studies, the first to examine potential changes in $5HT_{1C}$ receptor mRNA levels in conjunction with down-regulation, showed that $5HT_{1C}$ receptor down-regulation by both (−)-DOB and mianserin occurred without a change in steady state levels of $5HT_{1C}$ receptor mRNA.

In conclusion, this report is the first account of $5HT_{1C}$ receptor regulation by agonists and antagonists in a cell culture model system. The choroid plexus epithelial cell cultures have been useful for eliminating indirect effects and also have ruled out an alteration in levels of $5HT_{1C}$ receptor mRNA as a mechanism involved in the regulation by antagonists or agonists. Future studies will need to address additional mechanisms. One possibility is that both agonists and antagonists induce conformational changes in the receptor protein that initiate the events involved in down-regulation. When the agonist interacts with the receptor, G protein coupling and activation of the phosphoinositide hydrolysis pathway occur, but antagonist binding to the receptor may induce a conformational change without receptor activation. Both changes in receptor conformation could lead to an alteration in receptor protein turnover, by as yet unidentified pathways. Studies using cell lines transfected with $5HT_{1C}$ receptor cDNA may be useful in addressing these questions.

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Send reprint requests to: Elaine Sanders-Bush, Department of Pharmacology, 432 Medical Research Building, Vanderbilt University School of Medicine, Nashville, TN 37232–6600.