Neurochemical Research, Vol. 27, Nos. 1/2, February 2002 (© 2002), pp. 113–120

Up-Regulation of 5-HT_{2B} Receptor Density and Receptor-Mediated Glycogenolysis in Mouse Astrocytes by Long-Term Fluoxetine Administration*

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(Accepted August 5, 2001)

The effects were studied of short-term (1 week) versus long-term (2–3 weeks) fluoxetine treatment of primary cultures of mouse astrocytes, differentiated by treatment with dibutyryl cyclic AMP. From previous experiments it is known that acute treatment with fluoxetine stimulates glycogenolysis and increases free cytosolic Ca^{2+} concentration ($[Ca^{2+}]_i$) in these cultures, whereas short-term (one week) treatment with 10 μM down-regulates the effects on glycogen and $[Ca^{2+}]_i$, when fluoxetine administration is renewed (or when serotonin is administered). Moreover, antagonist studies have shown that these responses are evoked by activation of a 5-HT_2 receptor that is different from the 5-HT_{2A} receptor and therefore at that time tentatively were interpreted as being exerted on 5-HT_{2C} receptors. In the present study the cultures were found by RT-PCR to express mRNA for 5-HT_{2A} and 5-HT_{2B} receptors, but not for the 5-HT_{2C} receptor, identifying the 5-HT_2 receptor activated by fluoxetine as the 5-HT_{2B} receptor, the most recently cloned 5-HT_2 receptor and a 5-HT receptor known to be more abundant in human, than in rodent, brain. Both short-term and long-term treatment with fluoxetine increased the specific binding of [^{3}H]mesulergine, a ligand for all three 5-HT_2 receptors. Long-term treatment with fluoxetine caused an agonist-induced up-regulation of the glycogenolytic response to renewed administration of fluoxetine, whereas short-term treatment abolished the fluoxetine-induced hydrolysis of glycogen. Thus, during a treatment period similar to that required for fluoxetine's clinical response to occur, 5-HT_{2B}-mediated effects are initially down-regulated and subsequently up-regulated.

KEY WORDS: Astrocytes; glial cells; glycogenolysis; 5-HT_{2B} receptor; mesulergine; serotonin; serotonin-specific reuptake inhibitors.

INTRODUCTION

Binding of serotonin to primary cultures of astrocytes was first shown by Hertz, Baldwin and Schousboe (17), who also found that serotonin binding could be displaced by fluoxetine. Later, serotonin was found to cause a very potent increase in free cytosolic calcium concentration ($[Ca^{2+}]_i$), a response which based on its potency was interpreted as being exerted on high-affinity 5HT_{2C} receptors (8,53), co-existing with

*Special issue dedicated to Dr. Arne Schousboe.

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0364-3190/02/0200-0113/0

lower-affinity 5-HT_{2A} receptors, known to be expressed in rodent astrocytes (13,36,39). It is in support of this concept, that there was a tendency towards a biphasic concentration dependence of a serotonin-induced stimulation of glycogenolysis, with one peak around 1 nM and a second peak around 1 μM serotonin, i.e., a similar concentration dependence as the increase in $[Ca^{2+}]_i$ (8).

Acute exposure to fluoxetine also increases $[Ca^{2+}]_i$ and stimulates glycogenolysis in astrocytes, albeit with much lower potency ($EC_{50} \sim 1$ μM). This effect is inhibited by the non-specific 5-HT_2 antagonist mesulergine, but not by the 5-HT_{2A}-specific antagonist, ketanserine (53). For this reason, and because the concentration dependence for the increase in $[Ca^{2+}]_i$ and the stimulation of glycogenolysis again was similar, both these effects were also interpreted as being due to stimulation of 5-HT_{2C} receptors. This conclusion is consistent with the observation that fluoxetine's affinity specifically for the 5-HT_{2C} receptor is high enough that concentrations of fluoxetine around 1 μM can be expected to act directly on 5-HT_{2C} receptors (24,35,37,52).

Fluoxetine has later been found to increase $[Ca^{2+}]_i$ in glioma cells (48) and in lymphocytes (14). However, it is an antagonist of membrane currents elicited by serotonin in xenopus oocytes expressing cloned 5-HT_{2C} receptors (35), and it counteracts serotonin-mediated phophatidyl inositide (PI) hydrolysis in rat choroid plexus, which expresses only 5-HT_{2C} receptors (37). The antagonist activity of fluoxetine in the latter preparations, versus its agonist effect on cultured astrocytes suggest that fluoxetine may not exert its effect on 5-HT_{2C} receptors in astrocytes, but rather on another high-affinity 5-HT_2 receptor. This concept is reinforced by the demonstrations that cultured rodent astrocytes express the more recently cloned 5-HT_{2B} receptors (22,42), and that the 5-HT_{2B} receptor is the 5-HT_2 subtype with the highest affinity for serotonin (25). Moreover, lymphocytes not only show a fluoxetine-induced increase in $[Ca^{2+}]_i$, but also express 5-HT_{2B} receptors, rather than 5-HT_{2C} receptors (46).

In rat choroid plexus 5-HT_{2C} receptor density, as indicated by mesulergine binding, is increased after treatment with fluoxetine for 14 days (31). In contrast, one week of exposure of cultured astrocytes to 10 μM fluoxetine resulted in reduced increase in $[Ca^{2+}]_i$ and decreased stimulation of glycogenolysis, when the cells were subsequently exposed to 10 μM fluoxetine or to serotonin concentrations low enough (10 nM) to have no effect on 5-HT_{2A} receptors (8). Although these contrasting findings are consistent with the antagonist effect of fluoxetine on choroid plexus cells and its agonist effects on astrocytes, there is also the possibility that the responses depended upon the length of the treatment period with fluoxetine. Moreover, receptor density, measured as mesulergine binding, might be affected different than down-stream signalling effects by fluoxetine. For these reasons we have in the present study measured both mesulergine binding and glycogenolytic effect of fluoxetine in primary cultures of astrocytes after short term (one week) and long-term (2–3 weeks) treatment with 10 μM fluoxetine. In addition it was determined that the only 5-HT_2 receptor mRNA species found in astrocytes treated with dBcAMP are those of the 5-HT_{2A} and 5-HT_{2B} receptors, and that there is no expression of the serotonin transporter. These observations greatly facilitate the interpretation of the effects of fluoxetine, since it does not interact with 5-HT_{2A} receptors (53).

EXPERIMENTAL PROCEDURE

Cell Cultures. Primary cultures of astrocytes were prepared from the cerebral hemispheres of new-born Swiss mice as previously described (19,20,26). The neopallia (the tissues dorsal and lateral to the lateral ventricles) were aseptically isolated, vortexed to dissociate the tissue, filtered through Nitex nylon meshes, diluted in a slightly modified Dulbecco's tissue culture medium (26) and planted in Falcon Primaria 35-mm culture dishes (20 dishes per brain). From the age of 2 weeks, 0.25 mM dBcAMP was included in the medium in all cases except for studies of mRNA expression in cells that had not been treated with dBcAMP. In contrast to previous claims (15), dBcAMP does not lead to the formation of reactive astrocytes (50), but it has a differentiating effect (32,45). More than 95% of the cell population in the cultures consists of astrocytes, and no neurons are present. Acute experiments were carried out using cultures, which were about 4 weeks old. Chronic treatment with fluoxetine (donated by Lilly Laboratories, Indianapolis, IN) started at the age of 3–4 weeks.

RT-PCR (Reverse Transcription-Polymerase Chain Reaction). mRNA expression was studied by reverse transcription polymerase chain reaction (RT-PCR) in cells that had been treated with dBcAMP and in corresponding cultures, which had not received such treatment. A cell suspension was prepared by discarding the culturing medium, adding Trizol (Gibco BRL, Life Technologies Inc., NY, USA) to cultures on ice (1 ml/100-mm culture dish), and triturating the cells by repeated pipetting. The resulting cell suspension was incubated at room temperature for 5 min (to complete cell lysis), 200 μl of chloroform was added, the mixture was vigorously vortexed and centrifuged at 12,000 *g* for 15 min at 4°C. The upper, aqueous layer was removed, 500 ml isopropanol was added, the solution was incubated at −80°C for 1 hr and centrifuged at 12,000 *g* for 10 min at 4°C. The supernatant, was washed twice with 75% ethanol, the pellet containing the cellular RNA was air dried and dissolved in 10 μl sterile, distilled water, and an aliquot was used for spectrophotometric determination of the amount of RNA.

RT was initiated by incubating 1 mg of RNA at 65°C for 5 min with 3.85 ng/μl Random Hexamer (Promega) and dNTPs (a mixture of dATP, dGTP, dCTP and dTTP-Pharmacia Biotech, Sweden) at a final concentration of 0.77 mM, and sterile, distilled water added to total volume of 13 μl. The mixture was rapidly chilled on ice (for at least 2 min) and briefly spun, and 4 μl 5X First-Strand Buffer, 2 μl 0.1 M dithiotreitol and 1 μl RNaseOUT Recombinant RNase Inhibitor (40 U/μl) were added. After the mixture had been incubated at 42°C for 2 min, 1 μl (200 U) of SUPERSCRIPT II (Gibco) was added, and the incubation at 42°C continued for another 50 min. Subsequently the reaction was inactivated by heating to 70°C for 15 min, and the mixture was chilled on ice for at least 2 min and briefly centrifuged. Finally, RNA complementary to the cDNA formed during the reaction was removed by addition of 1 ml of *E. coli* RNaseH (2U) and incubation at 37°C for 20 min.

PCR was performed in a thermocycler (Robocycler, Stratagene, Amsterdam, Holland). Five μl PCR Buffer, pH 8.4 (Gibco), 1.5 μl 50 mM $MgCl_2$ (Pharmacia Biotech, Sweden), 1 μl 10 mM dNTPs, 2 μl 5 μM of one of the 5-HT$_{2A}$, 5-HT$_{2B}$, and 5-HT$_{2C}$ receptor and the 5-HT transporter primers (11) and shown in Fig. 1, or of a β-actin Primer (used as a housekeeping gene), 0.4 μl *Taq* polymerase (Gibco), and 0.5 μl cDNA template were mixed with sterile distilled water to a final volume of 50 μl. Initially the template was denatured by heating to 94°C for 2 min, followed by thirty 2-min amplification cycles, each consisting of three 40-sec periods, the first at 94°C, the second at 57°C, and the third at 72°C. The final step was extension at 72°C for 10 min. Eventually the PCR products were separated by 1% agarose gel electrophoresis.

In order to estimate whether comparable amounts of tissue were used for each determination, β-actin was used as a housekeeping gene. Positive controls for 5-HT$_{2A}$ and 5-HT$_{2C}$ receptor mRNA was brain tissue from adult mice, for 5-HT$_{2B}$ receptor it was stomach fundus of adult mouse, and for the 5-HT transporter brain stem of adult mouse, all used instead of cultured cells from the beginning of the RT-PCR procedure. Negative controls were prepared from the cultures by omission of the inverse transcriptase from the RT mixture. A DNA ladder was also included. The brightest band in its middle represents 500 bp, the other bands 400, 300, 250, 200, 150, 100 and 50bp.

Mesulergine Binding. Binding of [^{3}H]mesulergine (from NEN, Dupont. Boston, MA) was measured by washing intact cultures two times with tissue culture medium without serum and subsequently incubating them in such a medium for 15 min at 37°C in the presence of 12 nM [^{3}H]mesulergine (total binding) or 12 nM [^{3}H]mesulergine plus 10 μM non-labeled mesulergine (non-specific binding). Mesulergine itself was used for determination of non-specific binding, since ligands distinguishing between 5-HT$_{2B}$ and 5-HT$_{2C}$ receptors were not available. The 15-min time period is sufficient to reach a plateau in binding (results not shown). The binding experiments were terminated by aspiration of the medium, wash of the cultures three times with ice-cold medium, and addition of 1 ml of 1 M NaOH to dissolve the cultures. Specific binding of [^{3}H]mesulergine binding was calculated by subtraction of non-specific binding from total binding, and it was expressed as fmol/mg protein, calculated from the radioactivity in the dissolved cultures, the protein content, determined by the Lowry technique (33), and the specific activity of the incubation medium.

Glycogenolysis. For measurement of glycogenolysis, the cultures were preincubated for 30 min with [6-^{3}H]glucose (from NEN, Dupont, Boston, MA) in cell culture medium (25 μCi/ml) without fluoxetine. The glucose concentration in the medium was reduced to 3 mM in order to obtain a relatively high specific radioactivity. Such a reduction has previously been found to have no major short-term effect on glycogen synthesis. Subsequently, the cultures were washed and exposed during a ten-min period either to non-radioactive normal medium (control conditions) or non-radioactive normal medium containing 10 μM fluoxetine (9). The culture medium was then replaced with a 150 mM NaCl solution containing 3 mM glucose, the cultures were sonicated and heated to 95°C, and the contained glycogen precipitated on a Whatman chromatographic paper 31-ET and washed as described by Subbarao and Hertz (47). The radioactivity of the precipitate after incubation under control conditions and in the presence of fluoxetine was measured in a scintillation counter. This value was lower after exposure to the fluoxetine (X DPM/culture) than under control conditions (Y DPM/culture). The drug-induced glycogenolysis was expressed as a percentage of total radioactivity incorporated from [^{3}H]glucose into glycogen by the equation: $100 - (X/Y \times 100)$ %.

RESULTS

Expression of 5-HT Receptor Subtypes and Transporter. Figure 2 shows agarose gels displaying astrocytic cDNAs, obtained by RT-PCR using the Primers indicated by Choi et al. (11) for mouse 5-HT$_{2A}$ receptors (Fig. 2A), 5-HT$_{2B}$ receptors (Fig. 2B), 5-HT$_{2C}$ receptors (Fig. 2C) and the 5-HT-transporter (Fig. 2D), respectively. The first lane, to the far left, represents astrocytes that had not been treated with dBcAMP; the second lane dBcAMP-treated astrocytes; the third lane positive controls for each of the receptors and for the 5-HT transporter, as detailed in 'Methods'; the fourth lane a PCR-negative control; the fifth lane a DNA ladder, as described in 'Methods'; the sixth lane β-actin, used as a housekeeping gene, in cultures that had not been treated with dBcAMP; the seventh lane β-actin in dBcAMP-treated cells; the eighth lane a positive control for β-actin; and the ninth lane a PCR-negative control for β-actin. The approximate size of each RT-PCR product is indicated in Fig. 2.

The expression of β-actin was relatively similar in the different gels, especially when comparing dBcAMP

	Receptor	Sequence	Product size
A	5-HT2A Forward	AAGCCTCGAACTGGACAATTGATG	
	5-HT2A Reverse	AAGATTTCAGGAAGGCTTTGGTT	~470 bp
B	5-HT2B Forward	CTCGGGGGTGAATCCTCTGA	
	5-HT2B Reverse	CCTGCTCATCACCCTCTCTCA	~370 bp
C	5-HT2C Forward	CAGATCAGAAGCCACGTCGA	
	5-HT2C Reverse	GGCTTATAATCGCAGCGCAA	~318 bp
D	SerT Forward	ACATCTGGCGTTTTCCCTACAT	
	SerT Reverse	TTTTGACTCCTTTCCAGATGCT	~500 bp

Fig. 1. Primer sequences used for RT-PCR of mRNA for 5-HT$_{2A}$ (Fig. 1**A**), 5-HT$_{2B}$ (Fig. 1**B**), and 5-HT$_{2C}$ (Fig. 1**C**) receptors as well as for RT-PCR of the 5-HT transporter (Fig. 1**D**). The primer sequences were obtained from Choi et al., 1997.

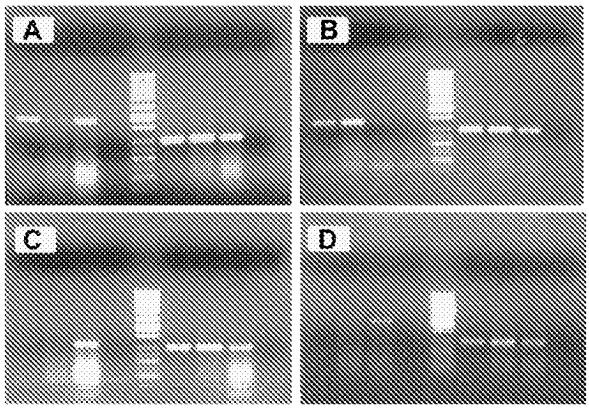

Fig. 2. Presence of 5-HT_2 family receptor mRNA and of the 5-HT transporter mRNA in astrocytes in primary cultures grown in the presence of dBcAMP or in its absence. Fig. **2A** represents a gel of the RT-PCR product of the 5-HT_{2A} receptor, using the Primer shown in Fig. 1A; Fig. **2B** a corresponding gel of the RT-PCR product of the 5-HT_{2B} receptor, using the Primer shown in Fig. 1B; Fig. **2C** a corresponding gel of the RT-PCR product of the 5-HT_{2C} receptor, using the Primer shown in Fig. 1C; and Fig. **2D** a corresponding gel of the RT-PCR product of the 5-HT transporter, using the Primer shown in Fig. 1A. In each Figure, the first lane, to the far left, represents the RT-PCR product from astrocyte cultures that had not been treated with dBcAMP, and the second lane the RT-PCR product from dBcAMP-treated astrocytes. The third lane is a positive control for each of the receptors and for the 5-HT transporter (mouse brain in A and C; mouse stomach fundus in B, and mouse brain stem in D). The fourth lane is a PCR-negative control. The fifth lane is a DNA ladder, in which the brightest band in the middle represents 500 bp, and the other bands 400, 300, 250, 200, 150, 100 and 50 bp. The sixth lane represents the RT-PCR product of β-actin, used as a housekeeping gene, in cultures that had not been treated with dBcAMP, and the seventh lane shows β-actin in dBcAMP-treated cells. The eighth lane is a positive control for β-actin, and the ninth lane a PCR-negative control for β-actin. The experiment was repeated a second time, with only the minor differences described in 'Results'.

treated and untreated cells. Thus, the intensity of the different bands may be used as a direct indication of receptor and transporter mRNA expression. The results indicate the presence of mRNA for the 5-HT_{2A} and the 5-HT_{2B} receptor, together with trace amounts of 5-HT_{2C} receptors in astrocytes, which had not been treated with dBcAMP. After treatment with dBcAMP the 5-HT_{2B} receptor showed a distinct increase, and the amount of 5-HT_{2B} receptor mRNA exceeded that of 5-HT_{2A} receptor mRNA. In contrast, the traces of 5-HT_{2C} receptor expression had disappeared in the dBcAMP-treated cells, and the expression of 5-HT_{2A} receptors was reduced. Serotonin transporter mRNA was neither present in dBcAMP-treated, nor in untreated cells, although a second experiment showed trace amounts of transporter cDNA in untreated cells, which had disappeared after treatment with dBcAMP. In all remaining experiments only dBcAMP-treated cells were used.

Mesulergine Binding. B_{max} for specific binding of mesulergine was 30–35 fmol/mg protein in cultures that had not been exposed to fluoxetine, and K_D was 12–13 nM (results not shown). Specific binding of 12 nM [^{3}H]mesulergine amounted to 15 fmol/mg protein (Fig. 3). Fluoxetine concentrations up to 0.1 μM had no effect on the binding, but higher concentrations strongly displaced specific binding of [^{3}H]mesulergine. The binding was abolished at 3 μM fluoxetine, but the magnitude of the response was determined with a considerable uncertainly, reflecting that the measured effect of fluoxetine on specific binding of mesulergine is a composite of individual measurements of total and non-specific binding of mesulergine both under control conditions and in the presence of fluoxetine. Half-maximum effect was seen at 1 μM of the drug.

After 1 week of treatment with 10 μM fluoxetine, specific binding of [^{3}H]mesulergine was more than two times higher in the fluoxetine-treated cultures (black column in Fig. 4A) than in sister cultures that had not received fluoxetine (white column in Fig. 4A), a statistically highly significant difference ($P < 0.01$). After fluoxetine treatment for 2–3 weeks (Fig. 4B) the binding remained greatly elevated ($P < 0.02$), although the apparent additional increase in specific binding between 1 week of treatment and 2–3 weeks of treatment

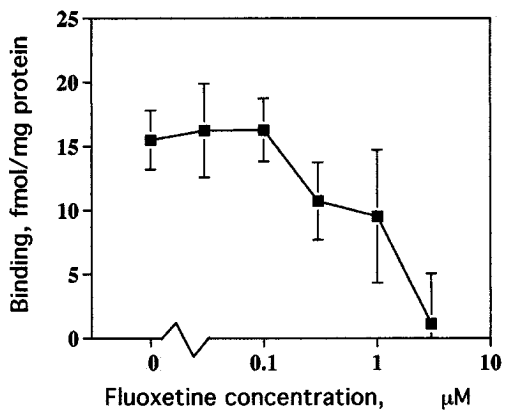

Fig. 3. Displacement of specific binding of 12 nM [^{3}H]mesulergine from intact primary cultures of mouse astrocytes by fluoxetine concentrations between 0.03 and 3 μM. The cultures were incubated for 15 min with 12 nM [^{3}H]mesulergine and different concentrations of fluoxetine (or no fluoxetine) in the presence (specific binding) or absence (non-specific binding) of 10 μM non-labeled mesulergine, and specific binding was determined as the difference between total and non-specific binding. Results are means ± SEM of 6 individual experiments, using cultures from 2 different batches.

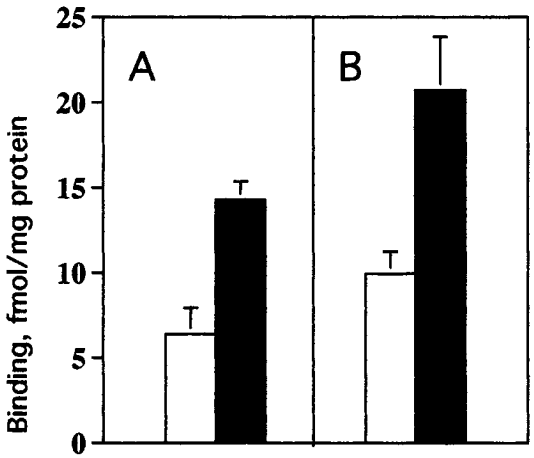

Fig. 4. Specific binding of 12 nM [^{3}H]mesulergine to intact primary cultures of mouse astrocytes, determined by subtraction of non-specific binding from total binding, in cultures treated with 10 μM fluoxetine for 1 week (black column in Fig. **4A**) or 2–3 weeks (black column in Fig. **4B**), compared to the specific binding in untreated control cultures from the same batches (white columns in Figs. **4A** and **B**), which were measured in the same experiments. Results are means ± SEM of 3–4 individual experiments, using cultures from 1 or 2 different batches. Both after 1 week and 2–3 weeks of treatment with fluoxetine, the binding of [^{3}H]mesulergine was significantly increased ($P < 0.02$ or better in student's t-test). The apparent increase in binding from 1 week to 2–3 weeks of treatment is not statistically significant, and the two control groups are also not significantly different from each other.

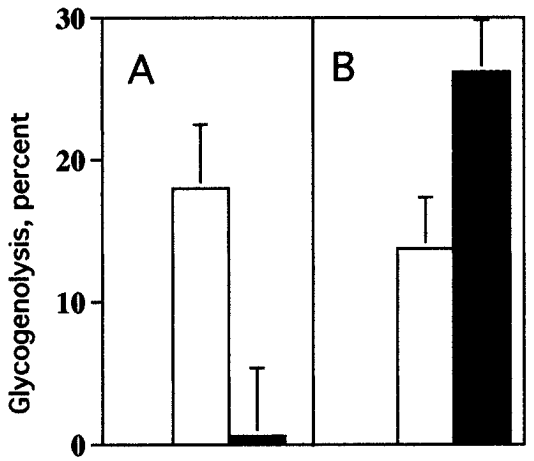

Fig. 5. Glycogenolysis, evoked by acute exposure to the 5-HT_{2B} agonist, fluoxetine (10 μM) for 10 min, and measured as reduction of previously incorporated radioactivity from [1-^{3}H]glucose into glycogen, in primary cultures of mouse astrocytes previously treated with 10 μM fluoxetine for 1 week (black column in Fig. **5A**) or 2–3 weeks (black column in Fig. **5B**), compared to fluoxetine-induced glycogenolysis in untreated control cultures from the same batches (white columns in Figs. **5A** and **B**), which were measured in the same experiments. Results are averages ± SEM values of 9–13 individual experiments, using cultures from at least two different batches. Both after 1 week and 2–3 weeks of treatment with fluoxetine, the glycogenolytic response was significantly different from that in control cultures ($P < 0.05$ or better in student's t-test). The difference between glycogenolysis after 1 week and 2–3 weeks of treatment with fluoxetine was statistically highly significant ($P < 0.0005$). The two control groups are not significantly different from each other.

was not statistically significant. The binding to sister cultures, that had not been treated with fluoxetine, did not change significantly during this time interval.

Glycogenolysis. The effect of chronic treatment with 10 μM fluoxetine was determined by measuring glycogenolysis in fluoxetine-treated cultures and in sister cultures from the same culture batches that had not received fluoxetine. The incorporation of radioactivity during the 30-min exposure to [6-^{3}H]glucose was similar in the two groups of cultures (results not presented).

In untreated control cultures, fluoxetine-induced glycogenolysis during a 10-min period amounted to 15–20% of initially incorporated radioactivity into glycogen, with no statistically significant difference between the two groups of control cultures (open columns in Fig. 5A and B). In contrast, the glycogenolytic response to acute administration of fluoxetine was abolished in cultures treated for 1 week with fluoxetine (black column in Fig. 5A), but it was significantly increased in cultures that had been treated for 2–3 weeks (black column in Fig. 5B). Thus, after 2–3 weeks both receptor density and receptor-mediated glycogenolysis were upregulated, but after only one week of treatment the receptor-mediated stimulation of glycogenolysis was inhibited, although receptor expression was upregulated.

DISCUSSION

5-HT_2 Receptor and Serotonin Transporter Expression. Treatment with dBcAMP was found to reduce 5-HT_{2A} receptor gene expression, to increase 5-HT_{2B} receptor gene expression and to abolish the expression of 5-HT_{2C} receptors. In the light of this observation, the previous tentative conclusion, that dBcAMP-treated astrocytes express 5-HT_{2C} receptors must be revised to an expression of 5-HT_{2B} receptors. The pronounced expression of 5-HT_{2B} receptor mRNA is consistent with the observation of 5-HT_{2B} receptor protein in similar cultures (42) and also with a previous observation that the 5-HT_{2B} receptor is among multiple 5-HT receptors expressed in cultured rat astrocytes (22). It is disputed whether 5-HT_{2B} receptors are expressed in rat brain (3,51), but they are found at modest density in mouse brain (10, E. K. C. Kong,

L. Peng and A. C. H. Yu, unpublished experiments) and at greater abundance in human brain (3,5,30,43) and meninges (44). This species difference is consistent with a much higher density of glial cells in human brain than in mouse and rat brain (4). 5-HT_{2B} receptors also play a role during early differentiation of the nervous system (11,12).

The observation that dBcAMP treatment enhances 5-HT_{2B} receptor expression is consistent with the observation by Jerman et al. (25) that 5-HT_{2B} receptor expression is increased 15-fold in SH-SY5Y cells by treatment with butyrate. Butyrate is known to evoke a multitude of effects on gene expression and cell growth in cultured cells, primarily secondary to hyperacetylation of histone, resulting from inhibition of histone deacetylase (29). The effects include both increases (16) and decreases (7) in gene expression. However, there is also evidence that the dBcAMP-mediated increase in intracellular cAMP (34) and in serotonergic differentiation may play a role in 5-HT_{2B} receptor induction (12).

The present cultures showed no serotonin transporter expression, at least after treatment with dBcAMP, explaining the discrepancy between the observation of 5-HT transporter expression (13) and Na^+-dependent, serotonin uptake (23,27) in primary cultures of rat astrocytes on one hand, and the finding by Hertz et al. (18) that serotonin accumulation in the present cultures of mouse astrocytes is Na^+-independent. It is also unaffected by 5 μM fluoxetine (L. Hertz, unpublished observation).

Fluoxetine Effects on Mesulergine Binding and Glycogenolysis. Specific binding of mesulergine, a ligand for all three 5-HT_2 receptors (25), to dBcAMP-treated astrocytes occurred with a B_{max} value of 30–35 fmol/mg protein, and K_D was 12–13 nM. The B_{max} is consistent with that for serotonin in similar cultures, estimated to be ~50 fmol per mg protein (17), since astrocytes also express adenylyl cyclase-linked serotonin receptors (18,21). The K_D is in good agreement with a significant stimulation of glycogenolysis and increase in $[Ca^{2+}]_i$ in similar cultures by 1 nM serotonin (8) and with an EC_{50} for serotonin-induced increase in $[Ca^{2+}]_i$ in SH-SY5T cells transfected with 5-HT_{2B} receptors of 1.4 nM (25). The EC_{50} value of 1 μM displacement of mesulergine by fluoxetine is in agreement with previous concentration-response curves for fluoxetine's effects on $[Ca^{2+}]_i$ and glycogenolysis in similar cultures (8,53). It is interesting that fluoxetine also increases $[Ca^{2+}]_i$ in lymfocytes (14), since lymphoid tissue, like the present astrocyte cultures, express mRNA for 5-HT_{2B} receptors, but not for 5-HT_{2C} receptors (46). Thus, fluoxetine appears to be an agonist at 5-HT_{2B} receptors, which is in contrast to its antagonist effect at 5-HT_{2C} receptors. It is not unusual for serotonin ligands to act differently on 5-HT_{2B} and 5-HT_{2C} receptors (40,49).

It was unexpected that fluoxetine displaced the entire specific binding of mesulergine, since mesulergine also is a ligand at 5-HT_{2A} receptors, which are expressed in the present cultures, although they are quantitatively less prominent. However, there was a considerable uncertainty of the magnitude of the effect of 3 μM fluoxetine. Also, the presence of mRNA for the 5-HT_{2A} receptor does not automatically imply expression of the receptor protein. Finally, although fluoxetine does not activate 5-HT_{2A} receptors (53), its affinity for these receptors is high enough (24,52) that 3 μM fluoxetine may cause some displacement of mesulergine binding.

Chronic exposure to fluoxetine induced a significant increase in mesulergine binding both after 1 week and after 2–3-weeks of treatment. It cannot be excluded that this might partly include an up-regulation of 5-HT_{2A} receptors, since an increase in 5-HT_{2A} receptor density in rat brain has been reported after chronic treatment with fluoxetine (28). Because the present measurements were performed at a concentration corresponding to the K_D value for mesulergine binding, it can also not be concluded with certainty whether both receptor density and affinity were altered. However, a 2–3 times increase in binding density is not possible at a concentration similar to the K_D value without at least some increase in receptor density. The increased mesulergine binding is analogous to that occurring to choroid plexus after chronic exposure to fluoxetine (31). This is in spite of the fact that fluoxetine is an antagonist at choroid plexus 5-HT_{2C} receptors, but an agonist at 5-HT_{2B} receptors in the present cells (8,53). However, a 'paradoxical' agonist-induced increase in receptor binding has also been reported in the case of other 5-HT_2 receptors (1,6,8). The increase in mesulergine binding appeared to become more pronounced, when the exposure to fluoxetine was continued beyond one week, confirming a gradual increase in binding of the non-subtype-specific 5-HT_2 ligand, [+−]-1-[2,5-dimethoxy-4-iodophenyl]-2-aminopropane HCl (DOI) in hypothalamus during chronic treatment with fluoxetine (32).

In spite of the increase in mesulergine binding after both 1 and 2–3 weeks of chronic treatment with fluoxetine, the effect of fluoxetine on glycogenolysis was down-regulated after one week of treatment, but up-regulated after 2–3 weeks. This discrepancy

between binding and down-stream signalling suggests additional effects by chronic fluoxetine treatment on signal transduction mechanisms, as previously suggested for agonist-induced up-regulation (8). The extent to which such alterations contribute to the therapeutic effects of fluoxetine is unknown. So is, indeed, the contribution of 5-HT$_{2B}$ receptors to the acute and chronic effects of fluoxetine. However, it is gradually becoming obvious that fluoxetine, in contrast to most other serotonin-specific reuptake inhibitors, at the pharmacologically relevant concentration of ~1 μM (2) also interacts with some, but far from all 5-HT$_2$ receptors (38). An interaction with astrocytic 5-HT$_{2B}$ receptors might be of special interest on account of the preponderance of these receptors in human brain (3) and accumulating evidence of astrocytic dysfunction in depression (41), one of the key indications for the clinical use of fluoxetine. It is also noteworthy that 5-HT$_{2B}$-mediated effects are initially down-regulated and subsequently up-regulated by fluoxetine during a treatment period similar to that required for fluoxetine's clinical response to occur,

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