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Differential Regulation of Rat 5-HT_{2A} and 5-HT_{2C} Receptors after Chronic Treatment with Clozapine, Chlorpromazine and Three Putative Atypical Antipsychotic Drugs

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Interactions with 5-HT_{2A} and 5-HT_{2C} receptors may be important for the actions of atypical antipsychotic drugs, such as clozapine (CLOZ). In this study we characterized the interaction of chlorpromazine (CPZ) and three putative atypical antipsychotic drugs, risperidone (RIS), amperozide (AMP), and ORG 5222 (ORG) with the 5-HT_{2A} and the 5-HT_{2C} receptor. CLOZ was used as a reference agent. These agents had 5-HT_{2C} receptor-binding affinities (K_i values) in the following rank order: ORG (0.9 nM) > CLOZ (13.2 nM) ≥ CPZ (27.1 nM) > RIS (112 nM) > > AMP (2580 nM). RIS (1.9 nM) and AMP (75.6 nM) had clearly higher affinities for the 5-HT_{2A} than the 5-HT_{2C} receptor; otherwise the 5-HT_{2A} and 5-HT_{2C} receptor affinities were approximately the same. Phosphoinositide hydrolysis studies in the rat choroid plexus revealed that all these agents were 5-HT_{2C} receptor antagonists, with an approximately similar rank order of potency compared to the 5-HT_{2C} receptor-binding data. Quantitative receptor autoradiography was used to study the regulation of 5-HT_{2A} and 5-HT_{2C} receptors after chronic treatment (14 days, SC injections once a day) with CLOZ (25 mg/kg), CPZ (15 mg/kg), RIS (0.3 mg/kg), AMP (5 mg/kg), and ORG (0.1 mg/kg). In the doses used, CLOZ, CPZ, and ORG decreased the frontal cortical 5-HT_{2A} receptor binding of [^{3}H]ketanserin and [^{125}I]DOI by 40% to 60%. AMP also significantly decreased 5-HT_{2A} receptor [^{3}H]ketanserin binding by 30%, whereas RIS did not affect 5-HT_{2A} receptor binding. In contrast to 5-HT_{2A} receptors, only CLOZ significantly (by about 50%) decreased 5-HT_{2C} receptor [^{3}H]mesulergine and [^{125}I]DOI binding in the choroid plexus. For comparison, CPZ was the only drug to significantly upregulate striatal D_2 receptor-binding sites, whereas none of the drugs affected striatal D_1 receptors. The main finding in this study is that 5-HT_{2A} and 5-HT_{2C} receptors are differentially regulated after chronic treatment with CLOZ, CPZ, RIS, AMP, and ORG.
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KEY WORDS: *5-HT_{2A} receptor; 5-HT_{2C} receptor; Clozapine; Chlorpromazine; Risperidone; Amperozide; ORG 5222; Atypical antipsychotic drug*

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In addition to other hypotheses, the role of brain serotonergic system in the pathophysiology and treatment of schizophrenia has been the focus of intensive research for years. Clozapine (CLOZ), the prototype of atypical antipsychotic drugs, interacts in many ways with the brain serotonergic system (for a review, see Meltzer and Nash 1991). Especially, 5-HT_{2A} receptors have been considered important in the therapeutical actions of CLOZ and other putative atypical antipsychotic drugs (Deutch et al. 1991; Meltzer and Nash 1991). Recent studies have shown that, in addition to

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the 5-HT_{2A} receptor (Leysen et al. 1978; Meltzer et al. 1989), CLOZ binds with high affinity for the 5-HT_{2C} receptor (Canton et al. 1990; Kuoppamäki et al. 1993b; Roth et al. 1992), which has many similarities to the 5-HT_{2A} receptor (Julius et al. 1990; Sanders-Bush and Breeding 1988).* We have previously suggested that the ability of chronic CLOZ treatment to downregulate 5-HT_{2C} receptors may contribute to some of its atypical effects (Hietala et al. 1992; Kuoppamäki et al. 1993a, 1994). Preliminary clinical studies have also supported the idea that 5-HT_{2C} receptor antagonism may have a role in the therapeutic actions of CLOZ (Kahn et al. 1993a, b; Owen et al. 1993).

The present study was done to find out whether other antipsychotic drugs would also be potent 5-HT_{2C} receptor antagonists, as we have previously shown for CLOZ (Kuoppamäki et al. 1993b). The interaction with the 5-HT_{2C} receptor (binding affinities and functional activity) was studied using [^{3}H]mesulergine binding assay and 5-HT stimulated phosphoinositide (PI) hydrolysis in the rat choroid plexus. For comparison, 5-HT_{2A} receptor affinities were also determined. A classical antipsychotic drug, chlorpromazine (CPZ), and putative atypical antipsychotic drugs, risperidone (RIS), amperozide (AMP), and ORG 5222 (ORG) were selected for the study. RIS, AMP, and ORG were chosen to represent novel atypical antipsychotic drugs, as there are data (partly preliminary) according to which all of these agents have antipsychotic effects in humans with a reduced risk for EPS (Björk et al. 1992; Chouinard et al. 1993; Claus et al. 1992; Sitsen and Vrijmoed-de Vries 1992).

To further characterize the role of 5-HT_{2A} and 5-HT_{2C} receptors in the antipsychotic drug action, we studied the regulation of these receptors after chronic treatment with behaviorally and biochemically active doses of CLOZ, CPZ, RIS, AMP, and ORG. Quantitative receptor autoradiography (receptor binding measured 68 hours after the last drug injections) was used for this purpose. 5-HT_{2C} receptor-binding sites in the rat choroid plexus were measured with [^{125}I]DOI and [^{3}H]mesulergine. [^{125}I]DOI and [^{3}H]mesulergine binding sites are thought to represent agonist high-affinity states of the 5-HT_{2C} receptor and total population (agonist high- and low-affinity states) of 5-HT_{2C} receptors, respectively (Havlik and Peroutka 1992; Leonhardt et al. 1992). In turn, 5-HT_{2A} receptor-binding sites in the frontal cortex were measured with [^{125}I]DOI and [^{3}H]ketanserin, which putatively represent binding to 5-HT_{2A} receptor high-affinity states and total 5-HT_{2A} receptor population, respectively (Leonhardt et al. 1992; Teitler et al. 1990). For comparison, regulation of striatal D_1 and D_2 receptors was studied using [^{3}H]SCH 23390 and [^{3}H]spiperone as radioligands, respectively.

* The 5-HT_{2A} and 5-HT_{2C} receptors have been previously called as 5-HT_2 and 5-HT_{1C} receptors, respectively.

MATERIALS AND METHODS

Animals

Male Sprague-Dawley rats (Harlan Sprague-Dawley, Indianapolis, USA) weighing between 180 and 230 g at the beginning of the study were used. The mean weights of rats did not differ between different groups. Rats were housed in groups of three under standard laboratory conditions (temperature, 21°C; humidity, 55 ± 5%; lights on from 6.00 A.M. to 6.00 P.M.). Free access to standard pellet food and tap water was available at all times.

Drugs, Doses, and Treatments

CLOZ (Leponex 25 mg/ml, Sandoz Pharma AG, Basle, Switzerland) and CPZ (Klorpromazin 25 mg/ml, Dumex, Denmark) were used in the form of commercially available ampules. CPZ ampules were further diluted with distilled water. RIS, AMP, and ORG were generously provided by Janssen Research Foundation (Beerse, Belgium), Pharmacia LEO Therapeutics AB (Malmö, Sweden), and Organon International BV (Oss, The Netherlands), respectively. (See Figure 1.)

Six groups of rats (n = 6 per treatment group) received subcutaneous (SC) injections of CLOZ (25 mg/kg), CPZ (15 mg/kg), RIS (0.3 mg/kg), AMP (5 mg/kg), ORG (0.1 mg/kg), or an equal volume (1 ml/kg) of saline once a day for 14 days.

The drug doses were chosen according to previous studies suggesting sufficient blockade (in vivo occupancy and/or the inhibition of 5-HTP-induced head twitches) or downregulation of 5-HT_{2A} receptors. For CLOZ, 25 mg/kg of CLOZ was used as a "positive control" of 5-HT_{2A} and 5-HT_{2C} receptor downregulation (Hietala et al. 1992; Kuoppamäki et al. 1993a, 1994; Reynolds et al. 1983).

CPZ. 10 mg/kg of CPZ (21 days) has been reported to downregulate cortical 5-HT_{2A} receptors by 60% (Andree et al. 1986). The same dose also occupies about 60% of 5-HT_{2A} receptors in vivo (Matsubara et al. 1993). The ED_{50} value of CPZ to inhibit 5-HTP-induced head twitches has been reported to be 0.87 mg/kg (Maj et al. 1978).

RIS. There are many studies showing that RIS occupies 5-HT_{2A} receptors and antagonizes 5-HT_{2A} receptor-linked behaviors with very low doses (ED_{50} values < 0.1 mg/kg; Janssen et al. 1988; Leysen et al. 1993). The ED_{50} value of RIS to inhibit, for example, 5-HTP-induced head twitches is 0.016 mg/kg (Janssen et al. 1988). Matsubara et al. (1993) have reported cortical 5-HT_{2A} receptor occupancy of about 65% and 80% with 0.25 and 0.5 mg/kg of RIS, respectively.

AMP. The ED_{50} value for blocking cortical 5-HT_{2A} receptors is 1.1 mg/kg (Meltzer et al. 1992). 5 mg/kg/day

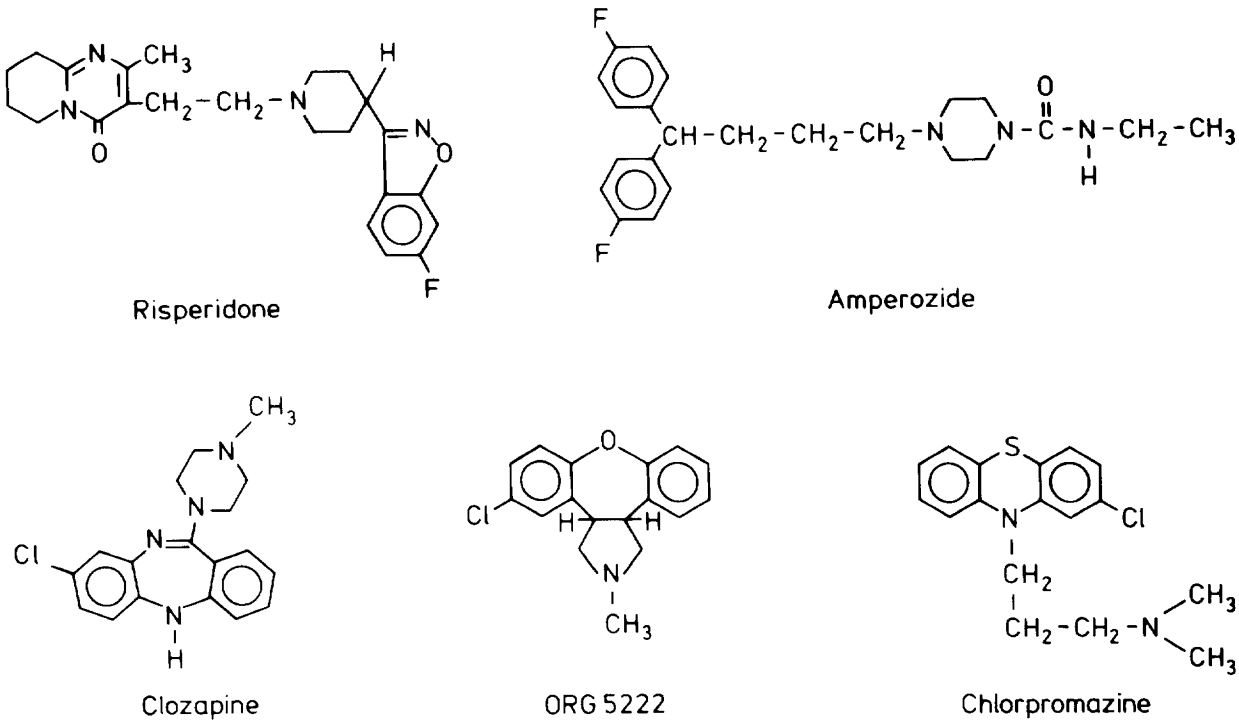

Figure 1. Chemical structures of the antipsychotic agents used in the present study.

PO for 21 days has been reported to significantly downregulate cortical 5-HT2A receptors (Svartengren 1993). According to Gustafsson and Christensson (1990), the ED_{50} value of AMP to inhibit 5-HTP-induced head twitches is about 0.3 to 0.4 mg/kg.

ORG. 0.25 mg/kg of ORG has been reported to occupy about 80% of cortical 5-HT2A receptors (Matsubara et al. 1993). ED_{50} values of ORG for the inhibition of 5-HT2A receptor-mediated head twitches and 5-HT2C receptor-mediated penile erection (both induced by ORG 10155) are 0.001 and 0.02 mg/kg, respectively (Broekkamp et al. 1990). In addition to sufficient 5-HT2A receptor blockade, the doses of CLOZ, CPZ, RIS, AMP, and ORG used are also behaviorally active and relevant (Broekkamp et al. 1990; Gustafsson and Christensson 1990; Janssen et al. 1988; Leysen et al. 1993).

Brain Dissection

Rats were decapitated 68 hours after the last injection. For receptor autoradiography, the brains were quickly removed after decapitation, frozen on tissue pedestals using dry ice and tissue glue (Tissue-Tek O.C.T. Compound, Miles Inc., Elkhart, IN, USA), and stored at −70°C. Coronal sections (16 μm) of choroid plexus (coordinates A 6860–A 6670 according to König and Klippel's atlas), frontal cortex (A 9820–A 9650), and striatum (A 9650–A 8920) were cut from the same brains with a cryostat microtome at −18°C. Sections were thaw-mounted on to gelatin-coated slides, air-dried at room temperature, and stored at −70°C with a desiccator until used. For the 5-HT2A and 5-HT2C receptor homogenate binding assay, choroid plexi and frontal cortices were quickly removed after decapitation, frozen on dry ice, and stored at −70°C. Fresh choroid plexi were used for 5-HT-induced phosphoinositide hydrolysis. For this purpose, choroid plexi were rapidly dissected after decapitation, and a single choroid plexus was placed into 5 ml of Krebs-bicarbonate (KRB) buffer.

5-HT2C Receptor Binding Assay

The 5-HT2C receptor-binding assay was performed with [^{3}H]mesulergine (81 Ci/mmol, Amersham) as previously described (Kuoppamäki et al. 1993b). Briefly, a [^{3}H]mesulergine concentration of 1.6 nM was used. The nonspecific binding was determined using 10 μM 5-HT, and 50 nM spiperone and 10 μM pargyline were included to occupy 5-HT2A receptors and inhibit monoamine oxidase activity, respectively.

5-HT2A Receptor Binding Assay

The 5-HT2A receptor-binding assay was performed with [^{3}H]ketanserin (64.1 Ci/mmol, New England Nuclear) as previously described (Kuoppamäki et al. 1993b). Briefly, [^{3}H]ketanserin concentration of 1.5 nM was used. Nonspecific binding was defined in the presence of 1 μM methysergide.

In 5-HT2A and 5-HT2C receptor-binding assays,

OptiPhase "Hisafe" 3 (LKB Wallac, Loughborough, England) was used as a scintillation fluid. Computer processing of the binding data [EBDA (equilibrium binding data analysis)/LIGAND] was performed as previously described (Hietala et al. 1990).

5-HT_{2C} Receptor-mediated Phosphoinositide (PI) Hydrolysis Assay

5-HT_{2C} receptor-mediated phosphoinositide hydrolysis was performed as previously described (Kuoppamäki et al. 1993b). Briefly, KRB buffer was used as incubation medium. The samples were labeled with 1 μCi of [^{3}H]myo-inositol (New England Nuclear) for 90 minutes in the presence of O_2/CO_2 (95:5). 10 μM pargylin and 10 mM lithium were added, and the incubation was continued for 15 minutes. The antagonists, if present, were added at the same time with pargyline and lithium. Thereafter, 100 nM 5-HT was added, and the samples were incubated for 30 minutes. A column of Dowex-1 anion exchange resin in the formate form was used to separate different inositol phosphates. Inositol monophosphate (IP) was eluted with 10 ml of 200 mM ammonium formate/0.1 M formic acid. OptiPhase "Hisafe" 3 (LKB Wallac, Loughborough, England) was used as scintillation fluid.

5-HT_{2C} Receptor Autoradiography with [^{3}H]Mesulergine

The 5-HT_{2C} receptor autoradiography with [^{3}H]mesulergine was performed as previously described (Kuoppamäki et al. 1994). Briefly, after preincubation, the sections were drop-incubated for 2 hours at room temperature with 100 μl of 170 mM Tris-HCl buffer (pH 7.5) containing a saturating concentration (5 nM) of [^{3}H] mesulergine (78 Ci/mmol, Amersham). Nonspecific binding was determined by incubating the adjacent sections in the presence of 5 μM methysergide. 100 nM spiperone was used to prevent binding to 5-HT_{2A} sites. The slides were apposed to Kodak XAR-5 x-ray films together with plastic [^{3}H]standards (American Radiolabelled Chemicals, St. Louis, MO, USA) for 32 days at 4°C.

The [^{3}H]autoabsorption (quenching) of the choroid plexus tissue is minimal (Geary and Wooten 1985). To avoid overestimation of the number of 5-HT_{2C} receptors (labeled by [^{3}H]mesulergine) and to compare absolute densities of [^{125}I]DOI and [^{3}H]mesulergine binding sites in the choroid plexus, [^{3}H]choroid plexus paste standards were prepared to convert the values of [^{3}H]plastic standards (μCi/g plastic) to pmol ligand bound per gram of tissue (Figure 2). Because of the small size of rat choroid plexus, pig choroid plexi obtained from a local slaughterhouse were used for this purpose. Prior to homogenization with a Teflon homogenizer, choroid plexi were rinsed in deionized water to remove excess blood and dried. Known concentrations of [^{3}H]mesulergine were added to tubes containing the tissue paste. Thereafter, the paste was mixed thoroughly and the tubes were centrifuged at 10,000 rpm for 40 seconds and frozen at −70°C. The paste blocks were removed from the tubes and frozen on tissue pedestals. Coronal sections (16 μm thick) were cut with the cryostat microtome at −18°C and thaw-mounted on gelatin-coated slides. A conversion factor was generated for choroid plexus paste standards and plastic standards by apposing both sets of standards to the same sheet of film for 32 days at 4°C.

5-HT_{2C} Receptor Autoradiography with [^{125}I]DOI

The 5-HT_{2C} receptor autoradiography with [^{125}I]DOI was done as previously described (Kuoppamäki et al. 1994). Briefly, after preincubation, the sections were drop-incubated for 1 hour at room temperature with 100 μl of 50 mM Tris-HCl buffer (pH 7.5) containing 10 mM $MgSO_4$, 0.1% (w/v) bovine serum albumin, and 0.3 nM [^{125}I]DOI (2,200 Ci/mmol, New England Nuclear). Nonspecific binding was determined by incubating the adjacent sections in the presence of 5 μM methysergide. 100 nM spiperone was used to prevent binding to 5-HT_{2A} sites. In pig choroid plexus, the 5-HT-$_{2C}$ receptor K_d value of [^{125}I]DOI has been reported to be 1.6 nM (Leonhardt et al. 1992), but a concentration of 0.3 nM of [^{125}I]DOI was used for practical reasons. In the case of CLOZ, it is assumed that there are no changes in K_d value of [^{125}I]DOI, as previously shown with [^{3}H]mesulergine (Kuoppamäki et al. 1993b) and that the binding primarily reflects changes in receptor density. The slides were apposed to Kodak XAR-5 x-ray films together with plastic [^{14}C]standards (American Radiolabeled Chemicals, St. Louis, MO, USA) for 1 to 2 days at 4°C.

5-HT_{2A} Receptor Autoradiography with [^{3}H]ketanserin

The 5-HT_{2A} receptor autoradiography with [^{3}H]ketanserin was done as previously described (Pazos et al. 1985), with minor modifications. The slides were preincubated for 15 minutes at room temperature in 170 mM Tris-HCl buffer (pH 7.5) and allowed to dry for 2 hours. Thereafter, the sections were drop-incubated for 2 hours at room temperature with 100 μl of Tris-HCl buffer containing a saturating concentration (4 nM) of [^{3}H]ketanserin (63.7 Ci/mmol, New England Nuclear, Boston, MA, USA). Nonspecific binding was determined by incubating the adjacent sections in the presence of 5 μM methysergide. 1 μM prazosin was included to prevent binding to α_1 receptors. The sections were then washed twice for 10 minutes in ice-cold buffer, dipped in ice-cold water to remove ions, and dried at

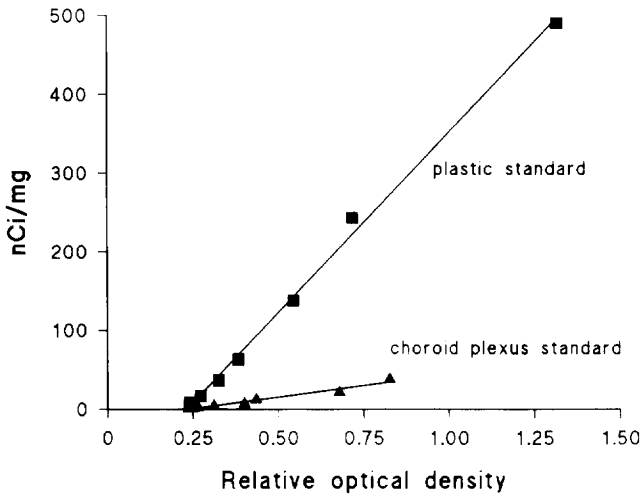

Figure 2. Radioactivities of [^{3}H]choroid plexus paste standards and [^{3}H]plastic standards against relative optical densities after 32-days' exposure. Note that radioactivities about tenfold lower in the choroid plexus than in plastic standards darken the x-ray films to a similar extent.

room temperature. The slides were finally apposed to Kodak XAR-5 x-ray films together with plastic [^{3}H]standards (American Radiolabelled Chemicals, St. Louis, MO, USA) for 13 weeks at 4°C.

5-HT$_{2A}$ Receptor Autoradiography with [^{125}I]DOI

The 5-HT$_{2A}$ receptor autoradiography with [^{125}I]DOI was done as described by Appel et al. (1990) with minor modifications. The slides were preincubated for 10 minutes at room temperature in 50 mM Tris-HCl buffer (pH 7.5) containing 10 mM $MgSO_4$ and 0.1% (w/v) BSA and allowed to dry for 2.5 hours. Thereafter, the sections were drop-incubated for 1 hour at room temperature with 100 µl of Tris-HCl buffer containing 0.3 nM [^{125}I]DOI (2,200 Ci/mmol, New England Nuclear). Nonspecific binding was determined by incubating the adjacent sections in the presence of 1 µM ketanserin. The sections were then washed twice for 15 minutes in ice-cold buffer, dipped in ice-cold water to remove ions, and dried at room temperature. The slides were finally apposed to Kodak XAR-5 x-ray films together with plastic [^{14}C]standards (American Radiolabelled Chemicals, St. Louis, MO, USA) for 2 to 3 days at 4°C.

D$_2$ Receptor Autoradiography with [^{3}H]spiperone

D$_2$ receptor autoradiography with [^{3}H]spiperone was performed as follows: The sections were preincubated for 10 minutes at room temperature (RT) in a 50-mM Tris-HCl buffer (pH 7.4) containing 120 mM NaCl, 5 mM KCl, 2 mM $CaCl_2$, and 1 mM $MgCl_2$. Sections were then dried for 30 minutes at RT and subsequently drop-incubated for 60 minutes at RT in 100 µl of a similar buffer containing a saturating concentration (1.5 nM) of [^{3}H]spiperone (119 Ci/mmol, Amersham) and 100 nM ketanserin (to block 5-HT$_{2A}$ receptors). Nonspecific binding was determined in the presence of 10 µM (−)sulpiride. Slides were then washed twice for 5 minutes in ice-cold buffer and dipped in ice-cold deionized water. After this, slides were dried overnight and finally apposed to Kodak XAR-5 x-ray films together with plastic [^{3}H]standards (American Radiolabelled Chemicals, St. Louis, MO, USA) for 32 days at 4°C.

D$_1$ Receptor Autoradiography with [^{3}H]SCH 23390

D$_1$ receptor autoradiography with [^{3}H]SCH 23390 was performed as previously described (Lappalainen et al. 1992). Briefly, after preincubation, the sections were drop-incubated for 45 minutes at RT with 100 µl of 50 mM Tris-HCL buffer (pH 7.4) containing 2 nM [^{3}H]SCH 23390 (71 Ci/mmol, New England Nuclear) and 120 mM NaCl, 5 mM KCl, 2 mM $CaCl_2$, and 1 mM $MgCl_2$. Nonspecific binding was determined by incubating the adjacent sections in the presence of 1 µM cis-flupentixol. The slides were apposed to Kodak XAR-5 x-ray films together with plastic [^{3}H]standards (American Radiolabelled Chemicals, St. Louis, MO, USA) for 3 weeks at 4°C.

Films were developed with an automated Fuji RG II x-ray film processor. For a comparison between treatment groups, all sections from all groups were exposed to the same sheet of film.

Image Analysis

The images were analyzed using a CCD Video Camera (Hamamatsu C3077, Hamamatsu Photonics K.K., Hamamatsu City, Japan) and a computerized image analysis system (MCID, M4 1.12, Imaging Research Inc., St. Catharines, Ontario, Canada) as previously described (Kuoppamäki et al. 1994). D$_1$ and D$_2$ receptors were measured from the whole striatum, 5-HT$_{2A}$ receptors from the layer IV of the frontal (cingulate) cortex and 5-HT$_{2C}$ receptors from the choroid plexi in lateral ventricles. Because of the differential [^{3}H]autoabsorption (quenching) of various brain areas, the values obtained with [^{3}H]plastic standards (µCi/g plastic) were converted to pmol of ligand bound per gram of tissue using conversion factors for the gray matter (American Radiolabeled Chemicals, St. Louis, MO, USA) in the case of 5-HT$_{2A}$ receptors, [^{3}H]choroid plexus paste standards in the case of 5-HT$_{2C}$ receptors, and [^{3}H]brain paste standards (Lappalainen et al. 1992) in the case of D$_1$ and D$_2$ receptors.

Table 1. Affinities (K_i, nmol/l) of CLOZ, CPZ, RIS, AMP, and ORG for Serotonin 5-HT_{2A} and 5-HT_{2C} Receptors[a]

Agent	5-HT_{2C}	5-HT_{2A}
CLOZ	13.2 ± 2.1	12.6 ± 3.3
CPZ	27.1 ± 2.9	10.5 ± 2.3
RIS	112 ± 0.4	1.9 ± 0.4
AMP	2580 ± 51	75.6 ± 14.4
ORG	0.9 ± 0.1	0.4 ± 0.1

[a] The radioligands for determining 5-HT_{2C} and 5-HT_{2A} receptor binding were [^{3}H]mesulergine (1.6 nM) and [^{3}H]ketanserin (1.5 nM), respectively. The tissues used to determine 5-HT_{2C} and 5-HT_{2A} receptor binding were choroid plexus and frontal cortex, respectively. Data represent mean ± SEM of three separate determinations. CLOZ data are taken from Kuoppamäki et al. (1993b).

Statistical Analysis

Statistical analysis of the data was carried out by one-way analysis of variance (ANOVA) followed by Tukey's test for post hoc analyses. A commercially available statistical software (Systat, Evanston, IL, USA) was used for this purpose. A p value lower than .05 was considered to be statistically significant.

RESULTS

5-HT_{2A} and 5-HT_{2C} Receptor Radioligand-Binding Studies

The results of radioligand-binding studies are summarized in Table 1. The rank order of affinities for the 5-HT_{2C} receptor was ORG > CLOZ ≥ CPZ > RIS > > AMP. In turn, the rank order of affinities for the 5-HT_{2A} receptor was ORG ≥ RIS > CPZ ≥ CLOZ > AMP. RIS and AMP had clearly higher affinities for the 5-HT_{2A} than the 5-HT_{2C} receptor; otherwise there were no major differences between the 5-HT_{2A} and 5-HT_{2C} receptor affinities.

5-HT_{2C} Receptor-Mediated Phosphoinositide (PI) Hydrolysis in the Choroid Plexus

Results of PI hydrolysis studies are summarized in Figure 3. 100 nM 5-HT stimulated the accumulation of inositol monophosphate (IP) four- to fivefold over basal values. This concentration of 5-HT was subsequently used in the antagonist studies. The rank order of potency to antagonize 5-HT-stimulated PI hydrolysis was ORG > CLOZ > RIS > CPZ > > AMP. None of the drugs did stimulate 5-HT_{2C} receptor-mediated PI hydrolysis themselves in concentrations from 100 nM to 33 μM (RIS, ORG, CPZ, CLOZ) and 10 μM to 100 μM (AMP). However, all the agents even decreased basal values of PI hydrolysis (by about 20% to 50%) with the above concentrations demonstrating negative intrinsic activity (data not shown).

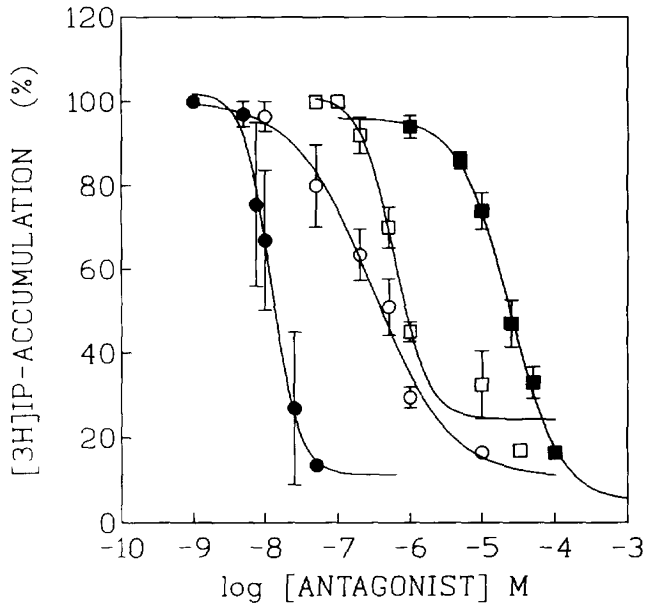

Figure 3. Inhibition of 5-HT-stimulated phosphoinositide hydrolysis in the rat choroid plexus by ORG (●), RIS (○), CPZ (□), and AMP (■). Increasing concentrations of antagonists were added to incubation tubes 15 minutes before the addition of 100 nM 5-HT. The IC_{50} values (with 95% CI) for ORG, RIS, CPZ, and AMP are 12 nM (6.5–22 nM), 300 nM (48–1900 nM), 600 nM (300–1100 nM), and 24 μM (11–51 μM), respectively. The IC_{50} value for CLOZ is 110 nM (46–260 nM; Kuoppamäki et al. 1993b). Each point represent the mean ± SEM of two to four determinations. All agents decreased also basal values of PI hydrolysis (in the absence of 5-HT) with concentrations from 100 nM to 33 μM (RIS, ORG, CPZ) and 10 μM to 100 μM (AMP), demonstrating negative intrinsic activity (data not shown). IP = inositol monophosphate.

Effects of Chronic Treatment with CLOZ, RIS, AMP, ORG, and CPZ on 5-HT_{2A} Receptor-Binding Sites in the Rat Frontal Cortex Labeled with an Agonist Radioligand, [^{125}I]DOI, and an Antagonist Radioligand, [^{3}H]ketanserin

The results are summarized in Figure 4. In the control group, 5-HT_{2A} receptor [^{3}H]ketanserin and [^{125}I]DOI bindings were 88.2 ± 9.6 and 1.77 ± 0.16 pmol/g tissue (mean ± SEM), respectively. The number of 5-HT_{2A} receptor-binding sites labeled with [^{3}H]ketanserin and [^{125}I]DOI were significantly decreased (by 50% and 62%, respectively) after chronic treatment with CLOZ. In addition, chronic treatments with ORG (0.1 mg/kg/day) and CPZ (15 mg/kg/day) significantly decreased (by about 40 to 55%) the binding of [^{3}H]ketanserin and [^{125}I]DOI to the 5-HT_{2A} receptors in the frontal cortex. AMP (5 mg/kg/day) significantly decreased 5-HT_{2A} receptor [^{3}H]ketanserin binding, but the decrease in [^{125}I]DOI binding did not reach statistical significance. Chronic treatment with RIS (0.3 mg/kg/day) had no significant effects on 5-HT_{2A} receptor binding.

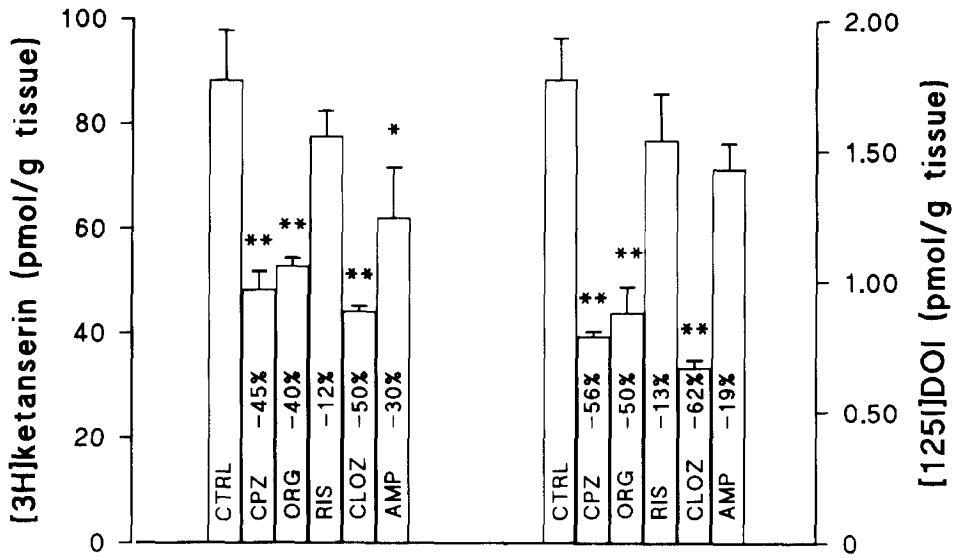

Figure 4. Effects of chronic (14 days) CLOZ (25 mg/kg), CPZ (15 mg/kg), ORG (0.1 mg/kg), RIS (0.3 mg/kg), and AMP (5 mg/kg) treatment on 5-HT2A receptor-binding sites in the layer IV of rat frontal (cingulate) cortex as determined by quantitative autoradiography with 0.3 nM [125I]DOI and 4 nM [3H]ketanserin. Percentage change from control group is given in each bar. Bars represent mean ± SEM, * $p < .05$ and ** $p \leq .001$ compared to control group. CTRL, control; CPZ, chlorpromazine; ORG, ORG 5222; RIS, risperidone; CLOZ, clozapine; AMP, amperozide.

Effects of Chronic Treatment with CLOZ, RIS, AMP, ORG, and CPZ on 5-HT2C Receptor-Binding Sites in the Rat Choroid Plexus Labeled with an Agonist Radioligand, [125I]DOI, and an Antagonist Radioligand, [3H]mesulergine

The results are summarized in Figure 5. In the control group, 5-HT2C receptor [3H]mesulergine and [125I]DOI bindings were 277 ± 12 and 2.27 ± 0.18 pmol/g tissue (mean ± SEM), respectively. As was the case with 5-HT2A receptors, chronic CLOZ treatment (25 mg/kg/day) significantly decreased the number of 5-HT2C receptor-binding sites labeled with [125I]DOI and [3H]mesulergine by 46% and 48%, respectively. No statistically significant changes were observed in the number of 5-HT2C receptor-binding sites after chronic treatments with RIS (0.3 mg/kg/day), AMP (5 mg/kg/day), ORG (0.1 mg/kg/day), and CPZ (15 mg/kg/day). RIS had a tendency toward increased 5-HT2C receptor binding, whereas other agents had an opposite trend.

Effects of Chronic Treatment with CLOZ, RIS, AMP, ORG, and CPZ on Striatal D_1 and D_2 Receptor-Binding Sites Labeled with [3H]SCH 23390 and [3H]spiperone, Respectively

The results are summarized in Figure 6. In the control group, D_1 receptor [3H]SCH 23390 and D_2 receptor [3H]spiperone binding were 190 ± 6.2 and 29.7 ± 2.3 pmol/g tissue (mean ± SEM), respectively. Chronic treatment with CPZ (15 mg/kg/day) significantly in-

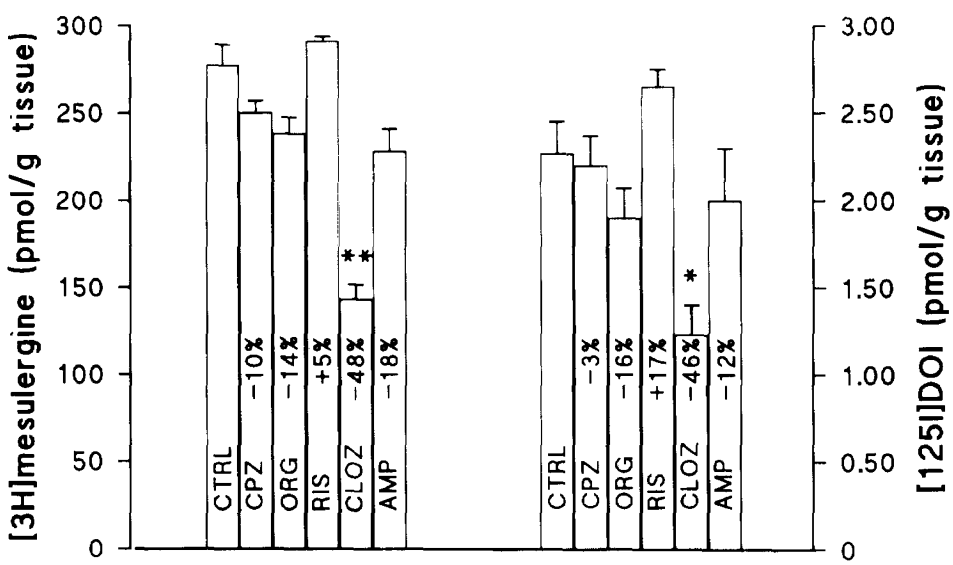

Figure 5. Effects of chronic (14 days) CLOZ (25 mg/kg), CPZ (15 mg/kg), ORG (0.1 mg/kg), RIS (0.3 mg/kg), and AMP (5 mg/kg) treatment on 5-HT2C receptor-binding site in the rat choroid plexus as determined by quantitative autoradiography with 0.3 nM [125I]DOI and 5 nM [3H]mesulergine. Percentage change from control group is given in each bar. Bars represent mean ± SEM, * $p < .01$ and ** $p < .001$ compared to control group. CTRL, control; CPZ, chlorpromazine; ORG, ORG 5222; RIS, risperidone; CLOZ, clozapine; AMP, amperozide.

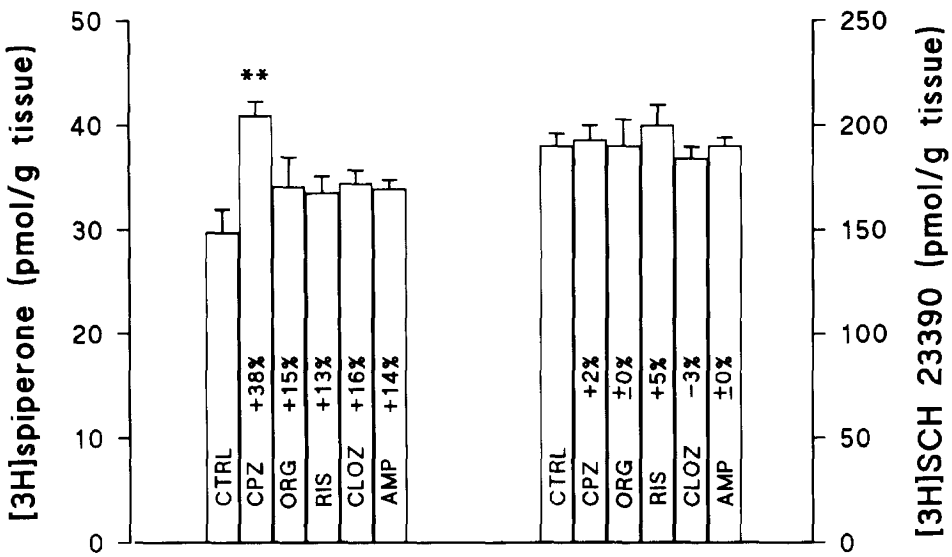

Figure 6. Effects of chronic (14 days) CLOZ (25 mg/kg), CPZ (15 mg/kg), ORG (0.1 mg/kg), RIS (0.3 mg/kg), and AMP (5 mg/kg) treatment on striatal D_1 and D_2 receptor-binding sites as determined by quantitative autoradiography with 2 nM [^{3}H]SCH 23390 and 1.5 nM [^{3}H]spiperone, respectively. Percentage change from control group is given in each bar. Bars represent mean ± SEM, ** $p < .01$ compared to control group. CTRL, control; CPZ, chlorpromazine; ORG, ORG 5222; RIS, risperidone; CLOZ, clozapine; AMP, amperozide.

creased the binding of [^{3}H]spiperone to the striatal D_2 receptors by 38%. There was a trend toward increased binding of [^{3}H]spiperone (13% to 16%) after repeated administration of CLOZ, RIS, AMP, and ORG. Binding of [^{3}H]SCH 23390 to striatal D_1 receptors was not significantly altered by any drug treatment.

DISCUSSION

Interaction of Antipsychotic Drugs with the 5-HT$_{2C}$ Receptor In Vitro

It is known that CLOZ has a nanomolar affinity for the 5-HT$_{2C}$ receptor (Canton et al. 1990; Kuoppamäki et al. 1993b; Roth et al. 1992) and that CLOZ acts as an antagonist at the 5-HT$_{2C}$ receptor (Kuoppamäki et al. 1993b). In the present study, ORG had even a higher affinity for the 5-HT$_{2C}$ receptor, the affinity of which (K_i, 0.9 nM) is one of the highest reported so far for the 5-HT$_{2C}$ receptor. In agreement with previous studies (Canton et al. 1990; Roth et al. 1992), a classical antipsychotic drug, CPZ, also had a relatively high affinity, whereas RIS and especially AMP had low K_i values for the 5-HT$_{2C}$ receptor. Roth et al. (1992) have reported slightly higher 5-HT$_{2C}$ receptor affinities using transfected COS-7 cells and [^{125}I]LSD as a radioligand. For comparison to the 5-HT$_{2C}$ receptor, the affinities of these drugs for the 5-HT$_{2A}$ receptor appeared to be rather similar, except in the case of RIS and AMP, which had higher affinities for the 5-HT$_{2A}$ receptor. It is evident that antipsychotic drugs cannot be divided into typical and atypical according to their in vitro affinities for the 5-HT$_{2C}$ receptor alone, as high affinity for the 5-HT$_{2C}$ receptor is not common for all putative atypical antipsychotic drugs (e.g., AMP); vice versa, CPZ, a classical antipsychotic drug, has a relatively high affinity for the 5-HT$_{2C}$ receptor. The same is true also for 5-HT$_{2A}$ receptors. Rather, an appropriate ratio of the 5-HT$_{2A/2C}$ receptor and some other receptor (e.g., 5-HT$_{2A}$/D_2 receptor ratio) may better differentiate typical and atypical antipsychotic drugs from one another (Meltzer et al. 1989).

Like CLOZ and its metabolites (Kuoppamäki et al. 1993b), CPZ, RIS, AMP, and ORG were shown to be 5-HT$_{2C}$ receptor antagonists, as they inhibited the 5-HT$_{2C}$ receptor-mediated PI hydrolysis in the choroid plexus but did not stimulate PI hydrolysis per se. In higher concentrations, all agents even decreased the basal PI hydrolysis demonstrating a negative intrinsic activity. We have seen the same before with CLOZ and its metabolites (Kuoppamäki et al. unpublished), and this seems to be the case also with many other 5-HT$_{2C}$ receptor antagonists (Barker et al. 1994). It remains to be seen which mechanisms are responsible for this phenomenon. Nevertheless, the capability to reduce basal 5-HT$_{2C}$ receptor-mediated PI hydrolysis appears to be equally shared by classical and atypical antipsychotic drugs.

Regulation of 5-HT$_{2A}$ Receptors

To further study the putative roles of 5-HT$_{2A}$ and 5-HT$_{2C}$ receptors in the antipsychotic drug actions, we studied the regulation of these receptors after chronic treatment with CLOZ, CPZ, RIS, AMP, and ORG. We had previous data that showed that chronic treatment with CLOZ (10 and 25 mg/kg/day) decreased the density (B_{max}) of 5-HT$_{2A}$ receptors in the homogenates of frontal cortex by 40% and 55%, respectively, without affecting the affinity (K_d) of [^{3}H]ketanserin (Kuoppamäki et al. unpublished). In the present study, rats were treated with behaviorally active doses (see

Methods) that have also been shown to cause high occupancy of cortical 5-HT$_{2A}$ receptors (occupancy determined after single injections). CLOZ potently decreased (by about −50% to −60%) the number of 5-HT$_{2A}$ receptor-binding sites in the frontal cortex (layer IV of cingulate region) labeled with [^{125}I]DOI and [^{3}H]ketanserin. This is in good agreement with previous findings (O'Dell et al. 1990; Reynolds et al. 1983; Wilmot and Szczepanik 1989). A classical antipsychotic drug, CPZ, and a putative atypical antipsychotic drug, ORG, also caused equal reductions (from −40% to −56%) in the number of 5-HT$_{2A}$ receptor-binding sites labeled with [^{125}I]DOI and [^{3}H]ketanserin. The same was true for AMP, although its effects on 5-HT$_{2A}$ receptor [^{125}I]DOI (−19%) and [^{3}H]ketanserin (−30%) binding sites were smaller than the effects of CPZ and ORG. We could not confirm the findings made by Svartengren (1993), who reported that AMP selectively downregulated agonist high-affinity states of the 5-HT$_{2A}$ receptor. In the present study, the alterations in the number of 5-HT$_{2A}$ receptor-binding sites labeled with [^{125}I]DOI and [^{3}H]ketanserin were generally very similar, suggesting that there are no major differences in the regulation of 5-HT$_{2A}$ receptor high- and low-affinity states. The most surprising result was that RIS, with a dose of 0.3 mg/kg that should potently antagonize 5-HT$_{2A}$ receptor-mediated head twitches (ED_{50} value 0.016 mg/kg) and occupy 5-HT$_{2A}$ receptors (ED_{50} value 0.067 mg/kg) in vivo (Janssen et al. 1988; Matsubara et al. 1993; Schotte et al. 1993) did not significantly alter the number of 5-HT$_{2A}$ receptor-binding sites. The reasons for this are not known. It may be that also other properties (shared by CLOZ, CPZ, ORG, and AMP but not RIS) than high 5-HT$_{2A}$ receptor occupancy are needed to elicit downregulation of 5-HT$_{2A}$ receptors in vivo in the frontal cortex. In addition, it has been recently reported that SR 46349B, a novel 5-HT$_{2A}$ receptor antagonist, upregulates cortical 5-HT$_{2A}$ receptors (Rinaldi-Carmona et al. 1993). It thus appears that treatment with structurally different chemical agents leads to also to 5-HT$_{2A}$ receptor adaptations other than downregulation.

Regulation of 5-HT$_{2C}$ Receptors

In addition to frontal cortical 5-HT$_{2A}$ receptors, chronic treatment with CLOZ (25 mg/kg/day) potently decreased the number of 5-HT$_{2C}$ receptor-binding sites in the choroid plexus labeled with [^{125}I]DOI and [^{3}H]mesulergine. We have shown this previously also with a lower dose (10 mg/kg) of CLOZ (Kuoppamäki et al. 1994). The reductions in 5-HT$_{2C}$ receptor [^{125}I]DOI and [^{3}H]mesulergine binding were of similar magnitude, although exact comparisons cannot be done because of the low (below K_d) concentration of [^{125}I]DOI. RIS and AMP did not significantly affect 5-HT$_{2C}$ receptor [^{125}I]DOI and [^{3}H]mesulergine binding, which is in good agreement with rather low in vitro affinities of RIS and AMP for the 5-HT$_{2C}$ receptor. Because ORG and CPZ had rather high 5-HT$_{2C}$ receptor affinities in vitro, alterations in the number of 5-HT$_{2C}$ receptor binding sites could have been expected. However, in doses that clearly downregulated 5-HT$_{2A}$ receptors, repeated administration of CPZ and ORG did not statistically significantly affect the binding of [^{125}I]DOI or [^{3}H]mesulergine to 5-HT$_{2C}$ receptors. This suggests that CPZ and ORG have at least some degree of selectivity for 5-HT$_{2A}$ receptors (vs. 5-HT$_{2C}$ receptors) in vivo during chronic treatment.

It is clear that doses used in the present study may be too low to cause alterations in 5-HT$_{2A}$ or 5-HT$_{2C}$ receptor binding (in the case there are no effects) and that higher doses may cause such alteractions. However, all the doses used in the present study are based on previous studies showing appropriate behavioral effects or effective in vivo blockade of 5-HT$_2$ receptors (see, e.g., Matsubara et al. 1993; Schotte et al. 1993). Most important, given an arbitrary estimate that drug metabolism in rats is in average about five times faster than that in humans (e.g., 25 mg/kg of a given drug for rats equals 5 mg/kg for humans), the present doses of drugs should also be quite relevant to clinically used therapeutic doses. In the case of 5-HT$_{2C}$ receptors in the choroid plexus, there is a receptor reserve of about 30% to 50% (Sanders-Bush and Breeding 1990). Because of this, almost full 5-HT$_{2C}$ receptor occupancy may be needed to elicit downregulation of 5-HT$_{2C}$ receptors. ED_{50} value for 5-HT$_{2C}$ receptor occupancy in vivo has been published only for CLOZ (1.8 mg/kg, SC) (Schotte et al. 1993). However, this value has been obtained after acute administration in a single time point, and receptor occupancy may be different in steady-state conditions during chronic treatment. Nevertheless, full dose-effect studies with these antipsychotic drugs would be needed to get a full picture of possible qualitatively or quantitatively different regulatory effects of 5-HT$_{2A}$ and 5-HT$_{2C}$ receptors.

Regulation of D_1 and D_2 Receptors

For comparison, regulation of striatal D_1 and D_2 receptors from the same brain samples was studied using [^{3}H]SCH 23390 and [^{3}H]spiperone, respectively. Only chronic treatment with CPZ caused a significant upregulation of striatal D_2 receptors, although other agents also had a trend toward increased [^{3}H]spiperone binding. This agrees with earlier studies. An other typical antipsychotic drug, haloperidol, unlike CLOZ, has also been reported to upregulate striatal D_2 receptors after chronic treatment (e.g., Kuoppamäki et al. 1993a; O'Dell et al. 1990). This minor effect of CLOZ and other putative atypical agents (compared to typical antipsychotic

drugs) on striatal D_2 receptors per se may contribute to their reduced propensity to induce EPS (Farde et al. 1992). Binding of [^{3}H]SCH 23390 to striatal D_1 receptors was not affected by any drug after chronic treatments. However, for example, O'Dell et al. (1990) have reported upregulation of D_1 receptors after chronic treatment with CLOZ (30 mg/kg IP for 21 days). Differences in the treatment protocol may explain the different results. Nevertheless, our study suggests only a minor role of striatal D_1 receptors in the actions of antipsychotic (typical or atypical) drugs.

CONCLUSIONS

CLOZ was the only drug in the present study that induced significant downregulation of 5-HT_{2C} receptors in the choroid plexus. However, in doses that failed to affect 5-HT_{2C} receptor binding, CPZ, AMP, and ORG significantly decreased the number of 5-HT_{2A} receptor-binding sites in the frontal cortex. In contrast to other agents used in the present study, RIS failed to alter 5-HT_{2A} receptor binding and had a tendency toward increased 5-HT_{2C} receptor binding. Thus, the effects of chronic treatment with RIS were clearly different from the effects of other agents. These results suggest that the regulation of 5-HT_{2A} and 5-HT_{2C} receptors are quantitatively different after chronic treatment with CLOZ, CPZ, RIS, AMP, and ORG. This is also true with compounds having equal 5-HT_{2A} and 5-HT_{2C} receptor affinities in vitro. Different adaptations of 5-HT_{2A} and 5-HT_{2C} receptors may contribute to differences in the clinical actions (therapeutic or adverse) of the presently studied drugs: We hypothesize that, among the 5-HT_2 receptor family, 5-HT_{2A} receptor effects of antipsychotic drugs (combined appropriately with D_2 receptor effects) primarily contribute to their reduced incidence of EPS. mCPP, which is a partial 5-HT_{2C} receptor agonist but a 5-HT_{2A} receptor antagonist (Conn and Sanders-Bush 1987) has been reported to have anxiogenic effects in rats and humans (Kennett 1992; Kennett et al. 1989; Murphy et al. 1989). These effects may be prevented by 5-HT_{2C} receptor antagonists (Kennett 1992; Kennett et al. 1989). Thus, the potent long-term effects of CLOZ (as an antagonist) on 5-HT_{2C} receptors may provide a theoretical basis for a suggested anxiolytic effect of CLOZ.

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