Dopamine Receptor Subtypes: Differential Regulation after 8 Months Treatment with Antipsychotic Drugs

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ABSTRACT

Regulation of dopamine receptor subtypes was determined after long-term (8 mo) administration of typical and atypical antipsychotic drugs using 3H-nemonapride, 3H-raclopride, 3H-spiradone, 3H-7-hydroxy-N,N-di-n-propyl-2-aminotetralin, 3H-SCH23390 and 125I-sulpiride in vitro receptor autoradiography. Drug-induced receptor upregulation was remarkably different across the various D2-like receptor radioligands. Chronic haloperidol treatment resulted in a strong increase in 3H-nemonapride, 3H-spiradone and 125I-sulpiride binding to striatal areas, whereas 3H-raclopride binding was marginally affected. Raclopride treatment elevated striatal binding of 3H-nemonapride and 3H-spiradone to a lesser extent, and did not alter 3H-raclopride binding. Clozapine treatment did not affect the binding of the tritiated radioligands. These differences suggest that 3H-nemonapride and 3H-spiradone are binding to an additional subset of D2-like receptors, not recognized by 3H-raclopride. 3H-Nemonapride binding in the presence of 300 nM raclopride uncovered a striatal binding site (designated as D₃-like receptor), that was up-regulated after chronic haloperidol, raclopride and clozapine treatment. The 125I-sulpiride binding sites in the prefrontal cortex were also up-regulated by the three antipsychotics. In contrast, 3H-spiradone binding sites were down-regulated in the prefrontal and dorsolateral cortical area. Chronic antipsychotic treatment did not affect D1-like or D₃ dopamine receptor subtype binding.

Dopamine receptors are differentiated into two major types: the D1-like receptors, which include the D₁ and D₅ receptors, and the D2-like receptors, which include the D₂, D₃ and D₄ receptors (Sibley and Monsma 1992; Sokoloff and Schwartz 1995; Baldessarini and Tarazi 1996). Chronic antipsychotic drug administration is a crucial component in the current treatment of schizophrenia. The observation that antipsychotics bind to and block striatal dopamine D2-like receptors in a direct correlation with their clinically effective antipsychotic doses (Creese et al., 1976; Seeman et al., 1976), implicates a major role of dopaminergic systems in schizophrenia. A number of animal studies reported an up-regulation of striatal D2-like receptors after subchronic (3–4 wk) drug administration (Burt et al., 1977; Seeman, 1980; O’Dell et al., 1990; Tarazi et al., in press), the period during which beneficial clinical effects of antipsychotics on patients are first noticed. However, the classical “typical” antipsychotics, including haloperidol, although effectively blocking psychoses, also cause extrapyramidal movement disorders, both transient (parkinsonism, dystonia, akathisia) and chronic (tardive dyskinesia, TD) (Jeste and Wyatt, 1979; Baldessarini and Tarsy, 1980). Atypical antipsychotic drugs, the prototype of which is clozapine, have been developed that are devoid of these extrapyramidal side effects. Short treatment periods of rats with clozapine is reported not to increase striatal D2-like receptor levels (O’Dell et al., 1990; Tarazi et al., in press). This suggests that the upregulation of striatal D2-like receptors is more likely associated with drug-induced extrapyramidal motor side effects rather than mediation of their antipsychotic action.

To gain further insight into the mechanisms of antipsychotic drug action and their involvement in the development of extrapyramidal side effects, an animal study examining the effects of prolonged antipsychotic treatment (6 mo or more), which mimicks the time frame of TD developing in antipsychotic-treated schizophrenic patients, would be preferable. Dopamine receptor up-regulation has been observed in striatal areas after prolonged drug treatment with anti-

ABBREVIATIONS: TD, tardive dyskinesia; 3H-7-OH-DPAT, 3H-7-hydroxy-N,N-di-n-propyl-2-aminotetralin; OD, optical densities; CP-M, caudate putamen (medial); CP-L, caudate putamen (lateral); HIPP, hippocampus; NA, nucleus accumbens; SCH23390, R(+)7-chloro-8-hydroxy-3-methyl-1-phenyl-2,3,4,5-tetrahydro-1H-3-benzazepine; SNpc, substantia nigra pars compacta; SNpr, substantia nigra pars reticulata; MPC, medial prefrontal cortex; DFC, dorsal frontal cortex; VTA, ventral tegmental area; Olf Tub, olfactory tubercle.
psychotic drugs (Clow et al., 1980; Owen et al., 1980; Murugargiah et al., 1983; Rupniak et al., 1984). However, a comparison between antipsychotics with respect to regional (extrastriatal) differences and degree of receptor up-regulation has not been made. Moreover, the recent cloning of several (D₁,₂) dopamine receptor subtypes (Sokoloff et al., 1990; Zhou et al., 1990; Sunahara et al., 1991; Van Tol et al., 1991), has led to the realization that the systems are more complicated than previously thought, with most of the available radioligands binding to more than one dopamine receptor subtype, therefore complicating interpretation of previous in vitro receptor binding studies. This is illustrated by the recent finding of D₂-like receptor up-regulation in postmortem striatal tissue from drug-naïve or antipsychotic-treated schizophrenic patients using ³H-nemonapride, but not ³H-raclopride, both D₂-like receptor radioligands (Seeman et al., 1993; Murray et al., 1995; Sumiyoshi et al., 1995).

We have examined whether chronic (8 mo) treatment with the typical antipsychotic (haloperidol), the atypical antipsychotic (clozapine) and the D₂/D₃ receptor antagonist raclopride which exhibits antipsychotic activity (Farde et al., 1988; Cookson et al., 1989) differentially affect dopamine receptor subtype binding measured by in vitro receptor autoradiography, in various brain regions in the rat using a number of radioligands with different dopamine receptor subtype affinities. Changes in dopamine receptor subtype binding will be interpreted as a functional index of in vivo antipsychotic drug action. Up- or down-regulation of a receptor subtype by both typical and atypical antipsychotics in a specific brain region will be suggestive of a common locus for antipsychotic drug treatment. Differences in region-specific or receptor subtype regulation by typical vs. atypical antipsychotics may indicate the involvement of a specific dopamine receptor subtype in the induction of extrapyramidal side effects.

Methods

Materials

Radioligands were obtained from New England Nuclear-Du Pont (Wilmington, DE) and Amersham (Arlington Heights, IL). Fluphenixol, ketanserin, eticlopride and sulpiride were purchased from Research Biochemical Inc. (Natick, MA). Clozapine was a gift from Sandoz (East Hanover, NJ), SCH23390 was a gift from Schering-Plough Research (Kenilworth, NJ) and raclopride was a gift from Astra Läkemedel AB (Södertälje, Sweden). All other compounds were purchased from Sigma Chemical Co. (St. Louis, MO).

Drug Treatment

Different groups of male Sprague-Dawley rats (Charles-Rivers, VA) weighing 200 to 220 g on delivery, were maintained under controlled light and temperature conditions but given free access to food and water. These rats were housed in the animal facility of Maryland Psychiatric Research Center (Baltimore, MD) and treated for 8 mo with three different drugs in the following doses: haloperidol (1.5 mg/kg/day), clozapine (25 mg/kg/day) and raclopride (10 mg/kg/day). The drugs were given in drinking water to mimic oral administration of antipsychotic drugs in patients with schizophrenia, and the control consisted of tap water adjusted to pH = 6.0. The solutions were made based on the average weekly weight of the rats and an estimation of their daily solution consumption.

Tissue Preparation

Immediately after drug treatment, rats were killed by decapitation, their brains were quickly removed, frozen by immersion in chilled isopentane and stored in liquid nitrogen until use. Coronal sections (16 μm) were cut in a cryostat at −20°C, thaw-mounted on gelatin-coated microscopic slides and stored at −80°C until use. On the day of the experiment, slides were thawed on a slide warmer and air dried at room temperature.

Receptor Binding

A number of ligands were used to quantify the different dopamine receptor subtypes. Four different radioligands were used to quantify the D₂-like receptors, ³H-nemonapride (previously known as ³H-YM-09151-2), ³H-spiperone, ³H-raclopride and ¹²⁵I-sulpiride. The first three radioligands were selected based on their high affinities for D₂-like receptors. Saturation and competition experiments showed that the binding of the three ligands to striatal sections was saturable and was inhibited by D₂-like receptor antagonists (Palacios et al., 1981; Köhler and Radesater, 1986; Yokoyama et al., 1994). Interestingly, these three ligands have different specificities for the different D₂-like receptors. ³H-nemonapride and ³H-spiperone bind with high affinity to the three D₂-like receptors (D₂, D₃ and D₄) in expression systems, although ³H-raclopride has high affinity for the D₂ and D₃ receptors and a much lower affinity for the D₄ receptor (Van Tol et al., 1991). ¹²⁵I-sulpiride was chosen to examine the response of extrastriatal (especially cortical) D₂-like receptors that are expressed at low levels to chronic antipsychotic treatment. D₃ receptors were quantified using ³H-7-OH-DPAT, the first selective radioligand that binds to the D₃ receptor with subnanomolar affinity compared to nanomolar affinities for D₂ and D₄ receptors in transfected cell lines (Levesque et al., 1992).

The D₁ receptor was quantified using ³H-SCH23390 according to the method of Dawson et al. (1986). This radioligand labels both the D₁ and D₃ receptors equally, so it is considered to be a D₁-like receptor ligand (Sunahara et al., 1991). Serotonin 5HT₂-receptor antagonists were quantified using ³H-ketanserin according to O’Dell et al. (1990).

Receptor Autoradiography

D₁-like receptor binding. Sections were preincubated for 1 hr in assay buffer (50 mM Tris-HCl, 120 mM NaCl, 5 mM KCl, 2 mM CaCl₂ and 1 mM MgCl₂; pH 7.4) at room temperature. Sections were then incubated for 1 hr at room temperature in assay buffer containing 1 nM ³H-SCH23390 (specific activity 73.2 Ci/mmol) and 10 nM ketanserin to block 5HT₂-like receptors. Nonspecific binding was determined in the presence of 1 μM flupenthixol. After incubation, slides were washed 2 × 5 min in ice-cold assay buffer, followed by a dip in ice-cold distilled water, then dried under a stream of cold dry air.

D₂-like receptor binding. ³H-nemonapride, ³H-spiperone and ³H-raclopride binding. Sections were preincubated for 1 hr at room temperature in assay buffer. Sections were then incubated in buffer containing either 1 nM ³H-nemonapride (specific activity 81.4 Ci/mmol), 1.2 nM ³H-spiperone (specific activity 116 Ci/mmol) in the presence of 40 nM ketanserin) or 5 nM ³H-raclopride (specific activity 86.5 Ci/mmol) for 1 hr at room temperature. Nonspecific binding was determined in the presence of 10 μM sulpiride (³H-nemonapride) or 1 μM flupenthixol (³H-spiperone and ³H-raclopride).

After each radioligand assay, slides were washed (2 × 5 min) in ice-cold buffer, followed by a quick dip in ice cold distilled water then dried under a stream of cold dry air.

¹²⁵I-sulpiride binding. Sections were preincubated for 1 hr at room temperature in 50 mM Tris-HCl buffer, pH 7.4. Sections were then incubated in Tris-HCl buffer containing 120 mM NaCl, 5 mM KCl, 1.5 mM CaCl₂, 4.5 mM MgCl₂, 0.01% ascorbic acid and 0.1 nM ¹²⁵I-sulpiride (specific activity 2000 Ci/mmol) for 1 hr at room temperature. Nonspecific binding was determined in the presence of 10
μM sulpiride. After incubation, slides were washed (3 × 4 sec) in Tris-HCl buffer then dried under a stream of cold dry air.

D<sub>4</sub>-like receptor binding. The optimal concentration of raclopride to completely block D<sub>2</sub>/D<sub>3</sub> receptors only was determined by competition experiments using unlabeled raclopride vs. 3H-nemonapride on striatal sections. Computer fitted curves showed that the raclopride curve is best fitted by assuming a two site model (P < .05, Florijn et al., 1994). A concentration of 300 nM raclopride completely displaced the high affinity binding site (D<sub>2</sub>/D<sub>3</sub> receptors), but did not block 15 to 22% of residual specific binding (which may represent D<sub>4</sub>-like receptors). Nonspecific binding was determined using different concentrations of butaclamol, sulpiride, flupenthixol and eticlopride.

Sections were preincubated for 1 hr at room temperature in assay buffer. Sections were then incubated in buffer containing 1 nM 3H-nemonapride in the presence of 300 nM raclopride for 1 hr at room temperature. Nonspecific binding was determined in the presence of 10 μM sulpiride. Slides were washed (2 × 5 min) in ice-cold buffer, followed by a quick dip in ice-cold distilled water then dried under a stream of cold dry air.

D<sub>3</sub> receptor binding. Sections were preincubated for 1 hr in 20 mM MOPS (3-[N-morpholino]propanesulfonic acid) buffer, containing 1 mM EDTA, 10 μM pargyline and 0.1% ascorbic acid; pH 7.2 at room temperature. Sections were then incubated in buffer containing 3 nM 3H-7-OH-DPAT (specific activity 116 Ci/mmol) for 1 hr at room temperature. Nonspecific binding was determined in the presence of 1 μM eticlopride. After incubation, slides were washed (2 × 3 min) in ice-cold buffer then dried under a stream of cold dry air.

Serotonin 5-HT<sub>2</sub>-like receptor binding. Sections were preincubated for 1 hr in assay buffer. Sections were then incubated in buffer containing 3.0 nM 3H-ketanserin in the presence of 1 μM prazosin (to block α<sub>1</sub>-adrenergic receptors) and 100 nM tetrahydrozine (to block a site associated with monoaminergic nerve terminals). Nonspecific binding was determined in the presence of 1 μM methysergide. After incubation, slides were washed (2 × 30 min) in ice-cold buffer, followed by a quick dip in ice-cold distilled water then dried under a stream of cold dry air.

 Autoradiography and image analysis. For the tritium radioligands, slides together with calibrated tritium standards (Amersham) were exposed to tritium sensitive films for 2 to 4 wk at 4°C. Films were then developed and fixed in D-19 Kodak (Eastman Kodak, NY). For the iodinated radioligand, standards of 10 mm<sup>3</sup> containing known concentrations of 125I-sulpiride were exposed along the slides to tritium sensitive films for 3 days (striatal sections), 7 days (nigral sections) or 30 days (cortical sections) at 4°C. OD of brain regions were measured using a computer-based densitometer, image analyzer (MCID-M1, Imaging Research Inc., Ontario, Canada). Brain regions of interest were outlined (fig. 1) and the OD of these regions were measured on two images representing total binding and two images representing nonspecific binding. The left and right sides of each region were measured separately and then averaged. The OD of the sampled regions were converted to nCi/mg using the calibrated standards. The values of nonspecific binding were subtracted from total binding to yield specific binding values, which were expressed in fmol/mg tissue (tritium-labeled ligands) or nCi/10 mm<sup>3</sup> (iodine-labeled ligand).

 Statistical analysis. An overall two-way analysis of variance using the four drinking-treated groups and different brain regions was first conducted. A significant two-way (P < .05) was followed by a one-way analysis of variance and post hoc Dunnett t test to identify statistically significant differences between the four different groups across brain regions.

Results

3H-Nemonapride and 3H-siperone binding sites were significantly elevated in CP (+53% and +41% respectively) and NA (+75% and +47%, respectively) of haloperidol-treated rats (Tables 1 and 2). 3H-nemonapride and 3H-siperone

Fig. 1. Diagramatic presentation of brain regions of interest used to quantify dopamine receptor subtypes.
binding sites were also increased, but at a smaller percentage, in CP (+32% and +19%, respectively) and NA (+34% and +20%, respectively) of chronic raclopride-treated rats. Table 2 shows an increase in specific 3H-spiperone binding sites in the SNpc and SNpr after chronic treatment with haloperidol (+72% and +69%, respectively) and raclopride (+38% and +48%, respectively). Another effect that was specific to 3H-spiperone, and not other radioligands, was the significant decrease of 3H-spiperone binding sites in the MPC by both spiperone, and not other radioligands, was the significant decrease (+50%) and (+31%) of haloperidol administration (+35%) and clozapine (+45%), whereas haloperidol, raclopride and clozapine treatment decreased 3H-spiperone binding in the DFC (−48%, −22% and −45%, respectively) (table 2). Surprisingly, an increase in 3H-raclopride binding sites was detected only in the CP (+25%) and NA (+26%) of haloperidol-treated rats (table 3).

The number of D2-like receptors (measured as the remaining 3H-nemonapride binding sites in the presence of 300 nM raclopride) were exclusively increased in CP and NA of all antipsychotic-treated rats (table 4). The increase was most profound after haloperidol administration (+78% and +82%, respectively), but was also significant after raclopride (+41% and +44%, respectively) and clozapine treatment (+33% and +35%, respectively).

Quantification of D2-like receptors using 125I-sulpiride revealed a significant increase in 125I-sulpiride binding in CP and substantia nigra of haloperidol- and raclopride-treated rats. In addition, 125I-sulpiride binding in MPC was significantly elevated by all three antipsychotics (table 5). 3H-ketanserin binding sites were significantly reduced in the cortex (dorsolateral: −55%, medial prefrontal cortex: −50%) of clozapine-treated rats only (table 6).

Chronic typical and atypical antipsychotics did not significantly change 3H-7-OH-DPAT binding to the D3 receptor (table 7) or 3H-SCH23390 binding to the D1-like receptors (table 8) in any brain region examined.

Discussion

Changes in serotonergic and catecholaminergic receptors have been detected in postmortem brain tissue from patient’s with schizophrenia (review: Seeman, 1992; Joyce, 1993), but because of previous drug treatment, it is unclear whether these changes are etiological in the disease or caused by the chronic exposure to antipsychotic drugs. Different ligands have also been used in positron emission tomographic studies to examine changes in dopamine D2-like receptors in the
brains of patients with schizophrenia in vivo (Wong et al., 1986; Farde et al., 1990). Increases in labeled spiperone and nemonapride, but not labeled raclopride, binding to the striata of patients diagnosed with schizophrenia have been reported in vivo and in vitro (Wong et al., 1986; Farde et al., 1990; Seeman et al., 1993; Murray et al., 1995; Sumiyoshi et al., 1995). We report an astonishing diversity in the apparent degree of dopamine receptor up- or down-regulation depend-
for cerebellar dopamine receptors (Janowsky et al., 1992), which have been reported to be exclusively of the D₂ receptor type (Sokoloff et al., 1990). Differences in receptor regulatory mechanisms, such as differences in G-protein coupling (Sokoloff et al., 1990) might be responsible for the lack in D₃ receptor up-regulation after functional blockade. Alternatively, endogenous dopamine has a high affinity for this receptor and may permanently occupy it, preventing the binding and subsequent receptor up-regulation by haloperidol and raclopride (Schotte et al., 1992). Thus, no conclusion can be drawn from these results concerning the role of the D₃ receptor in the treatment of schizophrenia with antipsychotic drugs. However, because all other dopamine receptor subtypes are subject to up-regulation after functional blockade, the role of D₃ receptors seems not to be important.

Dopamine D₂-like receptors were not, or only modestly, up-regulated in the CP of raclopride (+13%) or haloperidol (+28%) treated rats as measured by the D₂/D₃ subtype selective ligand ³H-raclopride. However, strong up-regulation of D₂-like receptors was detected using ³H-spiperone (+53%) and ³H-nemonapride (+41%), and to a lesser extent ¹²⁵I-sulpiride (+35%). This difference was not due to the presence of residual antipsychotics blocking ³H-raclopride binding sites because we found no significant change in the apparent affinity of ³H-raclopride between striatal sections of control vs. drug-treated rats (Scatchard analysis, data not shown), indicating the effectiveness of our preincubation step. Moreover, with ¹²⁵I-sulpiride [whose Kᵣ for the D₂ site is comparable to that of raclopride (Martres et al., 1985)], considerable receptor up-regulation is evident (table 5). Finally, our data are in agreement with a recent report (Schottes et al., 1995) where, in comparison with a significant increase in ³H-spiperone binding, a marginal change in the total number of binding sites was found for ³H-raclopride binding after a high dose regimen of haloperidol (5 mg i.p./day for 1 mo). Although their binding profiles to D₂ receptors expressed in cell lines, or from striatal homogenates, are in agreement with the classification of all of these radioligands as dopamine D₂ receptor antagonists (Zahniser and Dubocovic, 1983; Terai et al., 1989), a number of differences have been reported to exist between the binding characteristics of raclopride, nemonapride, sulpiride vs. spiperone, e.g., Na⁺ dependency,
differences in $B_{\text{max}}$ (Zahniser and Dubocovicv, 1983; Köhler and Radesater, 1986; Terai et al., 1989) that are probably due to the difference in molecular interaction with the extracellular amino acids forming the receptor pocket. Mutation analysis reveals that Asp 80, Asp 114 and His 394 are crucial for the binding of substituted benzamides, whereas only Asp 80 is necessary for $^3$H-spiiperone binding (Daniell and Strange, 1994; D'souza and Strange, 1995). A rather speculative explanation for our data could be that in the process of adaptation to receptor blockade by antipsychotics, subtle structural changes to the binding pocket of the receptor occur (for instance indirectly via receptor phosphorylation) differentially affecting the binding of the radioligands for the receptor, depending on the location of their binding sites at the receptor.

Alternatively, nemonapride and spiperone could be binding to an additional dopamine receptor subtype, not recognized by raclopride. Expression of the cloned $D_4$ receptor in cell lines revealed that raclopride differs from the former $D_2$-like receptor antagonists in its very low affinity for the $D_4$ receptor (Seeman and Van Tol, 1995). The number of $D_4$-like receptors in subcortical postmortem tissue from patients with schizophrenia, calculated by subtracting the number of binding sites defined with $^3$H-raclopride (a $D_2/D_3$ receptor antagonist) from the total binding defined with $^3$H-nemonapride (a nonselective $D_1/D_2/D_4$ antagonist), is greatly enhanced when compared to control values (Seeman et al., 1993; Murray et al., 1995; Sumiyoshi et al., 1995). Table 4 shows that in the presence of 300 nM raclopride, the optimal concentration to block $D_2/D_3$ receptors only (Florijn et al., 1994), the $D_4$-like receptor is up-regulated by the three antipsychotics, suggesting it may be a common site for antipsychotic drug action. Moreover, these data suggest that the reported up-regulation of $D_4$-like receptors in post mortem tissue from patients diagnosed with schizophrenia may be caused in part by antipsychotic treatment rather than being a component of the neuropathology of schizophrenia.

Because specific $D_4$ receptor drugs are not yet commercially available, it is not yet known if the additional up-regulated binding sites represent $D_4$ receptors. Caution is warranted, because these results imply a mismatch between $D_4$ receptor protein and mRNA levels: moderate levels of $D_4$-like receptors in contrast to negligible concentrations of $D_4$ receptor mRNA in the striatum (Schouts et al., 1995). However, higher levels of $D_4$ receptor mRNA have been detected in the cortex, thus the $D_4$-like receptors might be synthesized in the frontal cortex and subsequently be transported to presynaptic locations on corticostriatal terminals. However, haloperidol treatment did not significantly increase $D_4$ receptor mRNA in the cortex, whereas both striatal $D_4$ receptor protein and mRNA levels were increased 2-fold (Schouts et al., 1995), suggesting a regional-specific regulation of $D_4$-like receptors. To complicate matters, a recent study reported low levels of $D_4$ receptor mRNA in human striatum and cortex, and suggested that the subset of receptors defined by the receptor binding subtraction method is not $D_4$ receptors (Matsumoto et al., 1996), although another study localized $D_4$ receptors in GABAergic neurons of primate brains (Mrzljak et al., 1996). Improvements in the development of $D_4$ receptor protein assays, perhaps with specific radioligands or selective antibodies, will help to resolve these contrasting findings.

The mechanism by which raclopride strongly up-regulates $D_4$-like receptors but not, or only marginally, $D_2$ receptors is unclear since raclopride has low affinity for cloned $D_4$ receptors in expression systems (Seeman and Van Tol, 1995). Even at the dose of raclopride used in this study, which should result in a degree of $D_2$ receptor occupancy approximating that of haloperidol (Van Tol et al., 1991; Kakigi et al., 1995), a considerably lower percentage of receptor up-regulation was found with $^3$H-raclopride compared to that caused by haloperidol. This suggests that the effect of raclopride on $D_4$-like receptors may be via some indirect biochemical mechanism rather than by direct blockade of $D_4$-like receptors.

A number of studies have found that an increase in $D_2$-like receptor binding is not always accompanied by an elevated $D_2$ receptor mRNA level (Van Tol et al., 1990; Matsunaga et al., 1991; Creese et al., 1992). Instead, receptor degradation was decreased, presumably due to changes in posttranslational processing induced by persistent receptor blockade (Pich et al., 1988). It is tempting to speculate that raclopride’s affinity for the dopamine $D_2$ receptor is not sufficiently high to slow receptor degradation, resulting in functional blockade without apparent receptor up-regulation. Clozapine’s inability to up-regulate striatal $D_2$ receptors may be similarly explained by its lower affinity for this pool of receptors. However, this does not necessarily mean that dopamine receptor function is not affected. For instance, electrophysiological studies showed that clozapine at a similar dose reduced the number of spontaneously firing dopamine neurons in the ventral tegmental area (Chiodo and Bunney, 1983; White and Wang, 1983).

Differences between haloperidol, raclopride and clozapine can provide valuable information about their mechanism of action, for instance with reference to their liability to induce extrapyramidal side effects. Kakigi et al. (1995) investigated the occurrence of behavioral changes after chronic antipsychotic treatment with a similar drug dose and regimen as was used in our study. They reported that vacuous chewing movements (VCMs), thought to be analogous to human tardive dyskinesia (Gruner et al., 1986; Casey, 1991), are significantly elevated in chronic haloperidol- and raclopride-treated rats, but low after chronic clozapine treatment. At a clinically equivalent dose, raclopride (2 mg/kg) neither induces VCMs, nor up-regulates dopamine $D_2$ receptors or alters glucose metabolism (Ellison et al., 1987; Tarazi et al., 1993). Recently, Shirakawa and Tamminga (1994) compared the occurrence of VCM with dopamine D1-like receptor binding in the several brain areas in rats treated for 6 mo with haloperidol. Importantly, in rats with severe, but not mild VCMs, a significant decrease in $^3$H-SCH23390 binding was reported in the SNPr. In our study, no reduction in $^3$H-SCH23390 binding was observed in the SNPr or any other brain region examined in either haloperidol-, raclopride- or clozapine-treated rats (table 8). We did not, however, subdivide our drug-treated animals according to the frequency of VCM development, and this may have contributed to the discrepancy in $^3$H-SCH23390 binding in SNPr between the two studies.

Our data obtained from 8 mo treatment can be compared with those from 1-mo treatment (Tarazi et al., in press), due to identical drug treatment and receptor binding methodology. A remarkably comparable overall pattern of receptor regulation is found in 1- vs. 8 mo treatment. However, in the
NA, receptor up-regulation seems to develop more slowly: 3H raclopride binding is significantly elevated after haloperidol treatment and 3H-nenonapride binding is significantly increased after raclopride treatment only in the 8 mo-treated animals. It is striking that these subtle changes are found only in the NA, a brain region involved in mediating the clinical actions of antipsychotics.

Importantly, the D2 receptor subtype in the cortex of primates (Lidow and Goldman-Rakic, 1994) and rats (our data, Janowsky et al., 1992) appears to be a significantly up-regulated after haloperidol, raclopride or clozapine treatment. Thus, this response to antipsychotic treatment seems to occur across species. In contrast, down-regulation of the D1-like receptors in cortical areas by the three antipsychotics appears to be restricted to primates, because we failed to detect any changes in D1-like receptor binding (table 8). This discrepancy might result from the substantial differences between primate and rodent mesocortical laminar distribution and the occurrence of dopaminergic afferents and from differences in dopamine-neuropeptide co-localization (Berger et al., 1991). Also, striking differences in intrinsic activity and efficacy of several dopamine D1-like drugs between primates vs. rodents have been reported (Pifl et al., 1991) presumably due to molecular differences at the (post)receptor level (Vermeulen et al., 1994). Several behavioral and biochemical findings in animals have suggested that D1-like receptor antagonists may display antipsychotic activity (Altar et al., 1988; Coffin et al., 1989; Ellenhorn et al., 1991; Glenthoj et al., 1993). However, recent clinical trials using another D1-like receptor antagonist, SCH 39166, failed to show that D1-like receptor antagonists have significant antipsychotic actions (Debeaufreire et al., 1995; Karlsson et al., 1995). These clinical data, together with our results and other biochemical and electrophysiological effects (Hietala et al., 1990) argue against the involvement of D1-like receptors in mediating the clinical effects of antipsychotic drugs.

3H-Spiperone binding sites were down-regulated by the three antipsychotics in dorsolateral and medial prefrontal cortical areas. The nature of these binding sites is not clear, but serotonergic 5HT1-like receptors were ruled out because 3H-ketanserin binding was decreased only after chronic clozapine treatment (table 6, O’Dell et al., 1990). 3H-Spiperone apparently labels an additional, uncharacterized site. One study reported a similar reduction in cortical spiperone binding in schizophrenia (Arora and Meltzer, 1991). Thus, this site may be present in humans and may have been down-regulated after long-term antipsychotic therapy. Most likely, the increase in 3H-spiperone and 125I-sulpiride binding sites in the substantia nigra of haloperidol and raclopride-treated rats represents an increase in the dopamine D2 receptor subtype. Up-regulation of the D2 receptor is unlikely, since 3H-7-OH-DPAT binding in the substantia nigra was not affected.

In summary, careful analysis of various brain regions using different dopamine receptor radioligands revealed a perplexing heterogeneity in dopamine receptor response to chronic antipsychotic drug treatment. Our findings suggest that D1-like and D2 receptor subtypes are less likely to be involved in mediating the effects of antipsychotic drugs. A major aim of this study was to differentiate between structures involved in the antipsychotic action of typical and atypical antipsychotics vs. those involved in the side effects of typical antipsychotics. D2-like receptor radioligand binding in the presence of raclopride revealed the existence of an additional, formerly unnoticed, subset of dopamine D2-like receptors in striatum and nucleus accumbens. These D2-like receptors are strongly up-regulated after both typical and atypical antipsychotic treatment, suggesting them as possible common mediators of antipsychotic drug effects. We detected a common site of drug action for all three antipsychotics, D2 receptors in the MPC, presumably another important target for their antipsychotic effects. Similarly, all three antagonists decreased 3H-spiperone binding in the dorsolateral frontal cortex, the significance of which is not clear as this binding site has not been characterized. Finally, we detected a structure affected only by typical antipsychotics (D2 receptor subtype binding in the striatum) that possibly plays an instrumental role in the induction of tardive dyskinesia and other neuroleptic side effects.

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References


