ABSTRACT

Changes in members of the dopamine (DA) D1-like (D1, D5) and D2-like (D2, D3, D4) receptor families in rat forebrain regions were compared by quantitative in vitro receptor autoradiography after prolonged treatment (28 days) with the atypical antipsychotics olanzapine, risperidone, and quetiapine. Olanzapine and risperidone, but not quetiapine, significantly increased D2 binding in medial prefrontal cortex (MPC; 67% and 34%), caudate-putamen (CPu; average 42%, 25%), nucleus accumbens (NAc; 37%, 28%), and hippocampus (HIP; 53%, 30%). Olanzapine and risperidone, but not quetiapine, produced even greater up-regulation of D4 receptors in CPu (61%, 37%), NAc (65%, 32%), and HIP (61%, 37%). D1-like and D4 receptors in all regions were unaltered by any treatment, suggesting their minimal role in mediating actions of these antipsychotics. The findings support the hypothesis that antipsychotic effects of olanzapine and risperidone are partly mediated by D2 receptors in MPC, NAc, or HIP, and perhaps D3 receptors in CPu, NAc, or HIP, but not in cerebral cortex. Selective up-regulation of D2 receptors by olanzapine and risperidone in CPu may reflect their ability to induce some extrapyramidal effects. Inability of quetiapine to alter DA receptors suggests that nondopaminergic mechanisms contribute to its antipsychotic effects.

Dopamine (DA) exerts its effects in mammalian brain by interacting with several DA receptor types that include D1-like (D1, D5) and D2-like (D2, D3, D4) receptor families (Baldessarini and Tarazi, 1996). DA receptors, and particularly the D2-like family, have been implicated in the pathophysiology of psychotic disorders and, more directly, in the pharmacodynamic basis of beneficial effects of antipsychotic drugs (Baldessarini and Tarazi, 2001). They are also implicated in centrally mediated adverse effects typical of most neuroleptic-antipsychotics, including extrapyramidal neuroleptic reactions (particularly, parkinsonism and tardive dyskinesia) and hyperprolactinemia (Baldessarini and Tarsy, 1980; Baldessarini and Tarazi, 2001).

The introduction of the prototype “atypical” antipsychotic agent clozapine was a major step in developing drugs with less risk of the adverse neurological effects that are typical of standard antipsychotics but with similar or even superior beneficial effects. Clozapine is usually well tolerated, with very limited adverse extrapyramidal signs (EPS) or hyperprolactinemia and substantial evidence of superior antipsychotic effectiveness (Baldessarini and Frankenburg, 1991; Brunello et al., 1995). The pharmacological basis of the unusual clinical properties of this unique agent remains unclear. Clozapine interacts with high or moderate potency at serotonergic (5-HT2A, 5-HT2C, and others), acetylcholinergic (muscarinic), adrenergic (α1, α2, β2), histaminic (H1), and other neurohorm receptors. In contrast, it has only moderate affinity for both D1 and D2 DA receptors (Baldessarini and Frankenburg, 1991; Brunello et al., 1995; Baldessarini and Tarazi, 1996).

Clozapine also displays somewhat greater affinity for D4 than other DA receptors (Van Tol et al., 1991), suggesting that these receptors may represent potential sites of action of clozapine and perhaps other antipsychotic agents. D4 receptors also may be increased in postmortem brain tissue of schizophrenic patients (Seeman et al., 1993; Murray et al., 1995), although these findings remain inconsistent (Lahti et al., 1996). In addition, repeated administration of clozapine,
as well as other typical and atypical antipsychotics, increased D4 receptor abundance in rat caudate-putamen (CPu) and nucleus accumbens septi (NAC) (Schoots et al., 1995; Florijn et al., 1997; Tarazi et al., 1997a,c, 1998) and enhanced D4 mRNA expression in monkey striatum (Lidow and Goldman-Rakic, 1997). These agents also up-regulated D2 receptors in rat and monkey prefrontal cortex (Lidow and Goldman-Rakic, 1994; Florijn et al., 1997; Tarazi et al., 1997c, 1998). The findings support the view that D4 receptors in CPu and NAc, kic, 1994; Florijn et al., 1997; Tarazi et al., 1997c, 1998). The rat and monkey prefrontal cortex (Lidow and Goldman-Rakic, 1994). The selective increase in D2 receptor labeling in the extrapyramidal CPu probably reflects higher average occupancy of such receptor sites by typical neuroleptic agents and appears to parallel the risk of acute EPS as well as later-emerging tardive dyskinesia.

Despite its favorable characteristics, clinical use of clozapine is complicated by its high risk of potentially fatal bone marrow toxicity, as well as excessive sedation and dose-dependent risk of epileptic seizures (Baldessarini and Frankenburg, 1991). There is a keen interest in developing novel drugs with less adverse risk than clozapine but comparable antipsychotic effects. Several newer agents have emerged (Arnt and Skarsfeldt, 1998; Waddington and Casey, 2000; Baldessarini and Tarazi, 2001). Among them are the clozapine analogs olanzapine and quetiapine and the benzisoxazol derivative risperidone. Like clozapine, these compounds have multiple sites of molecular interaction and greater affinity for serotonin 5-HT2A than DA D2 receptors (Bymaster et al., 1996; Schotte et al., 1996; Gunasekara and Spencer, 1998). This receptor-interaction pattern may contribute to low EPS risk (Meltzer et al., 1989).

Olanzapine, quetiapine, and risperidone have undergone extensive pharmacological and behavioral characterization in animals (Arnt and Skarsfeldt, 1998; Tarazi et al., 2000; Waddington and Casey, 2000), but their long-term effects on DA receptors in mammalian forebrain are not well defined or quantitatively compared with those of other antipsychotics. Accordingly, we applied quantitative in vitro receptor autoradiography to assess regulation of D1-like, D2, D3, and D4 receptors in different forebrain regions following long-term treatment, slides were washed twice for 5 min in ice-cold buffer, dipped in air-dried (Florijn et al., 1997; Tarazi et al., 1997a,c, 1998).

Risperidone was determined with excess (120), KCl (5), CaCl2 (2), and MgCl2 (1), for the D1-like, D2, and D4 receptors, in different forebrain regions following long-term infusion of olanzapine, quetiapine, or risperidone in rats. Our working hypothesis was that these novel atypical antipsychotics would induce regionally selective changes in tissue levels of specific DA receptors more closely resembling those of clozapine than typical neuroleptics.

Experimental Procedures

Materials and Animal Subjects

Radioligands were from NEN Life Science Products, Inc. (Boston, MA): R,S,N[3H]-N-methyl-[3H]monamapride (86 Ci/mmol), S(-)[3H]meclopride (74 Ci/mmol), and R(-)[3H]H[2-3H]-chloro-8-hydroxy-2-phenyl-3,4,5,6-tetrahydro-1H-3-benzazepine (SCH-23390; 81 Ci/mmol); or from Amersham (Arlington Heights, IL): R(-)[3H]-4-(2,3-dihydroxy-N,N-di-n-propyl-2-amino-1,2,3,4-tetrahydrodronaphthalene (7-OH-DPAT; 116 Ci/mmol). Tritium-sensitive Hyperfilms were from Amersham. D-19 photographic developer and fixative were from Eastman Kodak (Rochester, NY).

Donated drugs included olanzapine (Eli Lilly, Indianapolis, IN), risperidone (Janssen, Beerse, Belgium), and quetiapine fumarate (Zeneca, Cheshire, UK). 1,3-Ditolyguanidine (DTG), S(-)-etiochlorine hydrochloride, cis-flupenthixol dihydrochloride, fluphenezine dihydrochloride, ketanserin tartrate, pindolol, and S(-)-spironolactone were purchased from RBI-Sigma (Natick, MA). Cation hydrochlorides, guanosine-5-triphosphate sodium (GTP), and tris-(hydroxy-methyl)-aminomethane (Tris) hydrochloride were from Sigma (St. Louis, MO).

Animals were male Sprague-Dawley rats (Charles River Laboratories, Wilmington, MA), initially weighing 200 to 225 g, maintained under artificial daylight (on, 7:00 AM–7:00 PM) in a temperature- and humidity-controlled environment with free access to standard rat chow and tap water in a USDA-inspected, veterinarian-supervised, small-animal research facility of the Mailman Research Center of McLean Hospital. Animal procedures were approved by the Institutional Animal Care and Use Committee (IACUC) of McLean Hospital, in compliance with pertinent federal and state regulations.

Drug Treatment and Tissue Preparation

Four groups (N = 7) of rats received control vehicle, olanzapine (5.0 mg/kg/day), risperidone (3.0 mg/kg/day), or quetiapine fumarate (10.0 mg/kg/day) by osmotic minipumps (Alzet, Palo Alto, CA) implanted subcutaneously to ensure continuous and steady infusion of drugs and overcome variations in tissue drug levels that would result from daily injections. Doses are based on typical behaviorally active doses in rats (Moore et al., 1992; Ellenbroek et al., 1996). After 4 weeks of treatment, rats were decapitated; brains were removed, quick-frozen in isopentane on dry ice, and stored at −80°C until autoradiographic analysis. Frozen coronal sections (10 µm) were cut in a cryostat at −20°C, mounted on gelatin-coated microscopic slides, and stored at −80°C until use. Tissue sections were obtained from CPu, NAC, hippocampus (HIP); areas of cerebral cortex including dorsolateral-frontal (DFC) and mesiofrontal (MFC), and entorhinal (EC) regions; islands of Calleja including the major island, and the olfactory tubercle. These selected cortical, limbic, and extrapyramidal brain regions mediate cognitive, emotional, and motor behaviors that are typically disturbed in patients with psychiatric disorders and altered by antipsyhcotic drug treatment (Benes, 2000; Baldessarini and Tarazi, 2001).

Receptor Autoradiography

Brain sections from all drug-treated rats were evaluated at the same time in each radioreceptor assay to minimize experimental variability. Sections were first preincubated for 1 h at room temperature (RT) in 50 mM Tris-Cl buffer (pH 7.4) containing (mM): NaCl (120), KCl (5), CaCl2 (2), and MgCl2 (1), for the D1-like, D2, and D4 receptors, or with slight modification for D3 assays (with 0.3 mM GTP, 40 mM NaCl, and no MgCl2 added).

D1-Like Receptors. Rat forebrain sections were incubated for 1 h at RT in the incubating buffer containing 1 nM [3H]SCH-23390 with 100 nM ketanserin to block 5-HT1A receptors. Nonspecific binding was determined with excess (1 µM) cis-flupenthixol. After incubation, slides were washed twice for 5 min in ice-cold buffer, dipped in ice-cold water, and dried under a stream of air (Florijn et al., 1997; Tarazi et al., 1997a, 1998).

D2 Receptors. Sections were incubated for 1 h at RT in the same buffer containing 1 nM [3H]monapride with 0.5 µM DTG and 0.1 µM pindolol to mask sigma (σ1,2) and 5-HT1A sites, respectively. Nonspecific binding was determined with 10 µM S(-)-spiroperidol. After incubation, slides were washed twice for 5 min in ice-cold buffer, dipped in ice-cold water, and air-dried (Tarazi et al., 1997b,c, 1998). Although the resulting radioligand binding may include traces of binding to D2 or D3 sites, most of the signal is believed to represent D2 receptors.
D₂ Receptors. Sections were preincubated for 1 h in Tris buffer modified as stated to minimize labeling of the high-affinity agonist binding state of D₂ receptors, then incubated for 1 h in the same buffer containing 3 nM [³H]7-OH-DPAT, with 5 μM DTG to mask sigma sites. Nonspecific binding was determined with 1 μM S(−)-eticlopride. After incubation, slides were washed twice for 3 min in ice-cold fresh buffer and dried (Tarazi et al., 1997c, 1998).

D₁ Receptors. Tissue sections were preincubated for 1 h at RT in the D₂ assay buffer, and then for 1 h with 1.0 nM [³H]nemonapride, 300 nM S(−)-raclopride to occupy D₂/D₃ sites, and other masking agents (0.5 μM DTG and 0.1 μM pindolol) used in the D₂ assay. Nonspecific binding was determined with 10 μM S(−)-sulpiride. The highly D₄-selective ligands L-745,870 and RBI-257 displaced approximately 80% of binding remaining in the presence of raclopride in adult CPu and NAc tissue, indicating that most of the raclopride-insensitive binding sites are D₄ receptors (Tarazi et al., 1997b,c, 1998).

Autoradiography and Image Analysis
Radiolabeled slides and calibrated ³H-standards (Amersham) were exposed to Hyperfilm (Eastman Kodak) for 2 to 5 weeks at 4°C. [³H]SCH-23390- and [³H]nemonapride-labeled brain sections were exposed for 2 (CPu and NAc) or 5 weeks (cortex and HIP) and [³H]7-OH-DPAT for 4 weeks. Films were developed in Kodak D-19 developer and fixative. Optical density (O.D.) in brain regions of interest was measured with a computerized densitometric image analyzer (MCID-M4, Imaging Research, St. Catharines, ON, Canada). Brain regions of interest were outlined (Fig. 1), and their O.D. was measured. Left and right sides of two contiguous sections represented total binding, and two other sections represented nonspecific binding; the four determinations were averaged for each subject (N = 7 rats/treatment). O.D. was converted to nanocuries per milligram of tissue with calibrated ³H-standards, and after subtracting nonspecific from total binding, specific binding was expressed as femtomoles per milligram of tissue.

Statistical Analysis
Two-way analysis of variance (ANOVA) was employed to evaluate overall changes across treatments and brain regions for each assay.

Results
Four weeks of continuous infusion of all test agents failed to alter tissue concentrations of D₁-like receptors in any brain region (Table 1). In contrast, olanzapine and risperidone significantly increased labeling of D₂ receptors in several forebrain areas, including MPC (by 67 and 34%, respectively), NAc (37 and 28%), CPu (by a lateral and medial average of 42 and 25%), and HIP (53 and 30%), but did not alter D₂ binding in DFC or EC (Table 2). Similar to D₁-like receptors, there were no changes in D₃-selective labeling in any brain region analyzed or with any agent (Table 3). Even more than signals representing mainly D₂ receptors (uncorrected for overlap with D₃ or D₄ receptors), D₄ labeling was up-regulated in several regions by treatment with olanzapine and risperidone, including NAc (by 65 and 33%, respectively), CPu (average of 61 and 37%), and HIP (61 and 37%), with no significant changes in several regions of cerebral cortex (Table 4). It is particularly noteworthy that quetiapine was anomalous and the only tested antipsychotic that did not alter expression of any DA receptor type in any brain region examined.

Discussion
Long-Term Effects of Newer Antipsychotics on the D₁ Receptor Family
Continuous subcutaneous infusion of olanzapine, quetiapine, or risperidone did not alter binding of [³H]SCH-23390 in...
TABLE 1

<table>
<thead>
<tr>
<th>Brain Region</th>
<th>Controls</th>
<th>Olanzapine</th>
<th>Risperidone</th>
<th>Quetiapine</th>
<th>Clozapine</th>
<th>Fluphenazine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cerebral cortex</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Medial-prefrontal</td>
<td>14.0 ± 0.5 (100)</td>
<td>13.3 ± 0.6 (95)</td>
<td>11.9 ± 1.5 (85)</td>
<td>15.5 ± 1.0 (107)</td>
<td>(93)</td>
<td>(102)</td>
</tr>
<tr>
<td>Dorsolateral</td>
<td>7.5 ± 0.9 (100)</td>
<td>6.2 ± 0.8 (82)</td>
<td>9.0 ± 0.8 (120)</td>
<td>8.2 ± 0.8 (109)</td>
<td>(100)</td>
<td>(96)</td>
</tr>
<tr>
<td>Nucleus accumbens</td>
<td>85.7 ± 4.1 (100)</td>
<td>86.0 ± 4.3 (100)</td>
<td>84.5 ± 7.3 (103)</td>
<td>81.1 ± 6.0 (95)</td>
<td>(92)</td>
<td>(96)</td>
</tr>
<tr>
<td>Caudate-putamen</td>
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<tr>
<td>Medial</td>
<td>87.6 ± 7.1 (100)</td>
<td>90.0 ± 10.3 (103)</td>
<td>78.0 ± 4.6 (99)</td>
<td>74.8 ± 7.2 (85)</td>
<td>(99)</td>
<td>(101)</td>
</tr>
<tr>
<td>Lateral</td>
<td>90.9 ± 7.9 (100)</td>
<td>98.8 ± 11.6 (109)</td>
<td>78.4 ± 4.3 (103)</td>
<td>83.3 ± 6.3 (92)</td>
<td>(91)</td>
<td>(104)</td>
</tr>
<tr>
<td>Hippocampus</td>
<td>7.5 ± 1.0 (100)</td>
<td>8.6 ± 0.5 (115)</td>
<td>8.3 ± 0.7 (111)</td>
<td>8.9 ± 0.6 (119)</td>
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</tr>
<tr>
<td>Entorhinal cortex</td>
<td>10.8 ± 0.7 (100)</td>
<td>11.3 ± 1.1 (105)</td>
<td>12.5 ± 0.6 (116)</td>
<td>12.6 ± 0.4 (117)</td>
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</tbody>
</table>

* Data (percentage of control) for clozapine (40 mg/kg/day) and fluphenazine (1 mg/kg/day) were determined previously (Tarazi et al., 1997c) and are shown for comparison.

TABLE 2

<table>
<thead>
<tr>
<th>Brain Region</th>
<th>Controls</th>
<th>Olanzapine</th>
<th>Risperidone</th>
<th>Quetiapine</th>
<th>Clozapine</th>
<th>Fluphenazine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cerebral cortex</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Medial-prefrontal</td>
<td>18.4 ± 0.6 (100)</td>
<td>30.8 ± 1.0 (167)*</td>
<td>24.2 ± 1.3 (134)*</td>
<td>20.1 ± 0.5 (109)</td>
<td>(160)*</td>
<td>(146)*</td>
</tr>
<tr>
<td>Dorsolateral</td>
<td>13.0 ± 0.7 (100)</td>
<td>14.9 ± 0.5 (107)</td>
<td>24.6 ± 0.4 (91)</td>
<td>14.7 ± 0.5 (106)</td>
<td>(111)</td>
<td>(92)</td>
</tr>
<tr>
<td>Nucleus accumbens</td>
<td>153.3 ± 7.2 (100)</td>
<td>210.4 ± 14.4 (137)*</td>
<td>196.8 ± 9.8 (128)*</td>
<td>164.4 ± 6.3 (107)</td>
<td>(103)</td>
<td>(167)*</td>
</tr>
<tr>
<td>Caudate-putamen</td>
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<td></td>
</tr>
<tr>
<td>Medial</td>
<td>169.9 ± 8.5 (100)</td>
<td>244.9 ± 21.7 (144)*</td>
<td>215.9 ± 12.6 (127)*</td>
<td>168.1 ± 11.1 (99)</td>
<td>(104)</td>
<td>(126)*</td>
</tr>
<tr>
<td>Lateral</td>
<td>224.5 ± 12.1 (100)</td>
<td>311.4 ± 16.3 (139)*</td>
<td>276.2 ± 18.0 (123)*</td>
<td>231.4 ± 17.4 (103)</td>
<td>(109)</td>
<td>(117)*</td>
</tr>
<tr>
<td>Hippocampus</td>
<td>35.3 ± 1.0 (100)</td>
<td>54.4 ± 3.5 (153)*</td>
<td>46.0 ± 2.1 (130)*</td>
<td>37.5 ± 2.6 (106)</td>
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<td></td>
</tr>
<tr>
<td>Entorhinal cortex</td>
<td>15.1 ± 1.0 (100)</td>
<td>16.4 ± 1.1 (108)</td>
<td>15.4 ± 1.2 (102)</td>
<td>16.9 ± 0.4 (112)</td>
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</tr>
</tbody>
</table>

* Data (percentage of control) for clozapine (40 mg/kg/day) and fluphenazine (1 mg/kg/day) were determined previously (Tarazi et al., 1997c) and are shown for comparison.

TABLE 3

<table>
<thead>
<tr>
<th>Brain Region</th>
<th>Controls</th>
<th>Olanzapine</th>
<th>Risperidone</th>
<th>Quetiapine</th>
<th>Clozapine</th>
<th>Fluphenazine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Islands of Calleja</td>
<td>36.9 ± 1.9 (100)</td>
<td>32.8 ± 1.9 (89)</td>
<td>34.5 ± 1.7 (93)</td>
<td>36.5 ± 1.8 (99)</td>
<td>(97)</td>
<td>(100)</td>
</tr>
<tr>
<td>Olfactory tubercle</td>
<td>18.6 ± 0.7 (100)</td>
<td>17.9 ± 1.7 (96)</td>
<td>20.0 ± 1.3 (108)</td>
<td>18.8 ± 1.0 (101)</td>
<td>(102)</td>
<td>(102)</td>
</tr>
<tr>
<td>Nucleus accumbens</td>
<td>17.9 ± 0.7 (100)</td>
<td>18.7 ± 1.3 (104)</td>
<td>17.4 ± 0.6 (97)</td>
<td>19.7 ± 0.8 (110)</td>
<td>(93)</td>
<td>(108)</td>
</tr>
<tr>
<td>Shell</td>
<td>7.9 ± 0.5 (100)</td>
<td>8.5 ± 0.4 (108)</td>
<td>9.0 ± 0.6 (114)</td>
<td>8.8 ± 0.3 (112)</td>
<td>(97)</td>
<td>(119)</td>
</tr>
<tr>
<td>Caudate-putamen</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Medial</td>
<td>3.4 ± 0.2 (100)</td>
<td>3.3 ± 0.2 (97)</td>
<td>3.3 ± 0.1 (97)</td>
<td>3.4 ± 0.2 (100)</td>
<td>(100)</td>
<td>(118)</td>
</tr>
<tr>
<td>Lateral</td>
<td>3.7 ± 0.3 (100)</td>
<td>3.2 ± 0.2 (86)</td>
<td>3.3 ± 0.1 (89)</td>
<td>3.6 ± 0.1 (97)</td>
<td>(100)</td>
<td>(118)</td>
</tr>
</tbody>
</table>

* Data (percentage of control) for clozapine (40 mg/kg/day) and fluphenazine (1 mg/kg/day) were determined previously (Tarazi et al., 1997c) and are shown for comparison.

any region of rat forebrain considered (Table 1). This radioligand binds both highly abundant D_1 receptors and rarer D_5 sites (Baldessarini and Tarazi, 1996). Lack of adaptive changes in D_1-like binding with the three newer antipsychotic agents tested agrees with similar previous findings following treatment with dissimilar agents including haloperidol, fluphenazine, raclopride, and clozapine (Florijn et al., 1997; Tarazi et al., 1997a, 1998). However, clinical trials have yielded no support for the hypothesis that D_1 antagonists may exert antipsychotic activity (Debeaurepaire et al., 1995). Lack of adaptive changes in D_1-like receptor levels after prolonged administration of olanzapine, risperidone, or quetiapine (Table 1) as well as clozapine and typical neuroleptics (Florijn et al., 1997; Tarazi et al., 1997a, 1998) adds to the impression that these receptors are not likely to play a major role in mediating effects of antipsychotic agents. Nevertheless, open-minded caution is required concerning the pharmacological potential of D_1 antagonists since current understanding of the physiology of these most abundant of the cerebral DA receptors remains remarkably limited (Baldessarini and Tarazi, 1996).

Long-Term Effects of Olanzapine, Risperidone, and Quetiapine on the D_2 Receptor Family

D_2 Receptors. D_2 receptor potency of tested agents ranks risperidone (K_i = 6.0 nM) > olanzapine (K_i = 31 nM) > quetiapine (K_i = 380 nM) (Schotte et al., 1996). Infusion of olanzapine and risperidone, but not quetiapine, increased binding of [³H]nemonapride in MPC (Table 2). Such increases mainly reflect up-regulation of D_2 receptors since
cortical D2 receptors did not increase significantly (Table 4), and D3 receptors are minimally expressed in rat frontal cortex (Sokoloff et al., 1990; Baldessarini and Tarazi, 1996). Similar D2 receptor up-regulation and increased D2 mRNA expression have been found in cerebral cortex of rats and nonhuman primates after repeated treatment with typical and atypical antipsychotics (Damask et al., 1996; Florijn et al., 1997; Lidow and Goldman-Rakic, 1997; Tarazi et al., 1997a,c, 1998). D2 receptor up-regulation in MPC by olanzapine and risperidone further supports the importance of D2 receptors in this region as a common target for both typical and atypical antipsychotics.

Prolonged treatment with olanzapine and risperidone also enhanced binding to D2 receptors in HIP but neither in DFC nor EC (Table 2). Such enhancement of D2 expression in HIP probably reflects an increase in the transcriptional activity of the D2 receptor gene since repeated treatment with clozapine increased D2 mRNA expression in HIP but not EC (Ritter and Meadow-Woodruff, 1997). D2 receptors in HIP, where DA innervation is limited, evidently are regulated differently from those in EC, where DA innervation is much denser (Goldsmith and Joyce, 1994). D2 receptors in HIP, but not EC, may act as additional common targets of olanzapine, risperidone, and perhaps other antipsychotics. Blockade and up-regulation of hippocampal D2 receptors by antipsychotics may contribute to improvement of delusions and hallucinations of psychotic patients by ameliorating DA hyperactivity postulated to occur in their HIP (Kriechhaus et al., 1992).

Repeated treatment with olanzapine and risperidone, but not quetiapine, also increased D2 receptor labeling in CPu (Table 2). Similar responses have been found after long-term treatment with typical neuroleptics but not clozapine (Florijn et al., 1997; Tarazi et al., 1997a,c, 1998). Up-regulation of D2 receptors in CPu may disturb neurotransmission in circuits involved in regulating movement and posture (Albin et al., 1989) and generally correlates with EPS risk. Similar responses to newer antipsychotics (olanzapine, risperidone) with relatively low EPS risk were, therefore, unexpected.

Additional indications that these drugs can occupy D2 receptors in human basal ganglia come from positron emission tomography studies showing that relatively high, but clinically encountered, doses of olanzapine and risperidone lead to occupation of striatal D2 receptors similar to that produced by typical neuroleptics and much more than that produced by clozapine (Farde et al., 1989; Kapur et al., 1999) or quetiapine (Gefvert et al., 1998). Quetiapine has the most benign clinical EPS risk of the three novel agents tested, ranking well below risperidone and probably also olanzapine (Gunasekara and Spencer, 1998; Leucht et al., 1999; Baldessarini and Tarazi, 2001; Tarsy et al., 2001). The lack of clinical EPS with quetiapine is paralleled by its low D2 affinity (Schotte et al., 1996) and weak antagonism of behavioral effects of DA microinjected directly into rat CPus (Campbell et al., 1991). Risperidone shares many characteristics of typical neuroleptics, including severe hyperprolactinemia and dose-dependent EPS risk (Fleischhacker and Marder, 2000; Baldessarini and Tarazi, 2001; Tarsy et al., 2001). In addition to its ability to occupy striatal D2 receptors, olanzapine has relatively potent antimuscarinic properties compared with risperidone or quetiapine (Bymaster et al., 1996; Schotte et al., 1996) that probably limits its risk of EPS effects.

Other studies using low daily doses of olanzapine (0.35–2 mg/kg) or risperidone (0.25–0.5 mg/kg) did not find D2 receptor up-regulation in rat or monkey striatum (Kuoppamaki et al., 1995; Lidow and Goldman-Rakic, 1997; Kusumi et al., 2000). In addition, long-term treatment with low doses of olanzapine (0.5–2 mg/kg) resulted in lesser oral chewing movements (a proposed animal model of tardive dyskinesia) than did haloperidol (Gao et al., 1998). Nevertheless, the present findings with higher doses of constantly infused olanzapine and risperidone did indicate significant apparent up-regulation of D2 receptors. Moreover, the present and previous results are consistent with clinical evidence that risks of acute EPS and of tardive dyskinesia with risperidone and olanzapine are substantially greater than with either quetiapine or clozapine (Tarsy et al., 2001).

D4 Receptors. These low-abundance proteins have a restricted distribution, with notable levels of expression in mammalian basal forebrain, most prominently in the islands of Calleja, followed by olfactory tubercle and NAc shell, with very low levels in CPu (Sokoloff et al., 1990; Levant, 1997; Table 3). Binding of [3H]7-OH-DPAT under D4-selective conditions was unchanged after prolonged exposure to all test agents in all regions examined. Even risperidone, which has relatively high D2 affinity (Schotte et al., 1996), failed to alter expression of D4 receptors, even in the islands of Calleja and NAc (Table 3), as was found with various other antipsychotics (Levesque et al., 1995; Florijn et al., 1997; Tarazi et al., 1997a,c, 1998).

Lack of response of D4 receptors to repeated antipsychotic treatment may reflect their unique molecular mechanisms, including absence of well defined interactions with G-proteins and signal transduction cascades (Sokoloff et al., 1990;...
Levant, 1997). Alternatively, the high avidity of D₃ receptors for DA and their selective protection from alkylolation by very low concentrations of DA (Zhang et al., 1999) suggests that occupation by endogenous DA may limit their interaction with potential antagonists to preclude up-regulation. It also follows that D₃ receptors are less likely to be critical for the actions of known or novel antipsychotics. Instead, D₄ receptors may be involved in stimulant reward and dependence (Pilla et al., 1999).

**D₄ Receptors.** As in previous studies (Tarazi et al., 1997b,c, 1998), D₄ receptors accounted for relatively high proportions (46–54%) of total D₂-like receptors in MPC, DFC, HIP, and EC, but much less (17–19%) in CPu and NAc (Tables 2 and 4). Prolonged administration of olanzapine and risperidone, but not quetiapine, significantly increased D₄ receptors in CPu and NAc (Table 4), presumably reflecting adaptive responses to D₄ blockade since both olanzapine and risperidone have much higher D₄ affinity than quetiapine (Schotte et al., 1996). D₄ receptors were found to increase in rat CPu and NAc after long-term administration of several typical and atypical antipsychotics (Florijn et al., 1997; Tarazi et al., 1997a,c, 1998), as well as olanzapine and risperidone (Table 4), supporting the impression that striatal limbic D₄ receptors represent a common target of dissimilar antipsychotics. Lack of D₄ up-regulation by quetiapine may, again, reflect its low affinity for all D₂-like receptors (Schotte et al., 1996; Baldessarini and Tarazi, 2001). D₄ up-regulation is also consistent with the proposal that reported increases in D₄like labeling in postmortem striatum tissue of patients diagnosed with schizophrenia (Seeman et al., 1993; Murray et al., 1995) may reflect responses to antemortem antipsychotic treatment rather than a neuropathology of schizophrenia (Baldessarini and Tarazi, 2001).

Long-term treatment with olanzapine and risperidone increased D₄ receptor labeling in HIP and not EC (Table 4), similar to responses of D₂ receptors (Table 2). This is the first evidence that D₄ receptors in HIP can be affected by novel antipsychotics. Moreover, these receptors may act in synchrony with D₂ receptors in HIP to mediate beneficial clinical actions of antipsychotics. Increased D₄ binding probably reflects post-transcriptional modifications or reduced D₄ receptor turnover since D₄ mRNA expression was decreased after repeated treatment of rats with haloperidol or clozapine (Ritter and Meadow-Woodruff, 1997). Lack of response of D₄ receptors in EC to antipsychotic treatment (Table 4) parallels the nonresponse of D₂ receptors in that relatively well DA- innervated region (Table 2). In spite of their profound up-regulating effects on D₄ receptor proteins in CPu and NAc, in frontal cerebral cortex, olanzapine and risperidone had only minor effects, and quetiapine had none (Table 4). Other antipsychotics also have had little effect on rat cortical D₄ receptors (Tarazi et al., 1997a,c). Regional differences in the regulation of D₄ mRNA transcription or protein synthesis may account for regional differences in adaptive increases of D₄ receptors and in responses to typical versus atypical antipsychotics. Consistent with this interpretation is a lack of increase in D₄ mRNA or receptor protein in rat cerebral cortex after repeated treatment with clozapine but significant increases in both with haloperidol (Schoots et al., 1995).

**Conclusions**

Similar to other typical and atypical antipsychotics, long-term treatment of rats with olanzapine and risperidone significantly up-regulated D₂ receptors in MPC, as well as D₄ receptors in NAc and CPu. These new findings add support to the hypothesis that these receptors and brain regions may be involved in the beneficial clinical effects of antipsychotics. In addition, both olanzapine and risperidone increased levels of D₂ as well as D₄ receptors in HIP (but not other cortical areas, including DFC and EC), suggesting a third possible site contributing to beneficial effects of atypical antipsychotics.

At behaviorally effective doses, olanzapine and risperidone also increased abundance of D₂ receptors in CPu, similar to the effects of long-term treatment with typical, but not atypical, antipsychotics. This finding parallels the moderate, dose-dependent risk of clinical EPS with these agents, consistent with their status as quantitatively atypical agents (Baldessarini and Tarazi, 2001; Tarsy et al., 2001). Lack of effects of olanzapine and risperidone on D₄-like or D₃ receptors in any brain region examined adds support to the view that these receptors are probably not prominently involved in the actions of various kinds of antipsychotics. Failure of quetiapine to alter abundance of any DA receptor type in any brain region examined is consistent with its low affinity for all DA receptors and suggests that nondopaminergic, and perhaps serotonergic or histaminergic, mechanisms may contribute to the clinical actions of this novel agent.

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