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Effects of risperidone on dopamine receptor subtypes in developing rat brain

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Abstract

The atypical antipsychotic risperidone is often prescribed to pediatric patients with neuropsychiatric disorders, though its effects on the developing brain remain unclear. Accordingly, we studied the effects of repeated treatment of risperidone on dopamine receptors in brain regions of juvenile rat. Levels of dopamine receptors (D_1, D_2, D_3, D_4) in forebrain regions of juvenile rats were quantified after 3 weeks of treatment with three different doses of risperidone (0.3, 1.0 and 3.0 mg/kg) and compared findings to those in adult rats treated with risperidone (3.0 mg/kg/day) previously. Risperidone (at 1.0 and 3.0 mg/kg/day) increased levels of D_1 receptors in nucleus accumbens and caudate-putamen of juvenile, but not adult rats. Conversely, all three doses of risperidone dose-dependently increased D_2 labeling in medial prefrontal cortex and hippocampus, and D_4 receptor in nucleus accumbens, caudate-putamen and hippocampus of juvenile animals as well as in adults. Only the high dose of risperidone (3.0 mg/kg) increased D_2 receptors in caudate-putamen in both juvenile and adult brain. D_3 receptors were not altered by risperidone in any brain region at any dose or age. The findings indicate dose-dependent effects of risperidone on dopamine receptors in developing animals, and that juvenile animals are more sensitive than adults to the cerebral effects of risperidone.

Keywords

Autoradiography; Caudate-putamen; Childhood-onset schizophrenia; Dopamine receptors; Frontal cortex; Risperidone

Introduction

Adult patients with psychotic and bipolar disorders are commonly treated with second-generation antipsychotic drugs [e.g. aripiprazole, clozapine, olanzapine, quetiapine, risperidone (RSP), ziprasidone], which have lower risks of adverse neurological effects than older neuroleptic drugs (Baldessarini and Tarazi, 2005). The newer antipsychotics are often prescribed to juvenile and adolescent patients with neuropsychiatric disorders despite a striking paucity of systematic investigations of their efficacy and safety in young patients. In particular, optimal doses for children and adolescents remain to be defined empirically. Such efforts are

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encouraged by evidence of marked, and probably pharmacodynamically based, maturation-dependent decreases in the potency of older neuroleptics including haloperidol and perphenazine (Campbell and Baldessarini 1981;Campbell et al, 1988). Moreover, juvenile patients appear to be at particularly high risk of adverse neurological and metabolic effects of some antipsychotic drugs (Lewis 1998;Findling and McNamara 2004;Baldessarini and Tarazi 2005). Well-designed clinical trials of the growing number of newer antipsychotic agents are required to determine optimally effective and safe doses in pediatric and adolescent patients.

The first modern antipsychotic agent since clozapine was the benzisoxazole derivative RSP. This agent has been particularly extensively studied and is widely used clinically, in pediatric patients. An early study found that RSP improved negative symptoms including emotional withdrawal, blunted effect, and cognitive impairment in adolescents diagnosed with various types of primary psychotic disorders (Grcevich et al. 1996). Another trial found that RSP improved both positive and negative psychotic symptoms in adolescents with schizophrenia (Armenteros et al. 1997). RSP treatment has also been successful in reducing aggressive behaviors in children with diverse diagnoses, including probable juvenile bipolar disorder (Schreier 1998;Frazier et al. 1999). In pervasive developmental disorders, which include autism, Asperger's syndrome, childhood disintegrative disorder, and Rett syndrome, RSP was effective in ameliorating the core symptoms, including hyperactivity, unstable mood, aggression and self-injurious behaviors (Perry et al. 1997;Barnard et al. 2002;McCracken et al. 2002;Erickson et al. 2005). In addition, RSP reduced the severity of motor and vocal tics and obsessive-compulsive features among patients with Gilles de la Tourette's syndrome (Bruggeman et al. 2001).

Regarding risks of adverse effects, children as well as adults can develop extrapyramidal side effects (EPS) and hyperprolactinemia, which are common dose-dependent adverse effects of RSP, and may be at greater risk for weight-gain and adverse metabolic effects of this agent than in older adolescents and adults (Tarsy et al. 2002;Fedorowicz and Fombonne 2005). Moreover, the bioavailability, absorption and metabolism of RSP in children have not been described. These considerations underscore the importance of establishing optimal doses of this antipsychotic agent so as to estimate maximal therapeutic efficacy with minimal adverse neurological and metabolic effects at specific ages during development.

RSP has multiple sites of molecular interaction. It shares with clozapine and olanzapine a greater affinity for serotonin 5-HT_{2A} than dopamine (DA) D_2 receptors, and has substantial affinity for DA D_3 and D_4 receptors, as well as adrenergic α_1 and α_2 receptors and histamine H_1 receptors (Schotte et al. 1996). RSP has undergone extensive pharmacological and behavioral characterization in adult animals (Arnt and Skarsfeldt 1998;Waddington and Casey 2000). In addition, we have assessed the effects of repeated doses of RSP and other antipsychotic agents on DA receptor subtypes in forebrain tissue of adult rats (Tarazi et al. 2001). However, long-term effects of RSP exposure on cerebral DA receptor subtypes in developing animals are unknown and require investigation. To provide such information, we assessed the regulation of DA D_1, D_2, D_3, and D_4 receptors in different forebrain regions following long-term administration of multiple doses of RSP, and compared the findings to previously reported effects of RSP-induced changes in DA receptors in adult rat brain (Tarazi et al. 2001).

Experimental Procedures

Materials and Animal Subjects

Radiochemicals were [N-methyl-^{3}H]R(+)-7-chloro-8-hydroxy-3-methyl-1-phenyl-2,3,4,5-tetrahydro-1H-3-benzazepine (SCH-23390, 81 Ci/mmol), and R,S(±)-[N-methyl-^{3}H] nemonapride (86 Ci/mmol) from New England Nuclear-Perkin-Elmer Corp. (Boston, MA), as

well as [2,3-^{3}H]R(+)-7-hydroxy-N,N-di-*n*-propyl-2-amino-1,2,3,4-tetrahydronaphthalene (7-OH-DPAT; 116 Ci/mmol) from Amersham (Arlington Heights, IL). Tritium autoradiography standards were from Amersham (Arlington Heights, IL). Tritium-sensitive Hyperfilm and D-19 photographic developer and fixative were from Eastman-Kodak (Rochester, NY).

Risperidone was donated by Janssen Pharmaceutica (Titusville, NJ). DTG (1,3-ditolylguanidine), *cis*-flupenthixol-di-HCl, fluphenazine-di-HCl, ketanserin tartrate, pindolol, and S(−)-sulpiride were obtained from Sigma–Research Biochemicals International (Sigma–RBI; Natick, MA). Cation hydrochlorides, guanosine-5′-triphosphate sodium (GTP), and *tris*-(hydroxymethyl)-aminomethane-HCL (Tris), were from Sigma Chemicals (St. Louis, MO).

Subjects were male Sprague-Dawley rats (Charles River Labs., Wilmington, MA) initially weighing 70–80 g at 22 d of age, weaned at 21 d, and maintained under artificial daylight (on, 07:00–19:00 h), in a temp.- and humidity-controlled environment with free access to standard rat chow and tapwater 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 local regulations.

In vitro dopamine receptor affinity

RSP was tested for affinity at the DA D_1 and D_2 receptors in juvenile (PD 30) and adult (PD 90) animals using membrane preparations from DA-rich corpus striatum (caudate putamen) tissue from rat forebrain. Sprague-Dawley rats were sacrificed by decapitation. Brains were quickly removed and dissected on ice. Tissue was homogenized in 50 mM Tris-HCl buffer (pH 7.4) containing 150 mM NaCl, washed twice and resuspended in the same buffer. For the D_1 receptor assay, homogenate was incubated with 300 pM [^{3}H]SCH-23390 for 30 min at 30° C; nonspecific binding was defined with 10 μM *cis*-flupenthixol. For the D_2 receptor assay, homogenate was incubated with 75 pM [^{3}H]nemonapride for 90 min at 30°C; nonspecific binding was defined with 10 μM haloperidol (Baldessarini et al. 1992). Binding was terminated by immersion in an ice bath. Tissue was rapidly separated from assay buffer mixtures on glass-fiber filter sheets (ISC BioExpress Co., Kaysville, UT) in a Brandel (Gaithersburg, MD) cell harvester, and washed with excess, ice-cold 150 mM saline. Samples on fiber sheets were punched out as discs and placed in minivials containing 4.5 ml Emulsifier-Safe (Packard Instruments, Meriden, CT), and samples were counted for tritium at 50% efficiency in a Beckman-Coulter liquid scintillation spectrophotometer (Fullerton CA). Assay included >10 different concentrations of RSP, in triplicate. $IC_{50} \pm SE$ was obtained with the ALLFIT program to fit percent inhibition of specific binding vs. drug concentration, and converted to Ki from the Cheng-Prusoff relationship, $Ki = IC_{50}/(1 + F/Kd)$, all as described previously (Kula et al. 1994).

Drug treatment and tissue preparation

Four groups of rats (N=6/group), at postnatal day 22 [PD 22]), received single, morning (10:00 h) intraperitoneal (i.p.) injections at 1 ml/kg body wt daily for 21 d. Groups of rats were given RSP in doses of 0.3, 1.0 or 3.0 mg/kg/d, or physiological saline (0.9% w/v) as a solvent control. RSP doses were guided by molecular and in vivo occupancy studies in adult animals. Lower doses of RSP did not elevate levels of striatal D_2 receptors, did not produce catalepsy and occupied D_2 receptors by less than 80%. In contrast, higher doses of RSP elevated striatal D_2 receptors, produced catalepsy and occupied more than 80% of striatal D_2 receptors (Kusumi et al. 2000;Tarazi et al. 2001;Kapur et al. 2003). A high dose of 3.0 mg/kg/d RSP was included for comparison with adults (Tarazi et al. 2001). No gross effects on motor behaviors and no significant changes in body weight were observed after repeated treatment of juvenile animals

with different doses of RSP compared to vehicle-treated animals. After 3 weeks of treatment, juvenile rats were sacrificed 24 hrs after the last injection of RSP or vehicle (PD 42) by decapitation; brains were removed, quick-frozen in isopentane on dry ice, and stored at −80° C.

Frozen sections (10 μm) were prepared in a cryostat at −20°C, mounted on gelatin-coated, glass microscope slides, and stored at −80°C until use. Coronal brain sections were taken through medial prefrontal (MPC) and dorsolateral-frontal (DFC) cerebral cortex, nucleus accumbens (NAc), hippocampus (HIP), and medial and lateral caudate-putamen (CPu). These cortical, limbic and extrapyramidal forebrain regions of interest are implicated in cognitive, emotional, and motor behaviors typically disturbed in young and adult patients with psychotic disorders and altered by antipsychotic drug treatment (Baldessarini and Tarazi 2005).

In vitro receptor autoradiography

Brain sections from all groups of 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-HCl buffer (pH 7.4) containing (mM): NaCl (120), KCl (5), $CaCl_2$ (2), and $MgCl_2$ (1), for the D_1-like, D_2 and D_4 assays, or with slight modification for D_3 assays (with 0.3 mM GTP, 40 mM NaCl, and no $MgCl_2$ added). Preincubation step is effective in minimizing the effects of endogenous DA and potential interference of residual RSP (Florijn et al. 1997).

D_1 Receptors—Rat forebrain sections were incubated for 1 h at RT in the incubating buffer containing 1 nM [^{3}H]SCH-23390 with 100 nM ketanserin to block 5-$HT_{2A/2C}$ 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 (Tarazi et al. 1998,2001).

D_2 Receptors—Sections were incubated for 1 h at RT in the same buffer containing 1.0 nM [^{3}H]nemonapride with 0.5 μM DTG and 0.1 μM pindolol to mask sigma ($\sigma_{1,2}$) and $5HT_{1A}$ sites, respectively. Nonspecific binding was determined with 10 μM S(−)-sulpiride. After incubation, slides were washed twice for 5 min in ice-cold buffer, dipped in ice-cold water, and air-dried (Tarazi et al. 1997,2001). Though the resulting radioligand binding may include traces of binding to D_3 or D_4-sites, most of the signal is believed to represent D_2 receptors.

D_3 Receptors—Sections were preincubated for 1 h in Tris buffer modified as stated to minimize labeling of the high-affinity agonist binding state of D_2 receptors, then incubated for 1 h in the same buffer containing 3 nM [^{3}H]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. 1997,2001).

D_4 Receptors—Tissue sections were preincubated for 1 h at RT in the D_2 assay buffer, and then for 1 h with 1.0 nM [^{3}H]nemonapride, 300 nM S(−)-raclopride to occupy D_2/D_3 sites, and other masking agents (0.5 μM DTG and 0.1 μM pindolol) used in the D_2 assay. Nonspecific binding was determined with 10 μM S(−)-sulpiride. D_4-selective ligands L-745,870 and RBI-257 displaced >85% of binding remaining in the presence of raclopride in adult CPu and NAc tissue, indicating that most of the raclopride-insensitive binding sites are D_4 receptors (Tarazi et al. 1997,1998,2001). In addition, transgenic mice lacking D_4 receptors showed quantitative absence of D_4 labeling by our autoradiographic assay method in comparison to wild-type mice (Gan et al. 2004).

Autoradiography and image analysis

Radiolabeled slides and calibrated [^{3}H]standards (Amersham) were exposed to Hyperfilm (Eastman-Kodak) for 2–5 weeks at 4°C. Films were developed in Kodak D-19 developer and fixative. Optical density (OD) in brain regions of interest was measured with a computerized densitometric image analyzer (MCID-M4, Imaging Research; St. Catharines, Ontario). Brain regions of interest were outlined and their OD was measured. OD was converted to nCi/mg of tissue with calibrated [^{3}H]standards and, after subtracting nonspecific from total binding, specific binding was expressed as fmol/mg tissue (Tarazi et al. 1997,1998,2001).

Statistical analysis

We used two-way analysis of variance (ANOVA) to evaluate overall changes across drug concentrations, receptor subtype and brain regions. Given overall significance of effects for drug dose, receptor subtype or brain region, Fisher post-hoc tests were used to test for significant differences in selected anatomical areas. Unless stated otherwise, data are presented as means ± SEM. Comparisons were considered significant at $p < 0.05$ in two-tailed tests, with degrees of freedom (df) based on N=6 subjects/treatment group.

Results

Experiments with rat brain homogenates indicated that RSP exhibits moderate to low affinity for D_1 receptors in juvenile and adult animals (Table 1). In contrast, RSP has high affinity for D_2 receptors in both aged groups, which is in agreement with other published reports (Table 1; Baldessarini and Tarazi, 2005). No significant differences were observed in RSP's affinity for either D_1 or D_2 receptors in developing vs. mature animals.

Three weeks of daily injections of 1.0 and 3.0 mg/kg of RSP, but not 0.3 mg/kg, to juvenile rats (from PD 22 to PD 42) significantly increased labeling of D_1 receptors in the NAc (by 34% and 64%, respectively; $F [df = 3; 20] = 12.9$, $p<0.001$) and CPu (by a lateral-and-medial average of 53% and 75%; $F [df=3; 20] = 16.5$, $p<0.001$) of juvenile rats (Table 2). In contrast, the three doses failed to alter the abundance of cortical and hippocampal D_1 receptors in developing animals at age 42 days (Table 2).

Repeated treatment with the three doses of RSP (0.3, 1.0 and 3.0 mg/kg) significantly increased concentrations of D_2 receptors in a dose-dependent fashion in the MPC (by 21%, 41% and 55%, $F [df=3; 20] = 16.7$, $p<0.001$) and HIP (by 24%, 57% and 90%, $F [df=3; 20] = 36.8$, $p<0.001$) of juvenile rats (Table 3). In addition, repeated treatment with 1.0 and 3.0 mg/kg of RSP increased D_2 receptor labeling in NAc (by 22% and 36%, $F [df=3; 20] = 87.7$, $p<0.001$). Only the highest dose of RSP (3.0 mg/kg) increased abundance of D_2 receptors in CPu (by a lateral-and-medial average of 20% and 37%; $F [df=3; 20] = 34.8$, $p<0.001$) of juvenile rats (Table 3).

There were no changes in D_3-selective labeling in any brain region analyzed after long-term administration of three doses of RSP (Table 4). In contrast, D_4 labeling was upregulated in several regions by treatment with 0.3, 1.0 and 3.0 mg/kg of RSP including NAc (by 28%, 31% and 32%, respectively; $F [df=3; 20] = 3.4$, $p<0.05$), CPu (average of 31%, 35% and 39%; $F [df=3; 20] = 3.3$, $p<0.05$), and HIP (by 28%, 31% and 32%, respectively; $F [df=3; 20] = 5.7$, $p<0.01$) with no significant changes in regions of cerebral cortex (Table 5).

Discussion

Effects of risperidone treatment on D_1 receptors

Repeated administration of 1.0 and 3.0 mg/kg of RSP for 21 days significantly increased D_1 receptor binding in NAc (by 33% and 64%, respectively), medial CPu (by 55% and 76%) and lateral CPu (by 50% and 78%) of juvenile rats (age 42 days; Table 2). The significant increases in striatal D_1 receptors in juvenile animals after repeated treatment with RSP contrasts with the lack of adaptive changes in striatal D_1 receptors found in adult animals after long-term treatment with 3.0 mg/kg of RSP (Table 2; Tarazi et al. 1997,1998,2001). Similar moderate to low affinity of RSP to D_1 receptors in juvenile and adult animals (Ki= 240 nM and 310 nM, respectively; Table 1) rule out the preferential direct blockade and upregulation of postsynaptic D_1 receptors in NAc and CPu of juvenile and not adult animals. It is plausible that downregulation of serotonin 5-HT_{2A} receptors after repeated RSP treatment (Tarazi et al. 2002) may disrupt the close functional and behavioral interactions reported to occur between D_1 and 5-HT_{2A} receptors in adult rat striatum (Bishop et al. 2003,2005). RSP-induced disruption of D_1/5-HT_{2A} interactions may extend to developing animals and trigger dose-dependent increases in striatal D_1 receptors.

Interestingly, repeated treatment of juvenile animals of the same strain, sex and age for the same period with fluphenazine, clozapine and olanzapine did not significantly alter levels of D_1 receptors in CPu and NAc of drug-treated animals vs. vehicle-treated controls (Moran-Gates et al. 2006). This provides a distinction in the mechanisms of action of RSP vs. other antipsychotic agents in developing animals, though the behavioral correlations of such molecular changes are not well defined. Earlier studies suggested that antipsychotic agents capable of blocking and upregulating striatal D_1 and D_2 receptors might be less likely to induce motor side effects, including tardive dyskinesia than those acting selectively on one or the other DA receptor subtype (Parashos et al. 1990;Marin et al. 1993). Accordingly, RSP-induced upregulation of striatal D_1 receptors might contribute to a lower incidence of undesirable motor side effects associated with D_2 receptor upregulation in CPu of developing animals (Table 3), and subsequently to a more benign neurological profile in pediatric patients treated with RSP.

In contrast, repeated treatment with three doses of RSP failed to alter concentrations of D_1 receptors in cerebral cortex in juvenile animals (Table 2). Lack of change in cortical D_1 receptors vs. selective increases in same receptors in CPu and NAc of developing rats after treatment with RSP may reflect different regulatory responses of cells expressing D_1 receptors in cortex vs. striatum, or perhaps differences in the types, neuronal localization or functions of D_1 receptors in these brain regions. These findings also contrast the significant downregulation of cortical D_1 receptors in similar juvenile animals treated with fluphenazine, clozapine and olanzapine (Moran-Gates et al. 2006). It is possible that the presynaptic D_1 receptors, which appear to be expressed transiently in cerebral cortex of developing animals and to contribute to antipsychotic-induced downregulation of cortical D_1 receptors (Teicher et al. 1991;Moran-Gates et al. 2006), are not modulated by RSP. These observations indicate further that there are age-related and agent-selective responses of D_1 receptors to repeated exposure to RSP and other antipsychotic drugs.

Effects of antipsychotic drug treatment on D_2 receptors

RSP displays high affinity for D_2 receptors in both juvenile and adult animals (Ki= 8.5 nM and 18 nM, respectively; Table 1). Prolonged treatment with three doses of RSP dose-dependently enhanced radioligand binding to D_2 receptors in MPC and not DFC of juvenile animals (Table 3). Similar D_2 receptor upregulation and increased D_2 mRNA expression have been found in cerebral cortex of adult rats and non-human primates treated with RSP and other antipsychotics (Damask et al. 1996,Lidow and Goldman-Rakic 1997;Tarazi et al. 1997,2001). These findings

further support the importance of D_2 receptors in MPC as common targets that mediate the actions of dissimilar antipsychotics in developing and mature animals and subsequently in juvenile and adult patients. Additional studies are in progress to clarify molecular mechanisms contributing to the observed increase in MPC D_2 receptors by evaluating the effects of multiple doses of RSP on D_2 mRNA expression in cortex and other brain regions of developing rat brain.

Repeated treatment with all three tested doses of RSP also increased, in dose-dependent fashion, binding of D_2 receptors in HIP of juvenile animals; this effect also occurs in adults (Table 3; Tarazi et al., 2001). However, a comparable dose of RSP (3.0 mg/kg) was more effective in enhancing D_2 receptor binding in HIP of juveniles (by 90%) vs. adults (30%), which further reflects the greater sensitivity of developing animals to the long-term molecular actions of RSP. Pharmacokinetic factors including differences in metabolism and absorption of RSP in juveniles vs. adults may have also contributed to the greater sensitivity of young animals to RSP treatment. This issue, however, requires further investigation since no pharmacokinetic data on RSP are available in developing animals or pediatric patients. Hippocampal D_2 receptor upregulation may lead to improvement of emotional behaviors mediated by HIP and other components of limbic system, which are typically disturbed in pediatric and adult patients (Tarazi and Kaufman 2005;Baldessarini and Tarazi 2005).

Repeated treatment with high dose of RSP (3.0 mg/kg) increased D_2 receptor labeling in CPu of juvenile animals (Table 3). Lower doses of RSP (0.3 and 1.0 mg/kg) tended to increase striatal D_2 receptors, but did not reach statistical significance (Table 2). Similar responses have been found after long-term treatment with the same agent and similar doses in adult animals (Kusumi et al. 2000;Tarazi et al. 2001). These differential D_2 receptor responses to different doses of RSP in juveniles correlate with differences in RSP-induced D_2 receptor occupancy in adult animals. Doses of 0.3–1.0 mg/kg RSP produced 50%–80% D_2 receptor occupancy, whereas a dose of 2.0 mg/kg or higher of RSP exceeded 80% occupancy of same receptor and produced catalepsy (Kapur et al. 2003). Therefore, doses up to 1.0 mg/kg of RSP in rats may correspond to the clinically comparable range in adult and perhaps in young patients. A dose of 3.0 mg/kg of RSP which upregulates D_2 receptors in CPu may disturb neurotransmission in circuits involved in programming and executing movement and may lead to the development of undesirable adverse EPS in adults as well as in juveniles (Albin et al. 1989). It is likely that risks of acute EPS and tardive dyskinesia in young patients treated with high doses of RSP are greater than with lower doses, as in adult patients (Tarsy et al. 2002).

Effects of risperidone treatment on D_3 receptors

D_3 receptor binding was unchanged after prolonged exposure of juvenile animals to the three tested doses of RSP in all regions examined (Table 4). These findings are consistent with the lack of changes in D_3 receptors or D_3 gene expression after repeated exposure to various dissimilar antipsychotics including RSP in adult animals. (Levésque et al. 1995; Tarazi et al. 1997,1998,2001). More recently, we found that other typical and atypical antipsychotics, including fluphenzaine, clozapine and olanzapine also failed to alter abundance of D_3 receptors in developing animals matched for strain, sex and age (Moran-Gates et al. 2006).

Signal transduction cascades, including interactions with G-proteins, in forebrain regions of adult and juvenile animals are not well defined (Ahlgren-Beckendorf and Levant 2004;Tang et al. 1994). Such unique receptor/effector coupling mechanisms could result in a lack of D_3 receptor upregulation in response to adequate receptor blockade by RSP. Alternatively, RSP may not affect the expression of brain-derived neurotrophic factor (BDNF) that controls the appearance of D_3 receptors during development (Sokoloff et al. 2002). A third possibility stems from the high avidity of D_3 receptors for DA, and their selective protection from alkylation by very low concentrations of DA in adult subjects (Zhang et al. 1999). This phenomenon may

also extend to developing animals and limit availability of D_3 receptors for occupancy and upregulation by even high doses of RSP.

Effects of risperidone treatment on D_4 receptors

Prolonged administration of the three tested doses of RSP significantly increased D_4 receptors in CPu and NAc (Table 5), possibly reflecting adaptive changes to direct D_4 receptor blockade since RSP has relatively high D_4 receptor affinity as determined in genetically transfected cells (Ki=16 nM; Schotte et al. 1996). Observed increases in D_4 receptors in NAc and CPu of juvenile animals is similar to that reported in same-age developing animals after repeated treatment with fluphenazine, clozapine and olanzapine (Moran-Gates et al. 2006), and to that in adult animals after prolonged administration of RSP and other typical and atypical antipsychotics (Tarazi et al. 1997,1998,2001).

These findings further support the hypothesis that striatolimbic D_4 receptors constitute common targets, which mediate the beneficial therapeutic effects of dissimilar antipsychotics in developing as well as mature subjects (Tarazi et al. 2004). Despite the failure of controlled trials with D_4-selective agents in adult patients diagnosed with schizophrenia (Kramer et al. 1997;Corrigan et al. 2004), the possibility exists that targeting D_4 receptors, in synchrony with other neurotransmitter receptors may mediate, at least in part, the beneficial therapeutic effects of RSP and other antipsychotics in juvenile and adult patients with early onset psychotic or cognitive disorders.

In spite of their relative abundance in cortical areas, D_4 receptor levels remained unchanged in MPC and DFC of juvenile animals after repeated treatment with RSP (Table 5). This finding agrees with the effects of dissimilar antipsychotic agents on cortical D_4 receptors in developing and mature animals (Tarazi et al. 1997,2001;Moran-Gates et al. 2006). Regional differences in the molecular mechanisms regulating D_4 mRNA transcription or protein synthesis in cortex vs. CPu and NAc, or perhaps different neuronal localization of D_4 receptors in these forebrain regions may account for regional differences in increases of D_4 receptors in CPu and NAc and not cerebral cortex after prolonged antipsychotic exposure to RSP and other antipsychotics in young and adult animals.

Repeated treatment with RSP significantly increased concentrations of D_4 receptors in HIP of juvenile animals dose-dependently (Table 5). In contrast, repeated treatment with fluphenazine, clozapine and olanzapine did not alter hippocampal D_4 receptors in same age group animals (Moran-Gates et al. 2006). These findings indicate that RSP exerts more potent effects than other antipsychotic agents on DA receptor subtypes in HIP of juvenile animals. The increase in hippocampal D_4 receptors in developing animals is similar to that observed in adult animals after long-term RSP administration (Tarazi et al. 2001). Potent pharmacological effects of RSP on D_4 receptors may render hippocampal D_4 receptors in developing and mature animals to become more sensitive to the prolonged actions of RSP. It is also possible that close functional interactions between D_2 and D_4 receptors lead to the elevation of both receptor subtypes in HIP of young and adult animals after RSP treatment.

Conclusions

Repeated administration of RSP to juvenile animals induced effects that were different from its effects in adult animals. Long-term treatment with higher doses (1.0 and 3.0 mg/kg) of RSP increased the abundance of D_1 receptors in NAc and CPu of juvenile animals, but not in adult animals. In addition, the three tested doses of RSP (0.3, 1.0 and 3.0 mg/kg) profoundly increased D_2 receptors in MPC and HIP of juvenile animals in a dose-dependent fashion and in greater magnitude than RSP-induced increases in D_2 receptors in same brain areas of adult rats of the same strain. Neurodevelopmental pharmacodynamic or pharmacokinetic factors

may have contributed to age-related differences in cortical and hippocampal D_1 and D_2 receptor responses to long-term RSP treatment. Common upregulation of corticolimbic D_2 and striatolimbic D_4 receptors suggest that these receptors are involved in the molecular actions of RSP and other antipsychotics in developing and mature animals, and perhaps in both young and adult patients. Selective increases of D_2 receptors in basal ganglia of both juvenile and adult rats after treatment with high dose (3.0 mg/kg) and not lower doses (0.3 and 1.0 mg/kg) of RSP may contribute to the development of motor side effects. Lack of change in D_3 receptors reflects its unique regulatory mechanisms in response to repeated treatment with RSP and other antipsychotics in juvenile and adult animals.

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Table 1

Affinity (K_i, nM ± S.E.) of risperidone at D_1 and D_2 receptors in juvenile vs. adult rat striatal tissue

Striatal tissue	D_1	D_2
Juvenile animals (PD 30)	240 ± 30	8.5 ± 1.4
Adult animals (PD 90)	310 ± 45	15 ± 2.2

Table 2

D_1 receptor binding in juvenile rats (PD 42) after three weeks of daily administration of risperidone

Brain region	Controls	RSP(0.3 mg/kg)	*Juvenile Rats* RSP(1.0 mg/kg)	RSP(3.0 mg/kg)	*Adult Rats* RSP(3.0 mg/kg)
Cerebral cortex					
Medical-prefrontal	31.2 ± 1.7 (100)	29.3 ± 1.6 (94)	32.5 ± 1.7 (104)	31.4 ± 1.0 (101)	(85)
Dorsolateral	22.7 ± 1.0 (100)	20.6 ± 1.2 (91)	21.1 ± 1.1 (93)	22.6 ± 0.4 (100)	(120)
Nucleus accumbens	177 ± 11.0 (100)	182 ± 12.5 (103)	236 ± 20.9 (**133**)*	290 ± 12.7 (**164**)*	(103)
Caudate-putamen					
Medial	168 ± 16.8 (100)	190 ± 19.0 (113)	261 ± 19.1 (**155**)*	295 ± 18.6 (**176**)*	(99)
Lateral	187 ± 16.9 (100)	206 ± 16.2 (110)	281 ± 19.0 (**150**)*	333 ± 13.8 (**178**)*	(103)
Hippocampus	19.7 ± 1.5 (100)	16.8 ± 1.7 (85)	18.3 ± 0.8 (93)	19.6 ± 0.7 (99)	(111)

Data are mean ± SEM values for binding (fmol/mg tissue, [% of control]), determined by quantitative autoradiography following daily i.p. injection of vehicle or risperidone (RSP) for 3 weeks, with significant differences from controls indicated in bold ([*] $p<0.05$, N=6 rats/group). Data (% of control) for RSP (3 mg/kg/d) in adult animals were reported previously (Tarazi et al., 2001) and are shown for comparison.

Table 3

D_2 receptor binding in juvenile rats (PD 42) after three weeks of daily administration of risperidone

Brain region	Controls	RSP(0.3 mg/kg)	*Juvenile Rats* RSP(1.0 mg/kg)	RSP(3.0 mg/kg)	*Adult Rats* RSP(3.0 mg/kg)
Cerebral cortex					
Medial-prefrontal	36.4 ± 1.6 (100)	44.1 ± 1.1 (**121**)*	51.4 ± 1.5(**141**)*	56.5 ± 3.5 (**155**)*	(**134**)*
Dorsolateral	30.3 ± 6.0 (100)	28.8 ± 4.2 (95)	34.3 ± 2.3 (113)	35.5 ± 3.4 (117)	(91)
Nucleus accumbens	157 ± 3.6 (100)	156 ± 1.6 (99)	192 ± 2.8 (**122**)*	213 ± 3.4 (**136**)*	(**128**)*
Caudate-putamen					
Medial	153 ± 6.2 (100)	160 ± 4.2(105)	180 ± 4.5 (118)	214 ± 5.5 (**140**)*	(**127**)*
Lateral	223 ± 4.8 (100)	227 ± 4.4 (102)	261 ± 5.2 (117)	299 ± 4.2 (**134**)*	(**123**)*
Hippocampus	37.2 ± 1.6 (100)	46.0 ± 1.4 (**124**)*	58.5 ± 3.3 (**157**)*	70.5 ± 2.7 (**190**)*	(**130**)*

Data are mean ± SEM values for binding (fmol/mg tissue, [% of control]), determined by quantitative autoradiography following daily i.p. injection of vehicle or risperidone (RSP) for 3 weeks, with significant differences from controls indicated in bold ([*] $p<0.05$, N=6 rats/group). Data (% of control) for RSP (3 mg/kg/d) in adult animals were reported previously (Tarazi et al., 2001) and are shown for comparison.

Table 4

D_3 receptor binding in juvenile rats (PD 42) after three weeks of daily administration of risperidone

Brain region	Controls	RSP(0.3 mg/kg)	*Juvenile Rats* RSP(1.0 mg/kg)	RSP(3.0 mg/kg)	*Adult Rats* RSP(3.0 mg/kg)
Islands of Calleja	46.2 ± 2.2 (100)	49.9 ± 1.3 (108)	44.7 ± 1.5 (97)	45.7 ± 1.9 (99)	(93)
Olfactory tubercle	30.9 ± 1.5 (100)	31.8 ± 2.3 (103)	29.0 ± 2.5 (94)	30.6 ± 0.8 (99)	(108)
Nucleus accumbens					
Shell	26.3 ± 0.9 (100)	28.5 ± 1.7 (108)	26.5 ± 0.8 (101)	25.6 ± 2.5 (97)	(97)
Core	19.9 ± 1.1 (100)	20.5 ± 1.4 (103)	18.9 ± 1.1 (95)	20.8 ± 1.0 (105)	(114)
Caudate-putamen					
Medial	14.0 ± 1.9 (100)	15.8 ± 2.1 (113)	14.3 ± 0.5 (102)	14.6 ± 1.1 (104)	(97)
Lateral	14.6 ± 1.0 (100)	15.3 ± 2.2 (105)	14.0 ± 0.8 (96)	15.1 ± 1.2 (103)	(89)

Data are mean ± SEM values for binding (fmol/mg tissue, [% of control]), determined by quantitative autoradiography following daily i.p. injection of vehicle or multiple doses of risperidone (RSP) for 3 weeks (N=6 rats/group). Data (% of control) for RSP (3 mg/kg/d) in adult animals were reported previously (Tarazi et al., 2001) and are shown for comparison.

Table 5

D_4 receptor binding in juvenile rats (PD 42) after three weeks of daily administration of risperidone

Brain region	Controls	RSP(0.3 mg/kg)	*Juvenile Rats* RSP(1.0 mg/kg)	RSP(3.0 mg/kg)	*Adult Rats* RSP(3.0 mg/kg)
Cerebral cortex					
Medial-prefrontal	19.8± 0.8 (100)	20.4 ± 1.4 (103)	20.2 ± 1.4 (102)	21.0 ± 1.1 (106)	(111)
Dorsolateral	16.4 ± 2.0 (100)	15.1 ± 0.9 (92)	16.4 ± 1.3 (100)	18.1 ± 1.5 (110)	(102)
Nucleus accumbens	30.6 ± 3.8 (100)	39.3 ± 2.9 (**128**)*	40.0 ± 2.5 (**131**)*	40.5 ± 2.6 (**132**)*	(**133**)*
Caudate-putamen					
Medial	31.1 ± 3.2 (100)	40.5 ± 2.8 (**130**)*	41.1 ± 2.8 (**132**)*	42.3 ± 1.9 (**136**)*	(**136**)*
Lateral	42.8 ± 3.1 (100)	56.4 ± 2.7 (**132**)*	59.2 ± 2.6 (**138**)*	60.4 ± 2.2 (**141**)*	(**137**)*
Hippocampus	19.2 ± 1.3 (100)	24.6 ± 1.3 (**128**)*	26.3 ± 1.9 (**137**)*	29.2 ± 2.4 (**152**)*	(**137**)*

Data are mean ± SEM values for binding (fmol/mg tissue, [% of control]), determined by quantitative autoradiography following daily i.p. injection of vehicle or risperidone (RSP) for 3 weeks, with significant differences from controls indicated in bold ([*] $p<0.05$, N=6 rats/group). Data (% of control) for RSP (3 mg/kg/d) in adult animals were reported previously (Tarazi et al., 2001) and are shown for comparison.