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Effects of risperidone on glutamate receptor subtypes in developing rat brain

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Abstract

Levels of ionotropic glutamate (Glu) *N*-methyl-D-aspartic acid (NMDA), 2-amino-3-(3-hydroxy-5-methyl-isoxazol-4-yl)propionic acid (AMPA), and kainic acid (KA) receptors in forebrain regions of juvenile rats (age 42 days) 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 0.3 mg/kg/day) did not alter levels of three ionotropic Glu receptors in all brain regions examined. Risperidone (at 1.0 and 3.0 mg/kg/day) significantly decreased NMDA binding in caudate-putamen of juvenile and adult animals. In contrast, the same two doses of risperidone decreased NMDA receptors in nucleus accumbens of juveniles and not adults. Risperidone (at 1.0 and 3.0 mg/kg/day) increased AMPA receptors in medial prefrontal cortex and caudate-putamen of juvenile animals, whereas risperidone (at 3.0 mg/kg) increased AMPA receptors in caudate-putamen and hippocampus of adults. Kainate receptors were not altered by any dose of risperidone in any brain region examined in developing and mature animals. The findings indicate that risperidone exerts dose-dependent effects on Glu receptor subtypes in developing animals, and that Glu receptor responses to repeated administration of risperidone are different in juvenile animals than adults.

Keywords

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

Introduction

Newer antipsychotic drugs are increasingly prescribed to pediatric patients with different neurospcyhiatric disorders, in spite of the absence of careful characterization of efficacy, safety and optimal doses of these drugs in young patients. Evidence suggests that juvenile patients are at a higher risk of developing neurological and metabolic side effects compared to adults (Lewis 1998; Findling and McNamara 2004). Accordingly, there is a great need to conduct well-controlled clinical trials to better evaluate the tolerability and safety of different classes of antipsychotic drugs in pediatric and adolescent neuropsychiatric patients.

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Among all newer antipsychotic agents, risperidone (RSP) has been most extensively examined and widely used in pediatric patients. RSP was reported to improve schizophrenia symptoms in adolescents (Armenteros et al. 1997) as well as enhance emotional withdrawal and cognitive impairment in psychotic adolescent patients (Grcevich et al. 1996). In addition, RSP was effective in alleviating hyperactivity, stabilizing mood swings and reducing aggression and self-injurious behaviors in patients with pervasive developmental disorders, including autism, Asperger's syndrome, and Rett syndrome (Perry et al. 1997; Barnard et al. 2002; McCracken et al. 2002; Erickson et al. 2005). More recently, RSP became the first FDA-approved drug for managing symptoms of autistic disorders. RSP also reduced motor and vocal tics among patients with Tourette's syndrome (Bruggeman et al. 2001). However, young patients treated with RSP are at greater risk for neurological, hormonal and metabolic adverse effects of this agent than adults (Tarsy et al. 2002; Fedorowicz and Fombonne 2005).

Dysfunction in glutamatergic neurotransmission may contribute to the pathophysiology of psychotic disorders including schizophrenia, and perhaps the early-onset form of the disease (Goff and Coyle, 2001; Tsai and Coyle, 2002). Glutamate (Glu) commonly mediates its actions by interacting with three major ionotropic receptor subtypes: *N*-methyl-D-aspartic acid (NMDA), 2-amino-3-(3-hydroxy-5-methyl-isoxazol-4-yl)propionic acid (AMPA), and kainate (KA; Ozawa et al. 1998). NMDA receptors have been implicated as a critical site of action of psychotomimetic agents including phencyclidine (PCP) and ketamine, which can produce behavioral and emotional deficits that resemble symptoms of psychotic disorders (Javitt and Zukin 1991; Tsai and Coyle 2002). The three ionotropic Glu receptor subtypes appear to be targeted and modulated by different classes of antipsychotic agents. Repeated treatment with dissimilar antipsychotic drugs including RSP altered the levels of the three ionotropic Glu receptors in adult rat forebrain regions, although the direction of reported changes has been inconsistent (Meshul et al. 1996; Tarazi et al. 1996; Giardino et al. 1997; McCoy et al. 1998; Spurney et al., 1999; Tarazi et al., 2003).

Long-term effects of RSP exposure on ionotropic Glu receptor subtypes in developing animals are unknown and require investigation. Accordingly, we assessed the effects of repeated administration of multiple doses of RSP on concentrations of NMDA, AMPA and KA in forebrain regions of young animals, and compared the findings to previously reported effects of RSP-induced changes in the three ionotropic Glu receptors in adult rat brain (Tarazi et al. 2003).

2. Experimental Procedures

2.1. Materials and Animal Subjects

Radiochemicals—[3-^{3}H](+)-5-methyl-10,11-dihydro-[5H]-dibenzo[a,d] cyclohepten-5,10-imine (MK-801; 23.9 Ci/mmol for NMDA receptors), [5-^{3}H]-2-amino-3-(3-hydroxy-5-methyl-isoxazol-4-yl)propionic acid (AMPA; 83.4 Ci/mmol for AMPA receptors), and [vinylidene-^{3}H]-kainic acid (Ci/mmol for KA receptors) were purchased from New England Nuclear-Perkin-Elmer Corp. (Boston, MA). Tritium autoradiography standards were from Amersham (Arlington Heights, IL). Kodak Biomax MR films and D-19 photographic developer and fixative were obtained from Eastman-Kodak (Rochester, NY).

Risperidone was donated by Janssen Pharmaceutica (Titusville, NJ). 6-Cyano-7-nitroquinoxaline (CNQX), ketamine hydrochloride, potassium thiocyanate (KSCN), and spermine tetrahydrochloride were obtained from Sigma–Research Biochemicals International (Sigma–RBI; Natick, MA), ethylenediaminetetraacetic acid (EDTA) from Fisher Scientific (Fairlawn, NJ), as well as L-glutamic acid (Glu), L-glycine hydrochloride, and *tris*-(hydroxymethyl)-aminomethane (Tris) hydrochloride 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.

2.2. In vitro glutamate receptor affinity

RSP was tested for affinity at the NMDA, AMPA and KA receptors in juvenile (PD 30) and adult (PD 90), using a rat brain preparation as detailed previously (Reynolds et al., 1987; Tarazi et al., 2003). For binding affinities of RSP to NMDA receptors, rat brain minus cerebellum was frozen, thawed and homogenized by Polytron (at 50% maximum power) in 3 vols buffer (20 mM HEPES containing 1mM EDTA, pH 7.4 at 4°C) for 0.5 min, and then centrifuged at 48,000 × *g* for 10 min, and rehomogenized and recentrifuged 5 more times. The resulting tissue pellet was suspended in buffer and frozen overnight, then thawed and centrifuged again 3 times in the same buffer without EDTA. The final pellet was suspended in the EDTA-free buffer at 200 mg/ml and stored at −70°C for use within 3 weeks. Thawed tissue was diluted with the same buffer to provide the equivalent of 15 mg of original wet weight of tissue per assay, and incubated with 1.7 nM final concentration of [^{3}H]MK-801 as the assay radioligand. Glutamate (50 μM), glycine (30 μM) and spermine (50 μM) were added to the HEPES buffer to achieve maximum binding affinity of the ligand (Tarazi et al., 2003). Specificity was determined by 200 μM ketamine. Assay tubes were incubated for 60 min at 23°C, filtered (32 S&S filters; ISC Bioexpress, Kaysville, UT), and counted in minivials containing 4.5 ml Emulsifier-Safe (Perkin Elmer, Boston, MA) in Beckman Coulter beta scintillation counter (Fullerton CA) at ca. 50% efficiency.

For binding affinities of RSP to AMPA and KA receptors, rat cortical tissue was prepared as stated above. Assay buffer for AMPA receptors contained 50 mM Tris-HCl (pH 7.3), 2.5mM $CaCl_2$ and 30 mM KSCN (Wullner et al., 1994; Tarazi et al., 2003), whereas the KA receptor assay buffer contained only 50 mM Tris-HCl (pH 7.3). Radioligands were [^{3}H]AMPA (6.4 nM) to label AMPA receptors, and [^{3}H]kainate (4.63 nM) for KA receptors. Nonspecific binding was defined using excess L-Glu (1 mM) for AMPA receptors and excess unlabeled kainate (100 μM) for KA receptors. Assay tubes were incubated for 60 min on ice, then filtered and counted as described above. The three assays included >10 different concentrations of RSP, in triplicate. IC_{50} ± 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).

2.3. 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, *in vitro* and *in vivo* occupancy studies in adult animals (Schotte et al. 1996; Kapur et al. 2003). A high dose of 3.0 mg/kg/d RSP was included for comparison with adults (Tarazi et al. 2003). 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 patients with psychotic disorders (Baldessarini and Tarazi 2005).

2.4. In vitro receptor autoradiography

Brain sections from all drug-treated rats, and matching controls, were evaluated at the same time in each radioreceptor assay to minimize experimental variability. Sections were first preincubated for 60 min at room temperature (RT) in the appropriate specified buffer before incubating them with the radioligand to remove endogenous Glu and wash out any residual RSP that may interfere with binding of the radioligands to Glu receptors.

2.4.1. NMDA Receptors—Sections were preincubated for 60 min at RT in 50 mM Tris-HCl buffer (pH 7.4), then incubated for 150 min at RT in fresh buffer containing 10 nM [^{3}H]MK-801 and 100 μM L-Glu, 100 μM glycine, 1 mM EDTA, and 75 μM spermine to enhance the binding of [^{3}H]MK-801 to its site within the open cation channels associated with NMDA receptors. Nonspecific binding was determined by including 20 μM ketamine. After incubation, slides were washed in ice cold 50 mM Tris-HCl buffer, twice for 20 min, and dried (Tarazi et al., 1996, 2003).

2.4.2. AMPA Receptors—Binding protocol was modified from Wullner et al., (1994) and used in our previous study (Tarazi et al., 2003). Sections were incubated for 60 min at RT in 50 mM Tris-HCl buffer (pH 7.2), then incubated in fresh buffer containing 30 nM [^{3}H]AMPA, 2.5 mM $CaCl_2$ and 30 mM KSCN. Nonspecific binding was determined with 30 μM unlabeled CNQX. After incubation, slides were washed in the ice-cold Tris buffer, 3 times for 10 sec, and dried.

2.4.3. Kainate Receptors—Sections were preincubated for 60 min at 4°C in 50 mM Tris-HCl buffer (pH 7.0) at 4°C, and then incubated in this buffer containing 20 nM [^{3}H]KA for 60 min at 4°C. Nonspecific binding was determined with 25 μM unlabeled KA. After incubation, slides were washed in ice-cold 50 mM Tris buffer, 3 times for 10 sec, and air-dried (Tarazi et al., 1996, 2003).

2.5. Autoradiography and image analysis

Radiolabeled slides and calibrated [^{3}H]standards (Amersham) were exposed to Biomax MR films 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. 1998, 2001, 2002).

2.6. 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 had very low affinity at NMDA, AMPA and KA receptors in both juvenile (PD 30) and adult (PD 90) rat brain tissue. At concentrations of 10–100 μM, RSP inhibited binding of all three radioligands by less than 10% (all Ki > 10 μM).

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 decreased labeling of NMDA receptors in the NAc (by 25% and 32%, respectively; $F [df = 3; 20] = 11.4$, $p<0.001$) and CPu (by a lateral-and-medial average of 27% and 28%; $F [df=3; 20] = 13.8$, $p<0.001$) of juvenile rats (Table 1). In contrast, the three doses failed to alter the abundance of cortical and hippocampal NMDA receptors in developing animals at age 42 days (Table 1).

Repeated treatment with the three doses of RSP (0.3, 1.0 and 3.0 mg/kg) significantly increased concentrations of AMPA receptors in the MPC (by 27% and 29%, $F [df=3; 20] = 10.3$, $p<0.001$) and CPu (by a lateral-and-medial average of 27% and 28%; $F [df=3; 20] = 6.7$, $p<0.01$) of juvenile rats (Table 2). The lowest dose of RSP (0.3 mg/kg) did not have any effect on AMPA receptors in forebrain regions of juvenile animals. There were no changes in kainate-selective labeling in any brain region analyzed after long-term administration of three doses of RSP (Table 3).

Discussion

Effects of risperidone treatment on NMDA receptors

At PD42, levels of NMDA receptors in control animals were comparable to that reported in other studies but higher than that observed in adult animals in all forebrain regions examined (Table 1; Insel et al. 1990;Tarazi et al. 2003). Repeated administration of 1.0 and 3.0 mg/kg of RSP for 21 days significantly decreased binding of [^{3}H]MK-801 in medial and lateral CPu of juvenile animals (Table 1). These effects were similar to the effects of RSP in adult animals (Tarazi et al., 2003). Reductions in NMDA receptor binding induced by RSP in the CPu may result from indirect interactions with other neurotransmission systems that closely interact with glutamatergic neurotransmission, such as serotonin (5-HT) and DA systems (Aghajanian and Marek, 2000;Carlsson et al., 2001), since RSP has very low affinity for MK-801 binding sites in juvenile rat brain tissue (Ki > 10 μM).

RSP displays high affinity for multiple 5-HT receptor subtypes (Baldessarini and Tarazi 2005), and continuous treatment with RSP increased concentrations of 5-HT_{1A} receptors and decreased 5-HT_{2A} receptor levels in frontal cortex of both juvenile and adult rat brain (Tarazi et al. 2002; Moran-Gates et al. unpublished observations). RSP-induced changes in cortical 5-HT_{1A} and 5-HT_{2A} receptors may suppress Glu neurotransmission in corticostriatal projections innervating CPu, and lead to decreased expression of NMDA receptors in CPu. Evidence suggests that RSP is capable, perhaps through 5-HT receptors, of altering NMDA-receptor mediated neurotransmission in rat prefrontal cortex and its projections (Konradsson et al., 2006). Alternatively, there are indications that NMDA and DA D_2 receptors are anatomically co-localized in striatal neurons in adult rat brain (Ariano et al. 1997; Tarazi et al. 1998), and they often display antagonistic functional and cellular interactions (Cepeda et al., 1993; Carlsson et al., 2001). It is highly possible that the close interactions between NMDA and D_2 receptors also extend to developing animals. Accordingly, blockade and upregulation of

D_2 receptors in CPu of juvenile rats after repeated administration of RSP (Moran-Gates et al., 2007) may contribute to the observed decreases in NMDA receptor labeling in CPu.

NMDA receptor activation in adult animals contributes to the development of extrapyramidal side effects (EPS) commonly associated with classical neuroleptic drug treatment. In contrast, NMDA receptor antagonism attenuates neuroleptic-induced catalepsy (Schmidt and Bubser, 1989; Yoshida et al., 1991) and blocks neuroleptic-induced expression of immediate early gene *c-fos* in striatal tissue of adult animals (Boegman and Vincent, 1996). No studies have evaluated the role of NMDA receptors in induction of EPS in young animals. However, it is possible that suppression of striatal NMDA receptor activity by RSP may contribute to its relatively benign impact on the extrapyramidal system in young animals.

RSP at 1.0 and 3.0 mg/kg decreased binding levels of NMDA in NAc of juvenile and not adult rat brain tissue (Table 1). RSP-induced reduction in NMDA receptors in NAc suggests that this atypical antipsychotic agent may alter glutamatergic neurotransmission in the mesolimbic system in the brains immature animals more potently than adult subjects. This finding indicates that there are age-related responses of NMDA receptors to repeated RSP exposure. Interestingly, RSP decreased NMDA receptor binding in HIPP of adult and not juvenile animals (Table 1; Tarazi et al. 2003). These findings further support our hypothesis that age and other developmental factors contribute to differences in NMDA receptor response to RSP treatment in juvenile vs. adult animals.

Effects of risperidone treatment on AMPA and KA receptors

Levels of AMPA receptors were significantly lower in drug-naïve developing animals (PD42) than in adult animals in all forebrain regions examined (Table 2; Tarazi et al. 2003). In contrast, levels of KA receptors in developing animals were comparable to that in adult animals (Table 3;Tarazi et al. 2003). Continuous treatment with 1.0 and 3.0 mg/kg of RSP increased concentrations of AMPA receptors in MPC of juvenile and not adult animals (Table 2;Tarazi et al., 2003). This finding provides another distinction in the mechanism of action of RSP in developing vs. mature animals, and further indicates that cortical AMPA receptors in young animals are more vulnerable to the long-term effects of RSP. Facilitation of glutamatergic neurotransmission via NMDA and AMPA receptors in pyramidal cells of MPC has been proposed as a unique action of atypical antipsychotic agents in rats (Ninan et al., 2003). RSP-induced enhancement of glutamatergic neurotransmission via increases of AMPA receptors in MPC of juvenile rats may contribute to the beneficial effects of RSP on cognitive functions in patients with childhood-onset psychotic disorders (Grcevich et al. 1996).

Continuous treatment with 1.0 and 3.0 mg/kg of RSP also increased labeling of AMPA receptors in medial and lateral CPu (Table 2). This is similar to the effects of RSP on AMPA receptors in CPu of adult animals (Table 2; Tarazi et al., 2003). This finding, based on labeling with the agonist [^{3}H]AMPA, contrasts to a previously reported lack of effect of long-term administration of haloperidol or clozapine on AMPA receptors labeled with the antagonist [^{3}H]CNQX (Tarazi et al., 1996). Differences in the AMPA receptor binding states may have contributed to this discrepancy. The agonist radioligand [^{3}H]AMPA selectively labels a high-affinity state, whereas the antagonist [^{3}H]CNQX binds to both high-and low-affinity states of AMPA receptors (Nielsen et al., 1990;Hall et al., 1993). Long-term treatment with RSP and other antipsychotics seem to selectively increase the high-affinity binding state of AMPA receptors in CPu of juvenile and adult animals. In contrast, it may be difficult to detect such an increase when labeling both binding states of AMPA receptors are radiolabeled with an antagonist. Other studies also found elevations of [^{3}H]AMPA binding, with minimal changes in [^{3}H]CNQX binding, after long-term administration of clozapine, risperidone, or haloperidol (McCoy et al., 1996,1998).

Our present findings also suggest that striatal AMPA receptors constitute a novel common site of action that may contribute to beneficial clinical effects of RSP in developing and mature animals. RSP-induced upregulation of AMPA receptors may restore cortico-striato-limbic Glu neurotransmission by normalizing hypoglutamatergic activity suggested as a pathophysiological contribution in schizophrenia (Goff and Coyle, 2001; Tsai and Coyle, 2002). In support of this hypothesis, ampakines, drugs that act as positive modulators of the AMPA receptor complex and enhance Glu neurotransmission via AMPA receptors, potentiated the suppression of conditioned avoidance response induced by low doses of RSP in rats (Olsen et al., 2006) and improved cognitive impairments in schizophrenia patients treated with clozapine (Goff et al., 2001), though a recent study contradicts this observation (Goff et al., 2008). It remains to be determined if amapkines can improve cognitive deficits in patients with childhood-onset psychotic disorders.

Indirect actions arising from the RSP's effects on the central 5-HT system, again, may contribute to the increased AMPA receptor binding found in CPu (Table 2), since RSP has very low affinity for AMPA receptors (Ki > 10 μM). We recently found that treatment with similar doses of RSP increased 5-HT_{1A} but decreased 5-HT_{2A} receptors in cerebral cortex of developing animals (Moran-Gates et al., unpublished observations). Additional evidence for a direct interaction between 5-HT_{1A}/5-HT_{2A}/AMPA receptors arises from studies finding that 5-HT_{2A} receptor stimulation increased AMPA-mediated release of Glu by pyramidal cells in layer-V of prefrontal cortex, which produces corticostriatal and corticotectal projections (Miller, 1988;Aghajanian and Marek, 2000). In contrast, stimulation of 5-HT_{1A} receptors decreased AMPA-evoked electrical stimulation in prefrontal cortex (Cai et al., 2002). The increases and decreases in cortical $5HT_{1A}$ and $5HT_{2A}$ receptors, respectively, after continuous treatment with RSP may alter corticostriatal AMPA-mediated Glu neurotransmission and lead to an increase in AMPA receptor levels in CPu of developing animals.

Alternatively, the observed increase in AMPA receptors in rat CPu may result from RSP-induced upregulation of D_2 receptors (Moran-Gates et al., 2007), since both receptors are expressed on the same striatal neurons in adult and perhaps young animals (Ariano et al., 1997; Tarazi et al., 1998). It is noteworthy that RSP-induced changes in 5-HT and DA receptors produced opposite effects on NMDA (*decrease*) and AMPA (*increase*) receptors in CPu, suggesting that the two ionotropic Glu receptor subtypes respond differently to long-term changes in forebrain 5-HT and DA neurotransmission during development; an effect that also extends into adulthood (Tarazi et al., 2003).

Long-term infusion of three doses of RSP did not alter the binding of [^{3}H]kainate to KA receptors in any brain region examined (Table 3). Lack of change in tissue levels of KA receptors may result from the very low affinity of RSP to KA receptors in juvenile rat brain tissue (Ki > 10 μM), or from a lack of indirect effects on secondary neural elements that may trigger changes in KA receptor binding. This finding agrees with previous autoradiographic studies that did not find changes in KA receptor levels after repeated administration of RSP and other dissimilar antipsychotic agents in adult animals (Spurney et al., 1999,Gao et al., 2000;Tarazi et al., 2003).

Several experimental manipulations have been reported to induce changes in concentrations of KA receptors. Long-term treatment with barbiturate reduced KA receptors in mouse cerebral cortex (Short and Tabakoff, 1993). In contrast, an increase in KA receptors was observed in rat hippocampus 24 hours after withdrawal from chronic treatment with PCP or ethanol (Gao and Tamminga, 1994; Carta et al., 2002), and in rat striatum after long-term nigrostriatal DA denervation (Tarazi et al., 2000). However, the current findings and that of other studies indicate that KA receptors have resisted adaptations to long-term treatment with RSP and dissimilar classes of antipsychotic drugs in juvenile and mature animals.

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 decreased NMDA receptor levels in NAc and increased AMPA receptors in MPC of juvenile and not adult animals. These new findings indicate that there are age-related differential responses to RSP in young vs. adult animals. However, there were similarities between both aged groups since the two doses of RSP (1.0 and 3.0 mg/kg) decreased the abundance of NMDA and increased levels of AMPA receptors in CPu of juvenile and adult animals. These new findings add support to the hypothesis that NMDA and AMPA receptor changes in the basal ganglia may contribute to the psychopharmacological actions of RSP across different age groups. Lack of change in KA receptors reflects its unique regulatory mechanisms in response to repeated treatment with RSP and other antipsychotics in juvenile and adult animals, and suggest that KA receptors are less likely to mediate the actions of RSP and other antipsychotic drugs in developing and mature animals.

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Table 1

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

		Juvenile Rats		*Adult Rats*
Brain region	**Controls**	**RSP (0.3 mg/kg)**	**RSP (1.0 mg/kg)**	**RSP (3.0 mg/kg)**	**RSP (3.0 mg/kg)**
Cerebral cortex					
Medial-prefrontal	291.2 ± 20.0 (100)	280.5 ± 25.0 (96)	302.6 ± 21.1 (104)	306.1 ± 29.2 (105)	(91)
Dorsolateral	324.1 ± 14.2 (100)	339.9 ± 18.3 (105)	344.1 ± 17.5 (106)	343.5 ± 23.7 (106)	(95)
Nucleus accumbens	245.7 ± 17.9 (100)	265.6 ± 14.2 (108)	183.3 ± 21.7 (**75**)*	168.3 ± 20.3 (**68**)*	(98)
Caudate-putamen					
Medial	264.6 ± 21.2 (100)	258.6 ± 17.4 (98)	188.5 ± 20.6 (**71**)*	179.9 ± 17.6 (**73**)*	(**67**)*
Lateral	273.8 ± 22.6 (100)	270.0 ± 21.7 (99)	208.1 ± 15.8 (**76**)*	193.9 ± 21.5 (**71**)*	(**65**)*
Hippocampus					
CA_1-Region	424.9 ± 16.5 (100)	392.3 ± 22.8 (92)	404.0 ± 25.2 (95)	415.1 ± 21.7 (98)	(**81**)*
CA_3-Region	328.2 ± 33.0 (100)	312.7 ± 31.9 (95)	314.7 ± 20.2 (96)	292.3 ± 18.8 (89)	(**78**)*
Entorhinal cortex	415.0 ± 25.2 (100)	403.9 ± 28.6 (97)	397.2 ± 44.5 (96)	412.4 ± 20.8 (99)	(98)

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., 2003) and are shown for comparison.

Table 2

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

		Juvenile Rats		*Adult Rats*
Brain region	**Controls**	**RSP (0.3 mg/kg)**	**RSP (1.0 mg/kg)**	**RSP (3.0 mg/kg)**	**RSP (3.0 mg/kg)**
Cerebral cortex					
Medial-prefrontal	223.3 ± 11.8 (100)	222.0 ± 10.3 (99)	284.5 ± 15.1 (**127**)*	287.6 ± 9.2 (**129**)*	(104)
Dorsolateral	227.9 ± 21.1 (100)	217.2 ± 11.1 (95)	236.3 ± 11.7 (104)	238.9 ± 10.6 (105)	(99)
Nucleus accumbens	255.7 ± 11.7 (100)	248.8 ± 10.9 (97)	269.8 ± 20.6 (106)	264.7 ± 14.7 (104)	(103)
Caudate-putamen					
Medial	194.8 ± 10.5 (100)	186.4 ± 13.6 (96)	253.2 ± 14.0 (**130**)*	245.9 ± 12.0 (**126**)*	(**130**)*
Lateral	199.3 ± 10.6 (100)	205.8 ± 13.5 (103)	255.7 ± 15.7 (**128**)*	250.0 ± 12.1 (**125**)*	(**131**)*
Hippocampus					
CA_1-Region	385.1 ± 27.7 (100)	369.3 ± 17.5 (96)	384.2 ± 29.7 (100)	372.1 ± 24.6 (97)	(104)
CA_3-Region	286.9 ± 19.6 (100)	260.5 ± 11.0 (91)	282.3 ± 11.5 (98)	283.0 ± 14.9 (99)	(101)
Entorhinal cortex	240.5 ± 10.0 (100)	236.1 ± 9.0 (98)	242.6 ± 7.7 (101)	245.3 ± 8.9 (102)	(107)

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., 2003) and are shown for comparison.

Table 3

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

		Juvenile Rats			*Adult Rats*
Brain region	**Controls**	**RSP (0.3 mg/kg)**	**RSP (1.0 mg/kg)**	**RSP (3.0 mg/kg)**	**RSP (3.0 mg/kg)**
Cerebral cortex					
Medial-prefrontal	120.8 ± 11.2 (100)	115.1 ± 12.1 (95)	118.0 ± 9.1 (98)	112.2 ± 9.3 (93)	(100)
Dorsolateral	96.8 ± 10.4 (100)	99.6 ± 9.8 (103)	91.7 ± 7.9 (95)	88.3 ± 7.0 (91)	(96)
Nucleus accumbens	136.6 ± 13.0 (100)	132.0 ± 11.5 (97)	131.4 ± 12.2 (96)	124.8 ± 11.2 (91)	(100)
Caudate-putamen					
Medial	111.1 ± 9.2 (100)	105.8 ± 7.5 (95)	106.0 ± 8.4 (95)	98.6 ± 10.7 (89)	(98)
Lateral	129.5 ± 11.8 (100)	120.5 ± 11.3 (93)	117.9 ± 10.8 (91)	115.6 ± 13.1 (89)	(99)
Hippocampus					
CA_1-Region	48.8 ± 5.2 (100)	51.7 ± 4.2 (106)	45.5 ± 4.7 (93)	44.3 ± 5.6 (91)	(101)
CA_3-Region	261.4 ± 15.6 (100)	252.0 ± 18.9 (96)	254.8 ± 14.5 (97)	258.6 ± 29.7 (99)	(104)
Entorhinal cortex	81.0 ± 7.1 (100)	81.8 ± 8.0 (101)	83.3 ± 6.1 (103)	82.5 ± 6.2 (102)	(96)

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., 2003) and are shown for comparison.