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Long-term effects of olanzapine, risperidone, and quetiapine on ionotropic glutamate receptor types: Implications for antipsychotic drug treatment

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Running Title:

Effects of newer antipsychotics on ionotropic glu receptors

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Abbreviations:

CPu, caudate-putamen (L, lateral and M, medial); DA, dopamine; DFC, dorsolateral-frontal cerebral cortex; EC, entorhinal cortex; Glu, glutamate; MPC, mesioprefrontal cortex; NAc, nucleus accumbens; 5-HT, serotonin.

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ABSTRACT

Levels of ionotropic glutamate (Glu) *N*-methyl-D-aspartic acid (NMDA), α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA), and kainic acid (KA) receptors in rat forebrain regions were compared by quantitative in vitro receptor autoradiography after continuous treatment for 28 d with the atypical antipsychotics olanzapine, risperidone, and quetiapine, or vehicle controls. All three treatments significantly decreased NMDA binding in caudate-putamen (CPu; by 30%, 34%, and 26%, respectively) but increased AMPA receptor levels in same region (by 22%, 30%, and 28%). Olanzapine and risperidone, but not quetiapine, also reduced NMDA receptor labeling in hippocampal CA_1 (21%, 19%) and CA_3 (23%, 22%) regions. KA receptors were unaltered by any treatment in the brain regions examined. These findings suggest that the antipsychotic effects of olanzapine and risperidone may be mediated in part by NMDA receptors in hippocampus, and perhaps AMPA receptors in CPu. The findings also support the hypothesis that down-regulation of NMDA receptors by atypical antipsychotic agents in CPu contributes to their low risk of extrapyramidal side effects. Inability of olanzapine, risperidone and quetiapine to alter KA receptors suggests their minimal role in mediating the CNS actions of these drugs.

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Glutamate (Glu), a major excitatory neurotransmitter in the mammalian central nervous system (CNS), exerts its neural effects by interacting with two major groups of Glu receptors, the ionotropic (coupled to ion channels) and metabotropic (coupled to intracellular second messengers) types (Conn and Pin, 1997; Ozawa et al., 1998). Three subtypes of ionotropic Glu receptors are defined by preferred ligands that selectively activate them: *N*-methyl-D-aspartic acid (NMDA), α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA), and kainate (KA; Ozawa et al., 1998).

Ionotropic Glu receptors have complex structures. NMDA receptors are comprised of four or five subunits that are encoded by genes NMDAR-1 and NMDAR-2A–NMDAR-2D (Hollman and Heinemann, 1994). The NMDAR-1 subunit is essential for expression of functional NMDA receptors and determines the pharmacology of receptor binding site (Hollmann and Heinemann, 1994). It has several critical sites including a phencyclidine (PCP) binding site located within the ion-channel that binds PCP, ketamine and other related compounds, a strychine-insensitive glycine binding site, and others for magnesium, zinc and polyamines (Javitt and Zukin, 1991; Hollman and Heinemann, 1994). AMPA receptors also are assembled from four or five subunits derived from a family of four genes (gluR1–gluR4). The pharmacological profile of the assembled AMPA receptor depends on the composition of each subunit (Hollmann et al., 1991; Hollmann and Heinemann, 1994). KA receptors are composed of different subunits derived from genes for the low affinity gluR5–gluR7 and high affinity KA1–KA2 subunits (Hollmann and Heinemann, 1994). Functional KA receptors are assembled from five identical or non-identical subunits into homomeric or heteromeric complexes that differ in their pharmacological properties (Lerma, 1998).

Dysfunction in glutamatergic neurotransmission may contribute to the pathophysiology of psychotic disorders including schizophrenia (Goff and Coyle, 2001;

Tsai and Coyle, 2002). Ionotropic Glu receptors, particularly of the NMDA type, have been implicated as a critical site of action of psychotomimetic agents including PCP, ketamine, and other anesthetics that can produce behavioral and cognitive deficits that resemble some symptoms of psychotic disorders (Javitt and Zukin, 1991; Tsai and Coyle, 2002). Agonists at the modulatory glycine binding site of the NMDA receptor complex are reported to improve *negative* (amotivation and cognitive) symptoms of schizophrenia (Goff and Coyle, 2001; Tsai and Coyle, 2002). In addition, pathological abnormalities and alterations in Glu receptor densities have been found in postmortem forebrain tissue from patients diagnosed with schizophrenia compared to healthy controls (Meador-Woodruff and Healy, 2000; Goff and Coyle, 2001). However, it is not clear whether observed changes in Glu receptors in such brain specimens reflect the neuropathology of schizophrenia or adaptation to antemortem drug exposure.

Preclinical studies indicate that the three ionotropic Glu receptors are altered by treatment with antipsychotic drugs (APDs), although the direction of reported changes has been inconsistent. Different studies report increases, decreases, or no change in levels of these receptors after long-term treatment with various APDs (Meshul et al., 1996; Tarazi et al., 1996; Giardino et al., 1997; McCoy et al., 1998; Spurney et al., 1999). Moreover, contradictory and often opposite findings have been reported in the expression of subunits composing different Glu receptors after chronic administration of APDs (Fitzgerald et al., 1995; Riva et al., 1997; Healy and Meador-Woodruff, 1997). Typical neuroleptics such as haloperidol, as well as the atypical antipsychotic agent clozapine were commonly employed in these studies. However, both types of APDs are associated with specific adverse neurological effects—extrapyramidal side effects (EPS), particularly parkinsonism, dystonia and tardive dyskinesia in case of typical neuroleptics, excessive sedation and dose-dependent risk of epileptic seizures with clozapine (Baldessarini and Tarazi, 2001; Tarsy et al., 2002).

In recent years, several APDs have emerged with low risks of EPS (Baldessarini and Tarazi, 2001; Waddington and Casey, 2000). Among them are the clozapine analogs olanzapine and quetiapine, and the benzisoxazole derivative risperidone. These compounds have undergone extensive pharmacological, neurochemical and behavioral characterization in animals (Arnt and Skarsfeldt, 1998; Waddington and Casey, 2000; Tarazi et al., 2001, 2002), as well as extensive clinical testing and application (Baldessarini and Tarazi, 2001; Tarsy et al., 2002). However, their long-term effects on ionotropic Glu receptors in mammalian forebrain are not well defined nor have they been compared quantitatively to those of other antipsychotics. Accordingly, we applied quantitative in vitro receptor autoradiography to assess regulation of NMDA, AMPA and KA receptors in selected forebrain regions of interest following long-term infusion of olanzapine, quetiapine or risperidone in rats. We hypothesized that these test agents would induce regionally selective changes in tissue levels of specific Glu receptors more closely resembling those of associated with treatment with clozapine than with haloperidol as a representative typical neuroleptic.

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Experimental Procedures

Materials and Animal Subjects. Radiochemicals from New England Nuclear, Perkin-Elmer (Boston, MA) were Glu receptor ligands: [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]-α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA; 83.4 Ci/mmol for AMPA receptors), and [vinylidene-^{3}H]-kainic acid (Ci/mmol for KA receptors). 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).

Donated drugs included olanzapine (Eli Lilly; Indianapolis, IN), risperidone (Janssen; Beerse, Belgium), and quetiapine fumarate (Zeneca; Cheshire, UK). 6-Cyano-7-nitroquinoxaline (CNQX), KA, 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 200–225 g, 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 Ionotropic Glu Receptor Affinity. The three antipsychotic drugs, olanzapine, risperidone and quetiapine initially were tested for affinity at the NMDA, AMPA and KA receptors, using a rat brain preparation as detailed previously (Reynolds et al., 1987; Wullner et al., 1994). For binding affinities of three APDs 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 x *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., 1998). 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 three APDs 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), 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

drugs were initially screened for affinity at NMDA, AMPA and KA receptors at a concentration of 10,000 and 100,000 nM, with lower concentrations added following initial inhibition of radioligand binding by at least 50%, to support estimates of IC_{50} and Ki.

Drug Treatment and Tissue Preparation. Four groups of rats (N=6) received control vehicle, olanzapine (5.0), risperidone (3.0), or quetiapine fumarate (10.0 mg/kg/d) by osmotic minipumps (Alzet Corp.; Palo Alto, CA) implanted subcutaneously (s.c.) on the upper back of each animal to provide continuous infusions for 28 d. Doses are based on those typically reported to be behaviorally and neurochemically active in rats (Moore et al., 1992; Ellenbroek et al., 1996; Tarazi et al., 2001, 2002). After 4 weeks of treatment, residual drug solution in each minipump was <5% of the original volume, as predicted, indicating adequate drug delivery. On day 28, rats were decapitated; brains were removed, quick-frozen in isopentane on dry ice, and stored at –80°C until autoradiographic analysis. Frozen sections (10 µm) were prepared in a cryostat at –20°C, mounted on gelatin-coated, glass microscopic slides, and stored at –80°C until use. Coronal brain sections were taken through caudate-putamen (CPu), nucleus accumbens septi (NAc), hippocampal regions CA_1 and CA_3, dorsolateral-frontal (DFC) and mesioprefrontal (MPC]) cerebral cortex, and the entorhinal cortical (EC) region. These selected extrapyramidal, limbic, and cortical brain regions-of-interest mediate cognitive, emotional, and motor behaviors that are typically disturbed in patients with psychotic disorders and believed to be altered by antipsychotic drug treatment (Baldessarini and Tarazi, 2001).

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 temp. (RT) in the appropriate specified buffer before incubating them with the radioligand to

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remove endogenous Glu and wash out any residual drug that may interfere with binding of the radioligands to Glu receptors.

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, 1998, 2000).

AMPA Receptors: Binding protocol was modified from Wullner et al., (1994). 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.

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, 1998, 2000).

Autoradiography and Image Analysis.

Radiolabeled slides and calibrated [^{3}H]standards (Amersham) were exposed to Hyperfilm (Eastman-Kodak; Rochester, NY). Radiolabeled slides and calibrated [^{3}H]standards were exposed to Hyperfilm for 21 ([^{3}H]AMPA and [^{3}H]KA), or 30 d ([^{3}H]MK-801) 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

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densitometric image analyzer (MCID-M4, Imaging Research; St. Catharines, Ontario). Brain regions of interest were outlined (Fig. 1) and their OD 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=6 rats/treatment). 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.

[Fig. 1 about here]

Statistical Analysis. Two-way analysis of variance (ANOVA) was employed to evaluate overall changes across treatments and brain regions for each assay. Given overall significance of effects for treatment, Fisher post-hoc tests were used to test for differences due to each drug treatment in preselected 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.

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Results

Experiments with rat brain homogenates indicated that olanzapine, risperidone and quetiapine all had very low affinity at NMDA, AMPA and KA receptors. At concentrations of 10–100 µM, olanzapine, risperidone and quetiapine inhibited binding of all three radioligands by only 0%–6% (all Ki > 10 µM).

The observed distribution of ionotropic Glu receptors accorded closely with our previous findings in rat brain (Tarazi et al., 1996, 1998) that NMDA and AMPA receptors are highly expressed in hippocampal areas (CA_1 > CA_3), followed by cerebral cortex, CPu and NAc (Tables 1, 2). In contrast, KA receptors were expressed selectively in the hippocampal CA_3 region, MPC, and NAc (Table 3). Two-way ANOVA measuring overall changes across drug treatments and brain regions for NMDA assay was highly significant ($p<0.001$). Four weeks of continuous infusion of olanzapine, risperidone and quetiapine reduced labeling of NMDA receptors in the medial (by 30%, 33% and 27%, respectively; $F [2; 20 df] = 8.7$, $p<0.001$) and lateral portions of caudate-putamen (by 31%, 35% and 24%, $F [2; 20 df] = 11.3$, $p<0.001$). In addition, olanzapine and risperidone, but not quetiapine, significantly decreased NMDA receptor binding in the CA_1 (by 21% and 19%, $F [2; 20 df] = 5.3$, $p<0.01$) and CA_3 (by 23% and 22%, $F=5.3$, $p<0.01$) regions of hippocampus (Table 1). There were no significant changes in NMDA receptor levels in cerebral cortical MPC, DFC and EC regions (Table 1).

Two-way ANOVA for AMPA receptor assay was also significant ($p<0.05$). Continuous administration of olanzapine, risperidone and quetiapine increased binding of AMPA receptors in medial CPu [by 19%, 30% and 26%, respectively, $F [2; 20 df] = 4.4$, $p<0.02$] and lateral [by 24%, 31% and 29%, $F = 4.9$, $p<0.001$] regions, with no significant changes in cortical or limbic brain regions (Table 2). Long-term infusion of all test agents failed to alter tissue concentrations of KA receptors in any brain region (Table 3).

[Tables 1-3 about here]

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Discussion

Long-Term Effects of Newer Antipsychotics on NMDA Receptors. Continuous treatment with olanzapine, risperidone and quetiapine significantly decreased binding of [^{3}H]MK-801 to NMDA receptors in medial and lateral CPu (Table 1). These effects were similar to previously reported effects of clozapine but not haloperidol (Tarazi et al., 1996). Another study also reported a trend to reduced NMDA receptor binding in striatum after chronic treatment with clozapine but not with haloperidol (Spurney et al., 1999). This effect of clozapine may result from its proposed antagonistic action at NMDA receptors (Lidsky et al., 1993). However, it is unlikely that the effects of olanzapine, risperidone, or quetiapine result from direct NMDA receptor blockade since the three drugs showed very low affinity for MK-801 binding sites (all Ki > 10 μM) based on our in vitro assays. Reductions in NMDA receptor binding induced by olanzapine, risperidone, and quetiapine in the CPu may arise indirectly from neurochemical changes initiated by known interactions of these drugs with other neurotransmission systems, including those for serotonin (5-HT) or DA, both of which may modulate glutamatergic neurotransmission (Aghajanian and Marek, 2000; Carlsson et al., 2001). Such mechanisms would seem to implicate post-transcriptional changes at the protein level since chronic treatment with olanzapine and quetiapine, was reported not to alter expression of mRNA levels for NMDA-forming subunits in rat striatum (Tascedda et al., 1999, 2001).

The three APDs tested in this study have potent interactions at serotonin (5-HT) receptors (Baldessarini and Tarazi, 2001), and continuous treatment with the same drugs increased concentrations of 5-HT$_{1A}$ receptors and decreased 5-HT$_{2A}$ receptor levels in rat frontal cortex (Tarazi et al., 2002). Drug-induced changes in availability and functional status of these 5-HT receptors in cerebral cortex may suppress Glu neurotransmission in corticostriatal projections innervating CPu, and lead to decreased expression of striatal NMDA receptors. There also is evidence that NMDA and DA D$_2$

receptors are co-expressed in the same striatal neurons (Ariano et al., 1997; Tarazi et al., 1998), and indications that close and often antagonistic functional, behavioral and cellular interactions occur between the same receptors (Cepeda et al., 1993; Carlsson et al., 2001). Accordingly, blockade and upregulation of D_2 receptors in rat CPu after continuous administration of olanzapine and risperidone (Tarazi et al., 2001) may contribute to the observed decreases in NMDA receptor labeling in that brain region.

More importantly, NMDA receptor activation may contribute to induction of the extrapyramidal side effects of typical neuroleptics. Conversely, NMDA receptor antagonism has reduced neuroleptic-induced catalepsy (Schmidt and Bubser, 1989; Yoshida et al., 1991) and blocked neuroleptic-induced expression of immediate early gene *c-fos* in striatal tissue (Boegman and Vincent, 1996). In contrast, NMDA receptor agonists potentiated haloperidol-induced catalepsy (Yoshida et al., 1991). Suppression of striatal NMDA receptor activity by the three APDs included in the present study may contribute to their relatively benign impact on the extrapyramidal system (Baldessarini and Tarazi, 2001; Tarsy et al., 2002).

Continuous treatment with olanzapine and risperidone decreased NMDA receptor binding in hippocampal CA_1 and CA_3 regions (Table 1). Functional impairment of Glu neurotransmission within the hippocampal formation might contribute to the pathophysiology of psychosis (Gao et al., 2000; Goff and Coyle, 2001; Tsai and Coyle, 2002). Lower levels of hippocampal NMDA receptors may act in synchrony with higher levels of hippocampal D_2 receptors induced by antipsychotic treatment (Tarazi et al., 2001), to improve psychotic symptoms by ameliorating hippocampal DA hyperactivity and restoring NMDA-sensitive Glu-mediated outputs from hippocampus to limbic and cortical brain areas (Krieckhaus et al., 1992; Gao et al., 2000). It is tempting to speculate that changes in hippocampal NMDA receptors may contribute uniquely to the beneficial clinical effects of olanzapine and risperidone since other antipsychotic agents, including

clozapine as well as quetiapine and haloperidol, did not induce such effects in hippocampus (Table 1; Tarazi et al., 1996).

Long-term effects of newer antipsychotics on AMPA and KA receptors. Continuous treatment with olanzapine, risperidone, or quetiapine significantly increased labeling of AMPA receptors in medial and lateral CPu, and not in other forebrain regions (Table 2). 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 binding sites or receptor-states labeled by each radioligand 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 with similar affinity (Nielsen et al., 1990; Hall et al., 1993). With AMPA receptors in CPu, long-term treatment with APDs seems to increase the high-affinity binding state selectively. This effect may be difficult to observe when 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). These changes in AMPA receptors probably reflect post-transcriptional modifications, since olanzapine and quetiapine did not alter expression of mRNA encoding different AMPA subunits in striatum (McCoy et al., 1998; Tascedda et al., 1999, 2001).

Our present findings also suggest that AMPA receptors represent a novel common site of action that may contribute to beneficial clinical effects of olanzapine, risperidone, and quetiapine. Antipsychotic-induced upregulation of AMPA receptors may restore cortico-striato-limbic Glu neurotransmission by normalizing reduced glutamatergic 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, have improved cognitive impairments in schizophrenia patients treated with clozapine (Goff et al., 2001).

Similar to NMDA receptors, it is unlikely that effects of olanzapine, risperidone, or quetiapine on AMPA receptors in CPu result from direct receptor blockade since we found all three APDs to have very low affinity for all three ionotropic Glu receptors (all Ki > 10 µM). However, indirect actions arising from the effects of these drugs on the central 5-HT system, again, may contribute to the increased AMPA receptor binding found in CPu (Table 2). These effects include opposite long-term effects of olanzapine, risperidone, and quetiapine on cortical 5-HT_{1A} (increases) and 5-HT_{2A} (decreases) receptors (Tarazi et al., 2002). Additional evidence for a direct interaction between 5-$HT_{1A/2A}$/AMPA receptors arises from studies finding that 5-HT_{2A} receptor stimulation increased release of Glu by pyramidal cells in layer-V of prefrontal cortex, which produces corticostriatal and corticotectal projections (Miller, 1988). The mechanism involved depends on stimulation of AMPA receptors (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 changes in cortical $5HT_{1A}$ (increase) and $5HT_{2A}$ receptors (decrease) after continuous treatment with the APDs included in the present study may alter corticostriatal AMPA-mediated Glu neurotransmission and lead to an increase in post-transcriptional expression of postsynaptic AMPA receptors in CPu.

Alternatively, the observed increase in AMPA receptors in rat CPu may result from antipsychotic-induced upregulation of D_2 receptors (Tarazi et al., 2001), since both receptors may be expressed on the same striatal neurons (Ariano et al., 1997; Tarazi et al., 1998). It is noteworthy that antipsychotic-induced changes in 5-HT and DA

receptors produced opposite effects on NMDA (*decrease*) and AMPA (*increase*) receptors in CPu, suggesting that these ionotropic Glu receptor subtypes respond differently to long-term changes in forebrain 5-HT and DA neurotransmission.

Long-term infusion of olanzapine, risperidone, or quetiapine 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 three APDs to KA receptors, as well as a lack of indirect effects of such treatment on secondary neural mechanisms 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 chronic administration of the dissimilar antipsychotic agents clozapine, haloperidol, and raclopride (Tarazi et al., 1996; Spurney et al., 1999, Gao et al., 2000). In contrast, long-term treatment of rats with haloperidol or clozapine increased KA2 mRNA levels in the CPu. Clozapine treatment also caused an increase in gluR7 mRNA expression, and a decrease in gluR3 mRNA expression in both cortex and striatum (Healy and Meador-Woodruff, 1997). However, these brain region-specific alterations in mRNA levels of KA receptor subunits was not associated with changes in KA receptor densities after treatment with haloperidol or clozapine, suggesting that post-trancriptional factors may also contribute to maintaining KA receptors at constant levels in brain tissue during exposure to APDs.

Changes in levels of KA receptors have been reported after various experimental manipulations in animals. Lower levels of KA receptors were found in mouse cerebral cortex after chronic barbiturate treatment (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). In addition, changes in the expression of KA receptor proteins or the mRNAs

encoding their different subunits have been observed in postmortem tissue from some patients with schizophrenia compared to healthy controls, although these findings have not been consistently replicated (Meador-Woodruff and Healy, 2000). It is likely that the reported abnormalities in KA receptors in postmortem schizophrenia brain tissue are not the result of antemortem drug exposure, since KA receptors have resisted adaptations to long-term treatment with typical, atypical and newer atypical antipsychotic agents, and are less likely to mediate the actions of dissimilar classes of APDs.

Conclusions. Similar to the actions of clozapine, and in contrast to a lack of effect of haloperidol, long-term treatment of rats with olanzapine, risperidone, or quetiapine significantly downregulated NMDA receptors in medial and lateral CPu (Table 1). These new findings add support to the hypothesis that these receptor decreases of NMDA receptors in the basal ganglia may contribute to the relatively benign profile of clinical EPS with these agents (Baldessarini and Tarazi, 2001; Tarsy et al., 2001). In addition, both olanzapine and risperidone decreased levels of NMDA receptors in hippocampal CA_1 and CA_3 regions but not other cortical areas (including DFC and EC), suggesting a possible common site contributing to beneficial effects of newer atypical antipsychotics.

At behaviorally and neurochemically effective doses, olanzapine, risperidone and quetiapine also increased abundance of AMPA receptors in medial and lateral CPu, indicating that AMPA receptors in these brain regions constitute common targets that mediate the actions of newer APDs. Failure of these atypical APDs to alter abundance of KA receptors in any rat brain region examined adds support to the view that this ionotropic Glu receptor type is unlikely to contribute to the clinical actions of various kinds of antipsychotic agents.

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References

Aghajanian GK and Marek GJ (2000) Serotonin model of schizophrenia: emerging role of glutamate mechanisms. *Brain Res Rev* **31**:302–312.

Ariano MA, Larson ER, Noblett KL, Sibley DR, and Levine MS (1997) Coexpression of striatal dopamine receptor subtypes and excitatory amino acid subunits. *Synapse* **26**:400–414.

Arnt J and Skarsfeldt T (1998) Do novel antipsychotics have similar pharmacological characteristics? A review of the evidence. *Neuropsychopharmacology* **18**:63–101.

Baldessarini RJ and Tarazi FI (2001) Drugs and the treatment of psychiatric disorders, in *Goodman and Gilman's The Pharmacological Basis of Therapeutics* (Hardman JG, Limbird LE, Molinoff PB, Ruddon RW and Gilman AG, eds) pp. 485-520, McGraw-Hill, New York.

Boegman RJ, Vincent SR (1996) Involvement of adenosine and glutamate receptors in the induction of c-fos in the striatum by haloperidol. *Synapse* **22**:70–77.

Cai X, Gu Z, Zhong P, Ren Y, and Yan Z (2002) Serotonin 5-HT_{1A} receptors regulate AMPA receptor channels through inhibiting Ca^{2+}/calmodulin-dependent kinase II in prefrontal cortical pyramidal neurons. *J Biol Chem* **277**:36553–36562.

Carlsson A, Waters N, Holm-Waters S, Tedroff J, Nilsson M, and Carlsson ML (2001) Interactions between monoamines, glutamate, and GABA in schizophrenia: new evidence. *Ann Rev Pharmacol Toxicol* **41**:237–260.

Carta M, Olivera DS, Dettmer TS, Valenzuela CF (2002) Ethanol withdrawal upregulates kainate receptors in cultured rat hippocampal neurons. *Neurosci Lett* **327**:128–132.

Cepeda C, Buchwald NA, Levine MS (1993) Neuromodulatory actions of dopamine in the neostriatum are dependent upon the excitatory amino acid receptor subtypes activated. *Proc Natl Acad Sci USA* **90**:9576–9580.

Conn PJ and Pin JP (1997) Pharmacology and functions of metabotropic glutamate receptors. *Ann Rev Pharmacol Toxicol* **37**:205–237.

Ellenbroek BA, Lubbers LJ, and Cools AR (1996) Activity of Seroquel (ICI-204,636) in animal models for atypical properties of antipsychotics, comparison with clozapine. *Neuropsychopharmacology* **15**:406–416.

Fitzgerald LW, Deutch AY, Gasic G, Heinemann SF, and Nestler EJ (1995) Regulation of cortical and subcortical glutamate receptor subunit expression by antipsychotic drugs. *J Neurosci* **15**:2453–2461.

Gao XM, Sakai K, Roberts RC, Conley RR, Dean B, and Tamminga CA (2000) Ionotropic glutamate receptors and expression of N-methyl-D-aspartate receptor subunits in subregions of human hippocampus: effects of schizophrenia. *Am J Psychiatry* **157**:1141–11499.

Gao XM, Tamminga CA (1994) An increase in NMDA-sensitive [^{3}H]glutamate and [^{3}H]kainate binding in hippocampus 24 hours after PCP. *Neurosci Lett* **174**:149–153.

Giardino L, Bortolotti F, Orazzo C, Pozza M, Monteleone P, Calza L, and Maj M (1997) Effect of chronic clozapine administration on [^{3}H]MK801-binding sites in the rat brain: a side-preference action in cortical areas. *Brain Res* **762**:216–218.

Goff DC, Coyle JT (2001) The emerging role of glutamate in the pathophysiology and treatment of schizophrenia. *Am J Psychiatry* **158**:1367–1377.

Goff DC, Leahy L, Berman I, Posever T, Herz L, Leon AC, Johnson SA, and Lynch G (2001) A placebo-controlled pilot study of the ampakine CX516 added to clozapine in schizophrenia. *J Clin Psychopharmacol* **21**:484–487.

Hall RA, Massicotte G, Kessler M, Baudry M, and Lynch G (1993) Thiocyanate equally increases affinity for two DL-alpha-amino-3-hydroxy-5-methylisoxazolepropionic acid (AMPA) receptor states. *Mol Pharmacol* **43**:459–464.

Healy DJ and Meador-Woodruff JH (1997) Clozapine and haloperidol differentially affect AMPA and kainate receptor subunit mRNA levels in rat cortex and striatum. *Mol Brain Res* **47**:331–338.

Hollmann M, Hartley M, and Heinemann S (1991) Ca^{2+} permeability of KA-AMPA--gated glutamate receptor channels depends on subunit composition. *Science* **252**:851–853.

Hollmann M and Heinemann S (1994) Cloned glutamate receptors. *Ann Rev Neurosci* **17**:31–108.

Javitt DC and Zukin SR (1991) Recent advances in the phencyclidine model of schizophrenia. *Am J Psychiatry* **148**: 1301–1308.

Krieckhaus EE, Donahoe JW and Morgan MA (1992) Paranoid schizophrenia may be caused by dopamine hyperactivity of CA1 hippocampus. *Biol Psychiatry* **31**:560–570.

Lerma J (1998) Kainate receptors: an interplay between excitatory and inhibitory synapses. *FEBS Lett* **430**:100–104.

Lidsky TI, Yablonsky-Alter E, Zuck L, Banerjee SP (1993) Anti-glutamatergic effects of clozapine. *Neurosci Lett* **163**:155–158.

McCoy L, Cox C, and Richfield EK (1996) Chronic treatment with typical and atypical antipsychotics increases the AMPA-preferring form of AMPA receptor in rat brain. Eur J Pharmacol 318:41–45.

McCoy L, Cox C, and Richfield EK (1998) Antipsychotic drug regulation of AMPA receptor affinity states and GluR1, GluR2 splice variant expression. *Synapse* **28**:195–207.

Meador-Woodruff JH and Healy DJ (2000) Glutamate receptor expression in schizophrenic brain. *Brain Res Rev* **31**:288-294.

Meshul CK, Bunker GL, Mason JN, Allen C, and Janowsky A (1996) Effects of subchronic clozapine and haloperidol on striatal glutamatergic synapses. *J Neurochem* **67**:1965–1973.

Moore NA, Tye NC, Axton MS, and Risius FC (1992) The behavioral pharmacology of olanzapine, a novel “atypical” antipsychotic agent. *J Pharmacol Exp Ther* **270**:713–721.

Miller MW (1988) Development of projection and local circuit neurons in neocortex, in *Cerebral Cortex, Development and Maturation of Cerebral Cortex,* (Peters A and Jones EG, eds) pp.133-175, Plenum Press, New York.

Nielsen EO, Drejer J, Cha JH, Young AB, and Honore T (1990) Autoradiographic characterization and localization of quisqualate binding sites in rat brain using the antagonist [^{3}H]6-cyano-7-nitroquinoxaline-2,3-dione: comparison with (R,S)-[^{3}H]alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid binding sites. *J Neurochem* **54**:686–695.

Ozawa S, Kamiya H, and Tsuzuki K (1998) Glutamate receptors in the mammalian central nervous system. *Prog Neurobiol* **54**:581–618.

Paxinos F and Watson C (1982) The Rat Brain in Stereotaxic Coordinates. New York, Academic Press.

Reynolds IJ, Murphy SN, Miller RJ (1987) ^{3}H-labeled MK-801 binding to the excitatory amino acid receptor complex from rat brain is enhanced by glycine. *Proc Natl Acad Sci USA* **84**:7744–7748.

Riva MA, Tascedda F, Lovati E, and Racagni G (1997) Regulation of NMDA receptor subunit messenger RNA levels in the rat brain following acute and chronic exposure to antipsychotic drugs. *Mol Brain Res* **50**:136–142.

Schmidt WJ, Bubser M (1989) Anticataleptic effects of the N-methyl-D-aspartate antagonist MK-801 in rats. *Pharmacol Biochem Behav* **32**: 621–623.

Short KR, Tabakoff B (1993) Chronic barbiturate treatment increases NMDA receptors but decreases kainate receptors in mouse cortex. *Eur J Pharmacol* **230**: 111–114.

Spurney CF, Baca SM, Murray AM, Jaskiw GE, Kleinmann JE, and Hyde TM (1999) Differential effects of haloperidol and clozapine on ionotropic glutamate receptors in rats. *Synapse* **34**:266–276.

Tarazi FI, Campbell A, Yeghiayan SK, and Baldessarini RJ (1998) Localization of glutamate receptor subtypes in caudate-putamen and nucleus accumbens septi: Comparison of NMDA, AMPA and kainate receptors. *Synapse* **30**:227-235.

Tarazi FI, Florijn WJ, and Creese I (1996) Regulation of ionotropic glutamate receptors following subchronic and chronic treatment with typical and atypical antipsychotics. *Psychopharmacology* **128**:371–379.

Tarazi FI, Zhang K, Baldessarini RJ (2000) Effects of nigrostriatal dopamine denervation on ionotropic glutamate receptors in rat caudate-putamen. *Brain Res* **881**:69–72.

Tarazi FI, Zhang K, and Baldessarini RJ (2001) Long-term effects of olanzapine, risperidone, and quetiapine on dopamine receptor types in regions of rat brain: Implications for antipsychotic drug treatment. *J Pharmacol Exp Ther* **297**:711–717.

Tarazi FI, Zhang K, and Baldessarini RJ (2002) Long-term effects of olanzapine, risperidone, and quetiapine on serotonin receptors 1A, 2A and 2C in rat forebrain *Psychopharmacology* **161**:263–270.

Tarsy D, Baldessarini RJ, Tarazi FI (2002) Atypical antipsychotic agents: Effects on extrapyramidal function. *CNS Drugs* **16**:23–45.

Tascedda F, Blom JM, Brunello N, Zolin K, Gennarelli M, Colzi A, Bravi D, Carra S, Racagni G, and Riva MA (2001) Modulation of glutamate receptors in response to the novel antipsychotic olanzapine in rats. *Biol Psychiatry* **50**:117–122.

Tascedda F, Lovati E, Blom JM, Muzzioli P, Brunello N, Racagni G, and Riva MA (1999) Regulation of ionotropic glutamate receptors in the rat brain in response to the atypical antipsychotic seroquel (quetiapine fumarate). *Neuropsychopharmacology* **21**:211-217.

Tsai G and Coyle JT (2002) Glutamatergic mechanisms in schizophrenia. Ann Rev Pharmacol Toxicol **42**:165–179.

Yoshida Y, Ono T, Kizu A, Fukushima R, Miyagishi T (1991) Striatal N-methyl-D-aspartate receptors in haloperidol-induced catalepsy. *Eur J Pharmacol* **203**:173–180.

Waddington JL and Casey D (2000) Comparative pharmacology of classical and novel (second-generation) antipsychotics, in *Schizophrenia and Mood Disorders* (Waddington JL and Buckley PF eds), pp 1–13, Butterworth-Heinemann, Oxford.

Wullner U, Testa CM, Catania MV, Young AB, and Penney JB (1994) Glutamate receptors in striatum and substantia nigra: Effects of medial forebrain bundle lesions. *Brain Res* **645**:98–102.

Footnotes

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Table 1.

NMDA receptor binding after four weeks of continuous infusion of antipsychotic drugs

Brain region	Controls	Olanzapine	Risperidone	Quetiapine	Clozapine[#]	Haloperidol[#]
Cerebral cortex						
Medial-prefrontal	266 ± 24.7 (100)	257 ± 17.7 (97)	242 ± 17.4 (91)	282 ± 23.3 (106)	(**77**)*****	(**83**)*****
Dorsolateral	238 ± 24.1 (100)	248 ± 13.0 (104)	227 ± 15.7 (95)	252 ± 15.1 (106)	(100)	(95)
Entorhinal cortex	327 ± 13.1 (100)	292 ± 5.8 (89)	317 ± 13.5 (97)	320 ± 18.6 (98)	(100)	(108)
Hippocampus						
CA_1 region	466 ± 18.5 (100)	368 ± 19.1 (**79**)*****	379 ± 16.8 (**81**)*****	446 ± 29.8 (96)	(96)	(106)
CA_3 region	306 ± 17.4 (100)	236 ± 19.0 (**77**)*****	240 ± 8.4 **(78)***	303 ± 32.0 (99)	(102)	(104)
Nucleus Accumbens	235 ± 16.6 (100)	219 ± 17.1 (93)	230 ± 14.6 (98)	225 ± 14.8 (96)	(91)	(98)
Caudate-putamen						
Medial	241 ± 13.6 (100)	170 ± 12.4 (**70**)*****	161 ± 12.8 (**67**)*****	175 ± 11.0 **(73)***	(**74**)*****	(98)
Lateral	247 ± 9.7 (100)	172 ± 8.6 (**69**)*****	162 ± 14.2 (**65**)*****	189 ± 12.3 **(76)***	(**85**)*****	(100)

Data are mean ± SEM values for binding (fmol/mg tissue and [% of control]), determined by quantitative autoradiography following continuous subcutaneous infusion of vehicle or antipsychotic drugs for 4 weeks, with significant differences from controls indicated in bold ([*****] $p<0.05$, N=6 rats/group), all as described in Methods. [#] Data (% of control) for clozapine (25 mg/kg/d) and haloperidol (1.5 mg/kg/d) were determined previously (Tarazi et al., 1996), and are shown for comparison.

Table 2.

AMPA receptor binding after four weeks of continuous infusion of antipsychotic drugs

Brain region	Controls	Olanzapine	Risperidone	Quetiapine	Clozapine[#]	Haloperidol[#]
Cerebral cortex						
Medial-prefrontal	437 ± 11.4 (100)	434 ± 23.7 (99)	454 ± 16.2 (104)	475 ± 18.2 (109)	(96)	(102)
Dorsolateral	382 ± 4.4 (100)	376 ± 29.4 (98)	380 ± 14.9 (99)	380 ± 16.3 (99)	(94)	(90)
Entorhinal cortex	435 ± 10.1 (100)	416 ± 8.5 (96)	464 ± 9.4 (107)	453 ± 18.2 (104)	(112)	(101)
Hippocampus						
CA_1 region	650 ± 12.3 (100)	633 ± 21.3 (97)	673 ± 14.1 (104)	672 ± 18.4 (103)	(106)	(102)
CA_3 region	448 ± 11.5 (100)	422 ± 19.0 (94)	454 ± 16.9 (101)	453 ± 22.6 (101)	(117)	(116)
Nucleus Accumbens	434 ± 12.0 (100)	438 ± 18.4 (101)	446 ± 13.0 (103)	461 ± 14.2 (106)	(100)	(99)
Caudate-putamen						
Medial	282 ± 5.3 (100)	335 ± 13.1 (**119**)*****	365 ± 21.0 (**130**)*****	356 ± 25.2 (**126**)*****	(102)	(97)
Lateral	284 ± 4.3 (100)	352 ± 12.6 (**124**)*****	373 ± 24.2 (**131**)*****	366 ± 24.5 (**129**)*****	(109)	(107)

Data are mean ± SEM values for binding (fmol/mg tissue and [% of control]), determined by quantitative autoradiography following continuous subcutaneous infusion of vehicle or antipsychotic drugs for 4 weeks (N=6 rats/group), all as described in Methods. [#] Data (% of control) for clozapine (25 mg/kg/d) and haloperidol (1.5 mg/kg/d) were determined previously (Tarazi et al., 1996), and are shown for comparison.

Table 3.

Kainate receptor binding after four weeks of continuous infusion of antipsychotic drugs

Brain region	Controls	Olanzapine	Risperidone	Quetiapine	Clozapine[#]	Haloperidol[#]
Cerebral cortex						
Medial-prefrontal	121 ± 21.6 (100)	123 ± 15.8 (102)	121 ± 19.7 (100)	111 ± 16.8 (92)	(95)	(101)
Dorsolateral	95.3 ± 14.3 (100)	91.0 ± 11.3 (95)	91.1 ± 15.0 (96)	85.8 ± 11.0 (90)	(99)	(100)
Entorhinal cortex	93.9 ± 12.4 (100)	90.5 ± 8.0 (96)	90.2 ± 8.4 (96)	87.5 ± 10.4 (93)	(99)	(104)
Hippocampus						
CA_1 region	50.8 ± 3.5 (100)	57.2 ± 4.9 (113)	51.2 ± 3.5 (101)	50.0 ± 6.5 (98)	(95)	(91)
CA_3 region	148 ± 17.9 (100)	146 ± 16.7 (98)	155 ± 24.1 (104)	152 ± 22.9 (103)	(107)	(102)
Nucleus Accumbens	131 ± 20.7 (100)	132 ± 15.2 (101)	131 ± 21.8 (100)	123 ± 19.3 (94)	(90)	(110)
Caudate-putamen						
Medial	108 ± 16.1 (100)	106 ± 12.0 (98)	101 ± 15.4 (93)	104 ± 12.5 (96)	(98)	(113)
Lateral	116 ± 17.9 (100)	115 ± 14.4 (99)	106 ± 16.3 (91)	112 ± 14.0 (96)	(98)	(109)

Data are mean ± SEM values for binding (fmol/mg tissue and [% of control]), determined by quantitative autoradiography following continuous subcutaneous infusion of vehicle or antipsychotic drugs for 4 weeks (N=6 rats/group), all as described in Methods. [#] Data (% of control) for clozapine (25 mg/kg/d) and haloperidol (1.5 mg/kg/d) were determined previously (Tarazi et al., 1996), and are shown for comparison.

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Figure Legend

Fig. 1.

Sites of autoradiographic analyses of rat brain regions sampled in 10 µm coronal sections from: **A** (A 3.2–4.2), **B** (A 1.7–2.2), **C** (A 0.7–1.2), and **D** (A 0.2–0.7 mm anterior to bregma, according to Paxinos & Watson (1982). Abbreviations: **NAc**, nucleus accumbens septi; **CPu**, caudate-putamen (**L**, lateral and **M**, medial); **DFC**, dorsolateral-frontal cerebral cortex; **EC**, entorhinal cortex; **CA_1 and CA_3**, hippocampal regions; **MPC**, medial prefrontal cortex.

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