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Differential Effects of Haloperidol and Clozapine on Ionotropic Glutamate Receptors in Rats

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ABSTRACT Despite multiple lines of investigation the effect of neuroleptics on glutamate-mediated neurotransmission remains controversial. To study the effects of typical and atypical neuroleptics on selected parameters of glutamate-mediated neurotransmission, male Sprague-Dawley rats were randomly assigned to a 21-day oral treatment course with vehicle, haloperidol (HDL), or clozapine (CLZ). Coronal slices of rat brain were then incubated with tritiated ligands to measure NMDA, AMPA, and kainate receptor, and glutamate reuptake site density. Regions of interest included the frontal cortex, anterior cingulate cortex, dorsal striatum, ventral striatum, and the nucleus accumbens. CLZ increased the density of AMPA receptors significantly in the frontal and anterior cingulate cortices compared with normal controls. In the dorsal and ventral striatum, and nucleus accumbens as a whole, CLZ-treated rats had a higher AMPA receptor density compared with both the HDL- and vehicle-treated controls. Additionally, within the nucleus accumbens, CLZ-treated rats had a higher density of AMPA receptors compared with the HDL group in the core, and at trend level in the shell. There was a group by region interaction for NMDA receptor density, primarily reflecting the tendency of HDL treated rats to have high receptor densities in the frontal and anterior cingulate cortices. Kainate receptors and glutamate reuptake site densities did not differ significantly across groups. These results suggest a critical role for glutamate in the mediation of atypical antipsychotic drug action in anatomically-specific regions, and further encourage the investigation of glutamate neurotransmitter systems in schizophrenia. **Synapse 34:266–276, 1999.** Published 1999 Wiley-Liss, Inc.†

INTRODUCTION

Neuroleptics are the mainstay of the pharmacological treatment for schizophrenia. The role of glutamate, an excitatory amino acid neurotransmitter, and the effects of neuroleptics on glutamatergic neurotransmission in the pathophysiology of schizophrenia, are controversial topics. Both typical and atypical neuroleptics have significant effects on glutamate-mediated neurotransmission, suggesting a role for glutamate in the pathophysiology of psychosis. Haloperidol (HDL), a typical neuroleptic, is a potent antagonist of the dopamine (DA) D_2 receptor (Seeman, 1980). Activation of D_2 receptors reduces glutamate release in the striatum (Crowder and Bradford, 1987; Mitchell and Daggett, 1980), whereas agonists to the NMDA receptor complex mostly increase DA release (Carter, 1988; Clow and Jahamandas, 1989; Imperato et al., 1990; Krebs et al., 1991). Chronic neuroleptic treatment has also been implicated in DA supersensitivity, via a decrease in intrastriatal glutamate transmission, which may be partially responsible for the antipsychotic effects of HDL (Freed, 1988). These findings collectively suggest that typical neuroleptics might have significant effects on the glutamate systems that may underlie some of their therapeutic actions.

Clozapine (CLZ) is an atypical but extremely potent neuroleptic effective in treating refractory schizophrenia. In contrast to HDL, CLZ treatment induces relatively few extrapyramidal side effects (Meltzer, 1991). CLZ has an unusual pharmacological profile compared to HDL and other typical neuroleptics (Altar et al., 1986; Goldstein et al., 1989; Huttunen, 1995; Meltzer et

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al., 1989; Wiesel et al., 1994). However, the precise characteristics that confer its atypical status remain speculative because CLZ acts on so many neurochemical systems (Deutch et al., 1991; Kerwin, 1994). A number of neurotransmitters, including glutamate, could contribute to CLZ's atypical profile and clinical efficacy (Yamamoto et al., 1994). A modulatory role for CLZ on glutamate neurotransmission can be inferred from its ability to suppress striatal responses from glutamate afferents even after DA depletion (Lidsky and Banerjee, 1992). Moreover, CLZ has been reported to differ from HDL in its effect on glutamate release in the striatum (Yamamoto and Cooperman, 1994). However, both HDL and CLZ can directly displace the binding of tritiated MK-801 from the NMDA receptor (Janowsky and Berger, 1989; Lidsky et al., 1993). The direct effect of CLZ on glutamate receptor density is controversial. Electrophysiologically, CLZ potentiates NMDA-mediated neurotransmission, while HDL depresses the AMPA-mediated response in rat prefrontal cortex (Arvanov et al., 1997). The effect of CLZ on the AMPA-mediated response showed a trend in the same direction. These findings suggest a differential effect of these drugs on central glutamate systems.

The glutamate hypothesis of schizophrenia was inspired by the finding of decreased glutamate levels in the cerebrospinal fluid (CSF) of schizophrenics (Kim et al., 1980). Subsequently, phencyclidine (PCP) was found to cause psychotic symptoms at least in part through NMDA receptor blockade, prompting a second-generation hypothesis of abnormal NMDA receptor function in schizophrenia (Anis et al., 1983). Recent data regarding a glutamate hypothesis has been limited and conflicting. Several studies failed to find decreased CSF levels of glutamate in schizophrenic patients (Gattaz et al., 1985; Korpi et al., 1987), although others found an inverse correlation between CSF glutamate levels and positive symptoms in schizophrenic patients (Faustman et al., 1999). A post-mortem study of glutamate levels did not find significant changes in the frontal cortex, caudate nucleus, putamen, thalamus, nucleus accumbens, or olfactory tubercle (Perry, 1982). Nevertheless, the psychotogenic effects of NMDA antagonists are well established, suggesting that glutamate may play a role in the generation of psychotic symptoms in schizophrenia.

Post-mortem studies of glutamate receptors in schizophrenia vary by region and receptor sub-type. Two reports noted increased kainate receptor density in the medial frontal and "eye movement" frontal cortices (Nishikawa et al., 1983; Toru et al., 1988). Another found increased kainate receptor density in the orbital frontal cortex of schizophrenics (Deakin et al., 1989). In separate studies, Kerwin and colleagues reported decreased kainate receptor levels in the hippocampus and parahippocampal gyrus (Kerwin et al., 1988, 1990). Increased levels of NMDA NR2 receptor subunit mRNA expression have been demonstrated in the prefrontal cortex of schizophrenics, suggesting that the frontal lobe deficits seen in schizophrenia may be related to decreased glutamate neurotransmission or receptor function (Akbarian et al., 1996). In 1989, Kornhuber and colleagues found a significant increase in NMDA receptor density in the putamen, among other regions (Kornhuber et al., 1989). This finding has been bolstered by a report of deficient glutamate reuptake sites in the basal ganglia (Simpson et al., 1992). Noga and colleagues did not find changes in basal ganglia NMDA or kainate receptor density of schizophrenic patients, but reported a significant increase in AMPA receptor density in the ventral caudate (Noga et al., 1997). No consensus has emerged regarding the site or type of glutamate receptor changes associated with schizophrenia. More importantly, the changes noted in post-mortem tissue may be secondary to neuroleptics rather than reflecting the primary pathology of schizophrenia.

Despite the notion that glutamate systems in general, and NMDA receptors in particular, may be involved in psychosis, the effects of the antipsychotics on ionotropic glutamate receptor systems have received little attention until recently. Several studies have examined the effects of neuroleptics on glutamate receptor systems, but a clear consensus is lacking (Fitzgerald et al., 1995; Hamid et al., 1998; Healy and Meador-Woodruff, 1997; Kakigi et al., 1992; McCoy et al., 1998; Oretti et al., 1994; Tarazi et al., 1996). To further elucidate the involvement of glutamate receptor systems in both typical and atypical neuroleptic actions, we investigated the effects of subacute HDL or CLZ treatment on glutamate ionotropic receptor densities and glutamate reuptake sites in selected regions of rat brain. Regions were selected for their putative involvement in the pathophysiology of psychosis. Antipsychotic drugs were delivered in drinking water at doses chosen to approximate those used clinically. We postulated that subacute CLZ and HDL treatments would differentially affect glutamate receptors due to their separate pharmacological profiles.

MATERIALS AND METHODS

Material

The ligands [vinylidene-^{3}H]-kainic acid (58.0 Ci/mmol), d-[2,3-^{3}H]-aspartic acid (11.5 Ci/mmol), (+)-[3-^{3}H]-MK-801 (20.3 Ci/mmol) and [5-^{3}H]-CNQX (26.7 Ci/mmol) were obtained from Dupont-NEN (Boston, MA). Kainate and d-aspartic acid were obtained from Research Biochemicals International (Natick, MA). L-Glutamate, ketamine and all other chemicals were provided by Sigma Chemical Company (St. Louis, MO).

Treatment groups

Male Sprague-Dawley rats (Zivic-Miller), 200–250 g, were housed 3/cage with food and water ad libitum on a

standard 12-hour dark/light cycle. Animals were randomly assigned to a 21-day treatment course with vehicle (VEH), haloperidol (HDL) or clozapine (CLZ). Antipsychotic drugs were delivered in drinking water. Clozapine has a very short half-life in rodents, and single daily intraperitoneal injections do not result in sustained levels of clozapine in the brain (Baldessarini et al., 1993). Animals received either VEH (dilute tartaric acid buffered to pH 7.3 with $NaHCO_3$), HDL (1 mg/kg/d) or CLZ (20 mg/kg/d). Both HDL and CLZ were first dissolved in 0.1 M tartaric acid, brought to volume with distilled water and buffered to pH 7.1–7.3 with 1 M $NaHCO_3$. Solutions were replenished every two days.

This experiment utilized three groups of rats: Group (1)—rats treated with VEH (n = 11), Group (2)—rats treated 21 days with HDL (n = 6), and Group (3)—rats treated 21 days with CLZ (n = 8). All procedures involving animal use were in strict compliance with the NIH Guide for the Care and Use of Laboratory Animals.

Preparation of brain tissue

After a 72-hour period of drug withdrawal, animals were sacrificed without anesthesia. Their brains were removed rapidly, quick-frozen on dry ice, and stored at −70°C. Twenty-micron thick coronal sections were cut rostrocaudally using a cryostat (Jung Frigocut, Nussloch, Germany) and thaw-mounted onto acid-scrubbed, double-subbed, gelatin-coated slides. Sections were taken at the level of the anterior commissure. Slide mounted sections were stored in slide boxes sealed in plastic at −70°C until used in assays.

Quantitative receptor autoradiography

Before experiments, all sections were thoroughly blown dry with cool air for 30 minutes. After experiments, they were blown dry with cool air for 3 minutes.

CNQX

Preincubation consisted of 15 minutes at 4°C followed by 15 minutes at 30°C in a 50 mM Tris-HCl buffer at pH 7.2. Sections were then incubated for 45 minutes at 4°C in buffer containing 50 nM ^{3}H-CNQX. Non-specific binding was determined using 1 mM L-glutamic acid (modified from Kerwin et al., 1988). Sections were then rapidly dipped three times in ice cold 50 mM Tris-HCl buffer.

MK-801

Sections were incubated for 150 minutes in a buffer containing 30 mM EPPS, 100 µM L-glutamate, 100 µM L-glycine, and 1 mM EDTA (pH 7.45) at room temperature. Total binding was determined by the addition of 16 nM ^{3}H-MK-801 and non-specific binding with the addition of 200 µM ketamine. Sections were then washed twice in fresh ice cold buffer that did not contain either glutamate or glycine for a total of 40 minutes (modified from Subramaniam and McGonigle, 1991).

Kainic acid

Preincubation consisted of 15 minutes at 4°C followed by 15 minutes at 30°C in a 50 mM Tris-HCl buffer at pH 7.0. Incubation occurred for 120 minutes at 4°C in buffer containing 24 nM ^{3}H-kainic acid. Non-specific binding was determined by the addition of 100 µM kainate. The sections were then rapidly dipped three times in fresh ice cold 50 mM Tris-HCl buffer with a final dip in ice cold distilled water (McGonigle, personal communication).

D-aspartate

Preincubation consisted of 10 minutes at 30°C in a 50 mM Tris HCl buffer containing 300 mM NaCl at pH 7.4. Sections were then incubated for 20 minutes at 4°C in a buffer containing 100 nM ^{3}H-d-aspartic acid. Non-specific binding was determined using 100 µM cold d-Aspartic acid (modified from Anderson et al., 1990). Sections were rinsed four times for a total of 30 seconds in ice cold 50 mM Tris-HCl and 300 mM NaCl buffer.

Image generation and analysis

All sections were placed in boxes along with desiccant overnight before being placed into cassettes with tritiated microscales (Amersham, Arlington Heights, IL) and apposed to [^{3}H] Hyperfilm (LKB Instruments, Gaithersburg, MD). Exposure times were four days for CNQX and kainic acid, 15 days for d-aspartate and 13 days for MK-801. Films were developed and scanned into a Macintosh computer (Apple Computers, Cupeztino, CA). Receptor densities were measured using an image analysis system (NIH Image 1.52, Wayne Rasband, RSB, NIMH).

Quantitative densitometry was used to measure receptor density in the frontal cortex, anterior cingulate cortex, striatum, and nucleus accumbens. The striatum was subdivided into dorsal and ventral regions, while the nucleus accumbens was subdivided into the core and shell [see Fig. 1]. Each slide contained two sections and the four measured areas from the films were averaged. Specific binding was determined by subtracting nonspecific binding from the total binding.

Statistics

Comparisons between groups were carried out using a repeated measures analysis of variance (rmANOVA) for each ligand, to look for a main effect of group, and the effects of region, and group by region interactions. If there was a main effect of group ($p < 0.05$), a one-way ANOVA was conducted for each brain region, followed by post-hoc Fisher's PLSD t-tests.

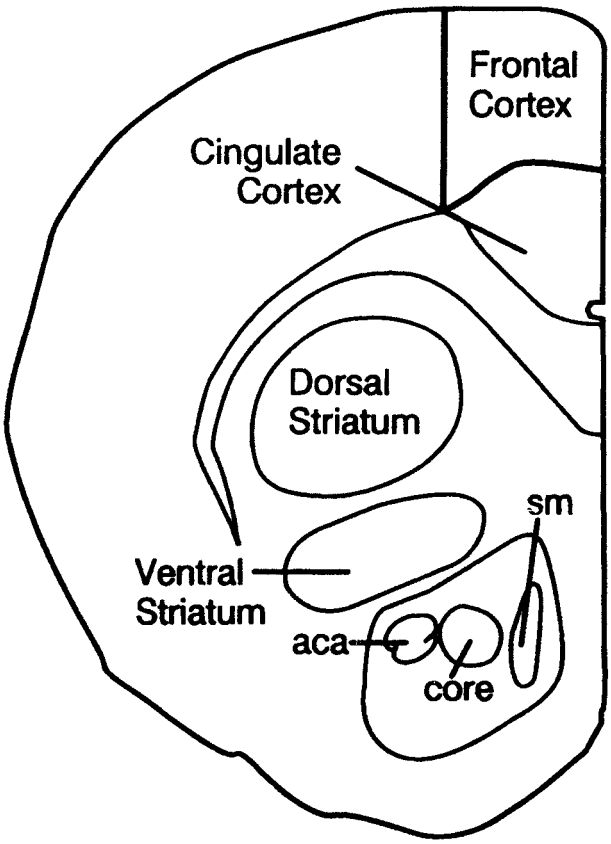

Fig. 1. Schematic of the regions measured in the rat brain to assess receptor binding levels.

RESULTS

AMPA receptors

By rmANOVA, there was a main effect of region (df = 5, 110; F = 76.1; $p < .001$). CNQX binding was highest in the anterior cingulate cortex (679 fm/mg), followed by the frontal cortex (644 fm/mg), accumbens-core (514 fm/mg), ventral striatum (505 fm/mg), accumbens-total (455 fm/mg), dorsal striatum (407 fm/mg), and accumbens-shell (402 fm/mg) (see Fig. 2).

Analysis of CNQX binding was also significant for a main effect of group (df = 2, 22; F = 4.35; $p = .026$) [see Fig. 3]. ANOVA analyses for group differences were significant in the frontal cortex ($p = .037$), anterior cingulate cortex ($p = .02$), dorsal striatum ($p = .007$), ventral striatum ($p = .014$), and nucleus accumbens as a whole ($p = .027$). Post-hoc t-tests revealed significantly higher AMPA receptor density in the frontal cortex and anterior cingulate of CLZ-treated rats relative to vehicle treated controls ($p = .011$ and .006, respectively). However, in the dorsal striatum, ventral striatum, and nucleus accumbens as a whole, CLZ-treated rats had a higher AMPA receptor density compared to both the HDL- and vehicle-treated controls ($p < .05$). Within the nucleus accumbens, CLZ-treated rats differed significantly from the HDL group in the core ($p = .02$), and at trend level in the shell ($p = .06$). Finally, there was a trend toward decreased AMPA receptor density in the accumbens shell following HDL treatment ($p = .07$).

There was also a group by region interaction (df = 10, 110; F = 3.02; $p = .002$); this interaction may reflect the consistently increased density of AMPA receptors across both cortical and subcortical structures following CLZ treatment, and a decreased density following HDL [see Fig. 4].

NMDA receptors

By rmANOVA, there was a main effect of region (df = 5, 110; F = 58.4; $p < .001$). MK-801 binding was highest in the anterior cingulate cortex (470 fm/mg), followed by the frontal cortex (354 fm/mg), ventral striatum (249 fm/mg), accumbens-total (222 fm/mg), accumbens-shell (209 fm/mg) and accumbens-core (202 fm/mg). There was no main effect of group, but there was a group by region interaction (df = 10, 110; F = 2.46; $p < .01$) [see Fig. 5]. This interaction may reflect the higher densities of NMDA receptors in cortical regions following HDL treatment compared to CLZ, as well as relatively equal binding densities in subcortical structures.

Kainic acid receptors

By rmANOVA, there was a main effect of region (df = 5, 110; F = 173.0; $p < .001$). Kainic acid binding was highest in the ventral striatum (199 fm/mg), followed by the dorsal striatum (172 fm/mg), accumbens-core (161 fm/mg), accumbens-total (152 fm/mg), anterior cingulate cortex (148 fm/mg), accumbens-shell (135 fm/mg) and frontal cortex (131 fm/mg) (see Fig. 2). No significant differences were seen between the groups, nor was there any group by region interaction (see Table 1).

Glutamate reuptake sites

By rmANOVA, there was a main effect of region (df = 5, 110; F = 27.8; $p < .001$). D-aspartic acid binding was highest in the anterior cingulate cortex (869 fm/mg) followed by the frontal cortex (853 fm/mg), ventral striatum (758 fm/mg), accumbens-core (752 fm/mg), dorsal striatum (708 fm/mg), accumbens-total (694 fm/mg), and accumbens-shell (657 fm/mg) (see Fig. 2). No significant differences were seen between the groups, nor was there any group by region interaction (see Table 1).

DISCUSSION

We examined the density of ionotropic glutamate receptors and reuptake sites in selected brain regions of normal rats treated for 21 days with orally administered HDL, CLZ, or vehicle. CLZ significantly increased the density of AMPA receptors in the frontal and

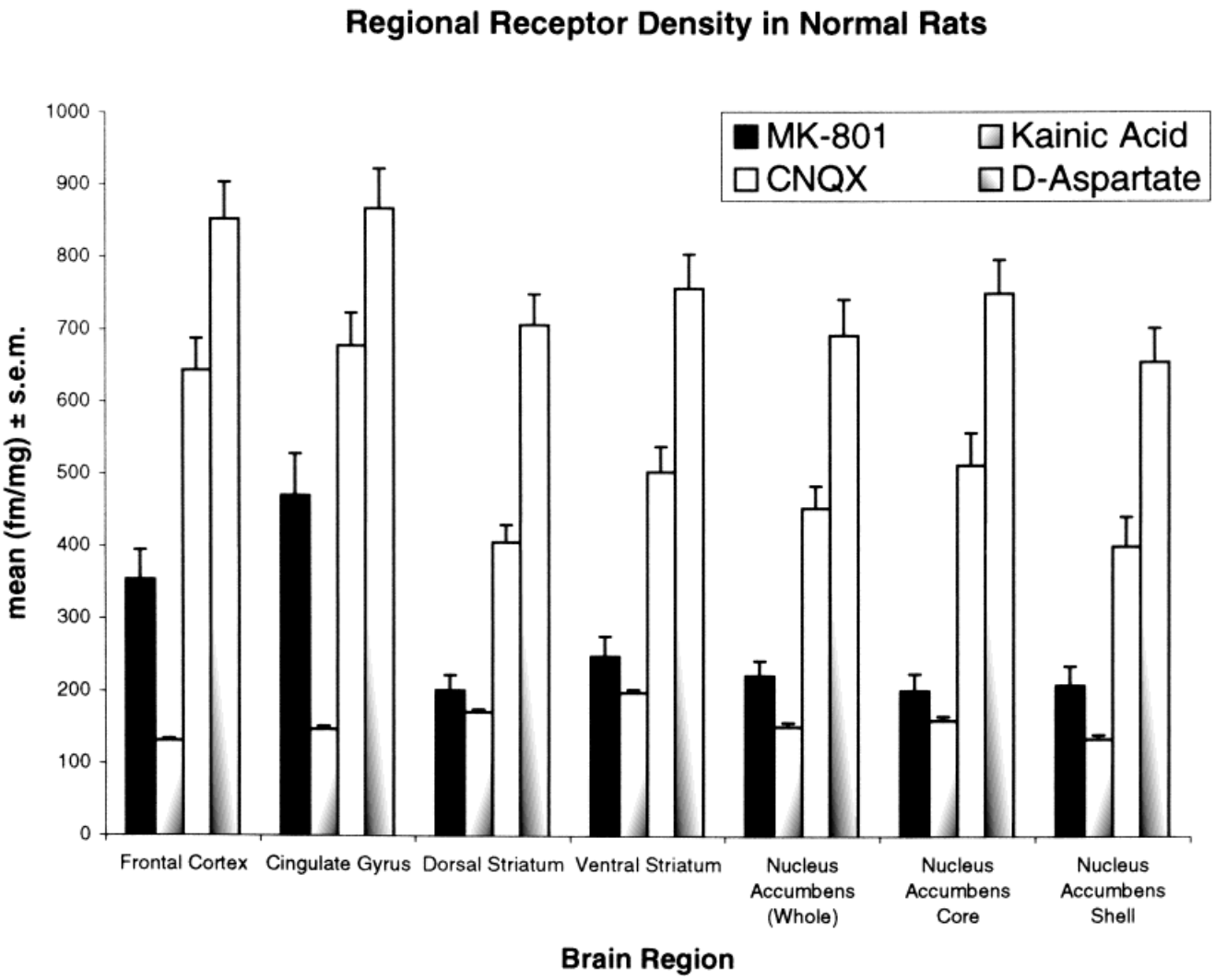

Fig. 2. Relative distribution of receptor density for NMDA, AMPA, kainate, and reuptake sites in vehicle treated control rats.

anterior cingulate cortices compared with normal controls. Moreover, in the dorsal striatum, ventral striatum, and nucleus accumbens as a whole, CLZ-treated rats had a higher AMPA receptor density compared to both the HDL- and vehicle-treated controls. Within the nucleus accumbens, CLZ-treated rats had a higher density of AMPA receptors compared to the HDL group in the core, and achieved trend level in the shell. There was a group by region interaction for NMDA receptor density, primarily reflecting the tendency of HDL-treated rats to have high receptor densities in the frontal and anterior cingulate cortices. Kainate receptors and glutamate reuptake site densities did not differ significantly across groups. These findings suggest a differential effect of CLZ and HDL on glutamate neurotransmission in both cortical and subcortical regions. The unique efficacy of CLZ may be derived, at least in part, from these differential effects.

There is no consensus regarding the effects of neuroleptic treatment on AMPA receptors. McCoy et al., 1998 found that HDL (0.5 mg/kg/day i.p. for 21 days) but not CLZ (20 mg/kg/day i.p. for 21 days) increased the density of AMPA receptors in the frontal cortex and the dorsal and ventral striatum. Tarazi et al., 1996 did not find significant changes in AMPA receptor density after treatment with HDL (1.5 mg/kg/day p.o. for 28 days or 8 months) or CLZ (25 mg/kg/day p.o. for 28 days or 8 months) (Tarazi et al., 1996). Kakigi and colleagues noted that chronic HDL treatment (6 months at 1.5 mg/kg/day p.o.) decreased AMPA receptor density in the nucleus accumbens, which our data tend to support (Kakigi et al., 1992). Clearly there is considerable dispute about the effects of neuroleptics on AMPA receptors.

AMPA receptors are aggregates of four types of subunits, designated GluR1–4. In support of our findings, CLZ appears to increase GluR2 mRNA expression

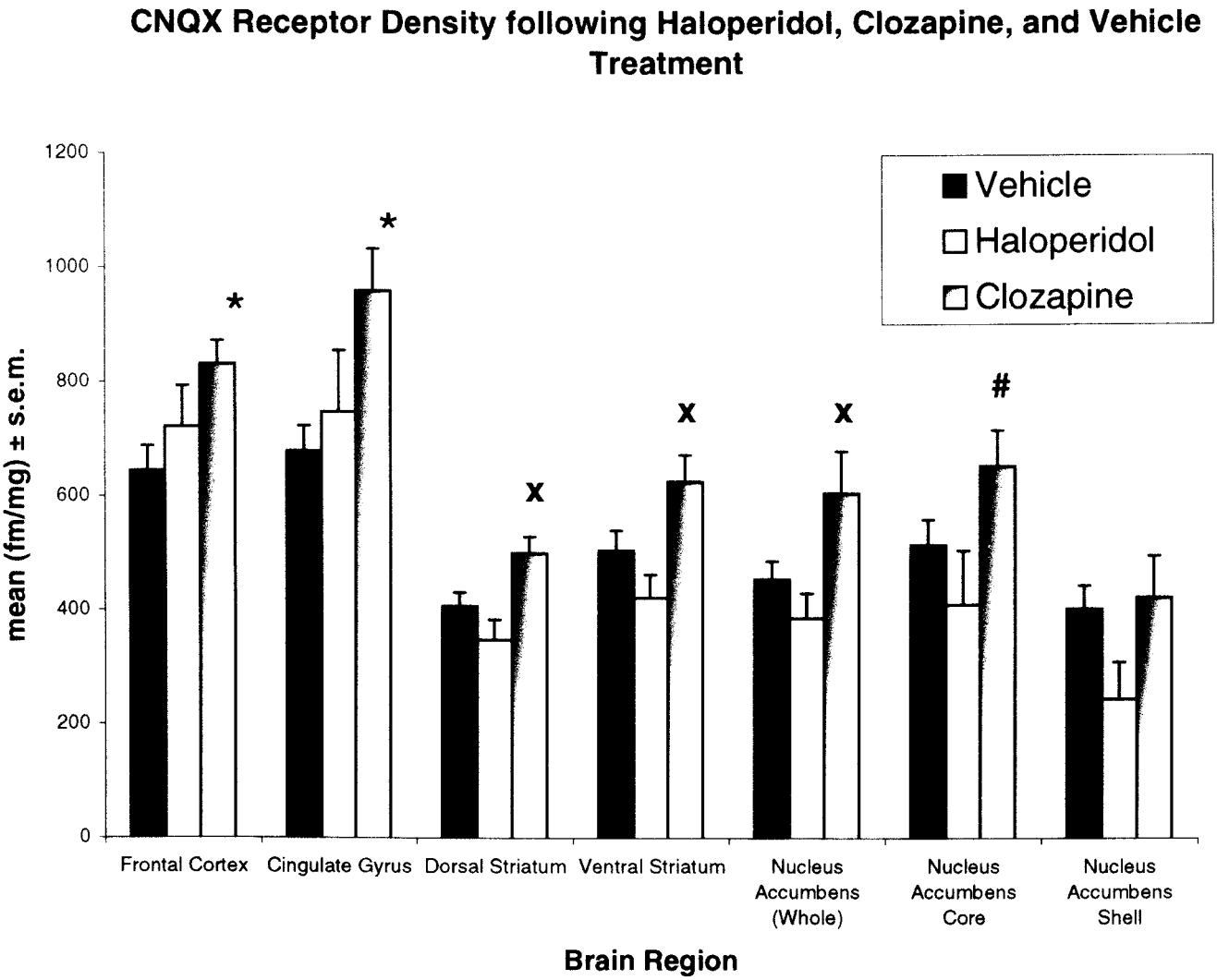

Fig. 3. AMPA receptor density was higher following CLZ treatment compared to vehicle treated controls in frontal and anterior cingulate cortices ($p < .05$) (*). In the dorsal and ventral striatum and nucleus accumbens, CLZ treated rats had a higher density of AMPA receptors compared to both HDL and vehicle-treated groups ($p < .05$) (**X**). In the core region of the nucleus accumbens, CLZ treated rats had a higher density than the HDL group ($p < .05$) (#).

in the frontal/parietal cortex, nucleus accumbens, and hippocampus (Fitzgerald, 1995), although this finding has been contested (McCoy et al., 1998; Oretti, 1994). Healy and Meador-Woodruff (1997) found that HDL (2 mg/kg/day s.c. for 14 days) actually decreased GluR2 and GluR4 mRNA expression in the frontal cortex and striatum, which our data tend to support. Counter to our report, Healy and Meador-Woodruff (1997) also reported that CLZ (20 mg/kg/day S.C. for 14 days) produced a decrease in GluR3 mRNA expression in the frontal cortex and striatum, and a decrease in GluR4 mRNA expression in the striatum, however, there is not always a direct correlation between receptor mRNA expression and protein levels in the central nervous system. Additionally, the subunit composition of the AMPA receptor might be altered by neuroleptic treatment without effecting the overall receptor density.

The conflicting results from studies of AMPA receptors following neuroleptic treatment may be secondary to methodological considerations. Differences in dose, route of administration, and duration of treatment must be considered. Oretti et al. (1994) administered HDL 1.5 mg/kg/day i.p., while McCoy et al. (1998) used HDL at a dose of 0.5 mg/kg/day i.p. or CLZ at 20 mg/kg/day i.p., for 21 days. Since HDL is subject to first-pass metabolism, after injection HDL levels are considerably higher than the same-but-orally-administered doses (Bianchetti et al., 1980). Other groups have used higher HDL doses: 2.0 mg/kg/day (Healy and Woodruff, 1997), 1.8 mg/kg/day (Fitzgerald et al., 1995) and 1.5 mg/kg/day (Kakigi et al., 1992; Tarazi et al., 1996), and CLZ doses: 35 mg/kg/day (Fitzgerald et al., 1995) and 25 mg/kg/day (Tarazi et al., 1996) than we employed. Differences in treatment regimens may un-

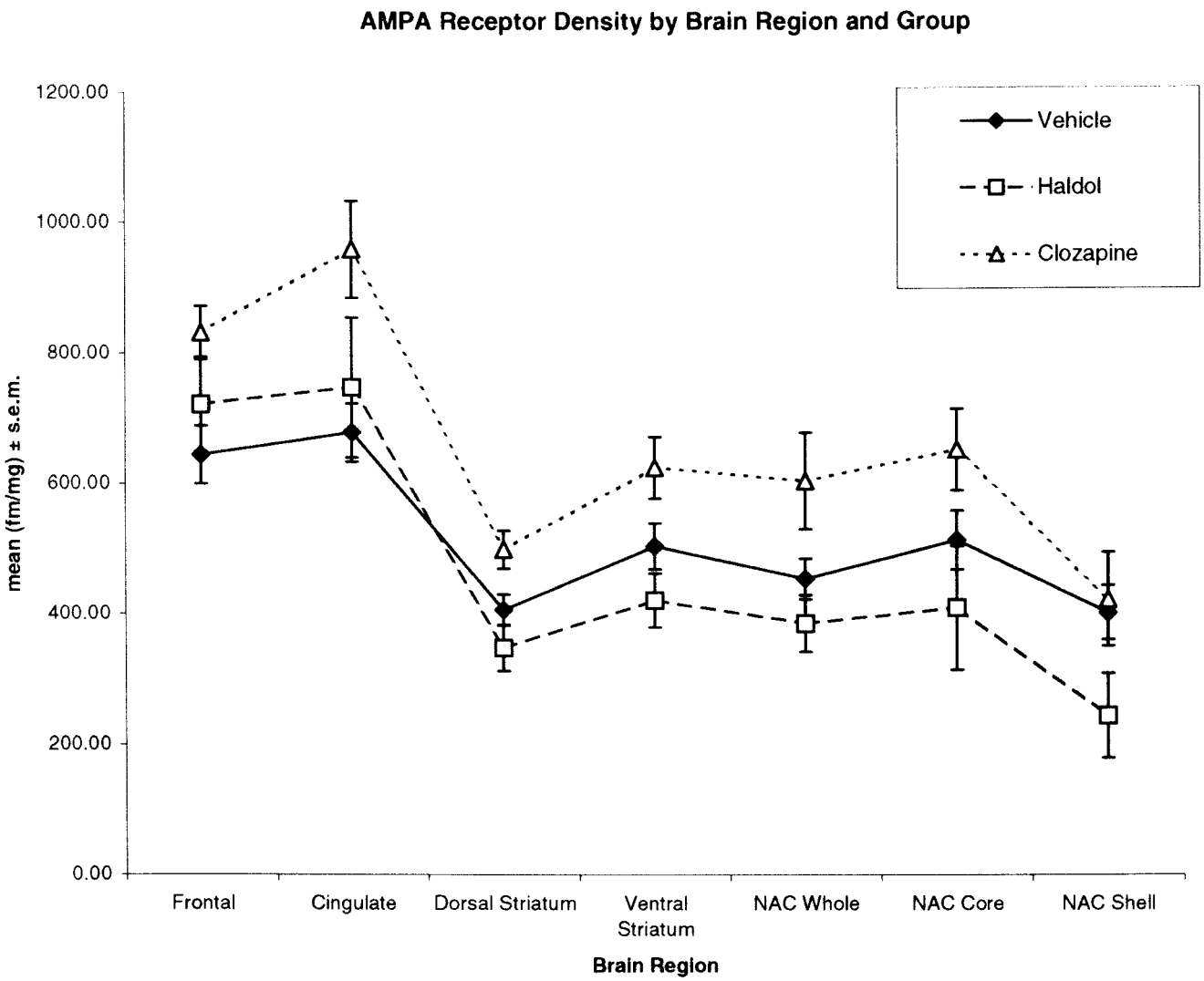

Fig. 4. Graphic representation of the binding density of AMPA receptors across selected brain regions following CLZ, HDL, and vehicle treatment. The group by region interaction is reflected in the relatively high density of AMPA receptors in the cortical regions following CLZ treatment, and the trend towards decreased basal ganglia receptor density following HDL treatment.

derlie the discrepancies between studies of glutamate receptor density and subunit mRNA expression levels.

Since compounds that disrupt NMDA receptor function induce psychotic symptoms, the mechanism of action of neuroleptics might involve an increase in the density of NMDA receptors. However, the absence of a significant group effect on NMDA receptor density in our study is consistent with the report of Meshul et al. (1996) and a previous report from our laboratory (Hamid, 1998). Meshul et al. (1996) did not find a change in the density of NMDA receptors in the striatum after a 28-day treatment with either HDL (0.5 mg/kg/day s.c.) or CLZ (30 mg/kg/day s.c.). We found no change in the striatum after HDL treatment (1 mg/kg/day i.p. for 21 days). In a study of Tarazi et al. (1996), treatment with CLZ (25 mg/kg/day p.o. for 28 days) actually caused a reduction of NMDA receptor density in the striatum. Giardino et al., 1997 treated rats for 21 days with CLZ (30 mg/kg/day i.p.), and found unilateral decreases in NMDA receptor density in the left anterior cingulate, frontoparietal motor, and frontoparietal somatosensory cortices. They did not detect changes in the prefrontal cortex, striatum, or nucleus accumbens. We did not fractionate our data by hemisphere, preventing direct comparison with their cortical findings; however, our subcortical results are largely in agreement. While there was a tendency for CLZ to reduce cortical NMDA receptor density in our study, it did not reach statistical significance. Larger cohorts may have revealed a difference. Although there is extensive data regarding NMDA receptor subunit mRNA expression following neuroleptic treatment, no consensus has emerged (Fitzgerald et al., 1995; Meshul et al., 1996; Oretti et al., 1994; Riva et al., 1997). Neuroleptic-

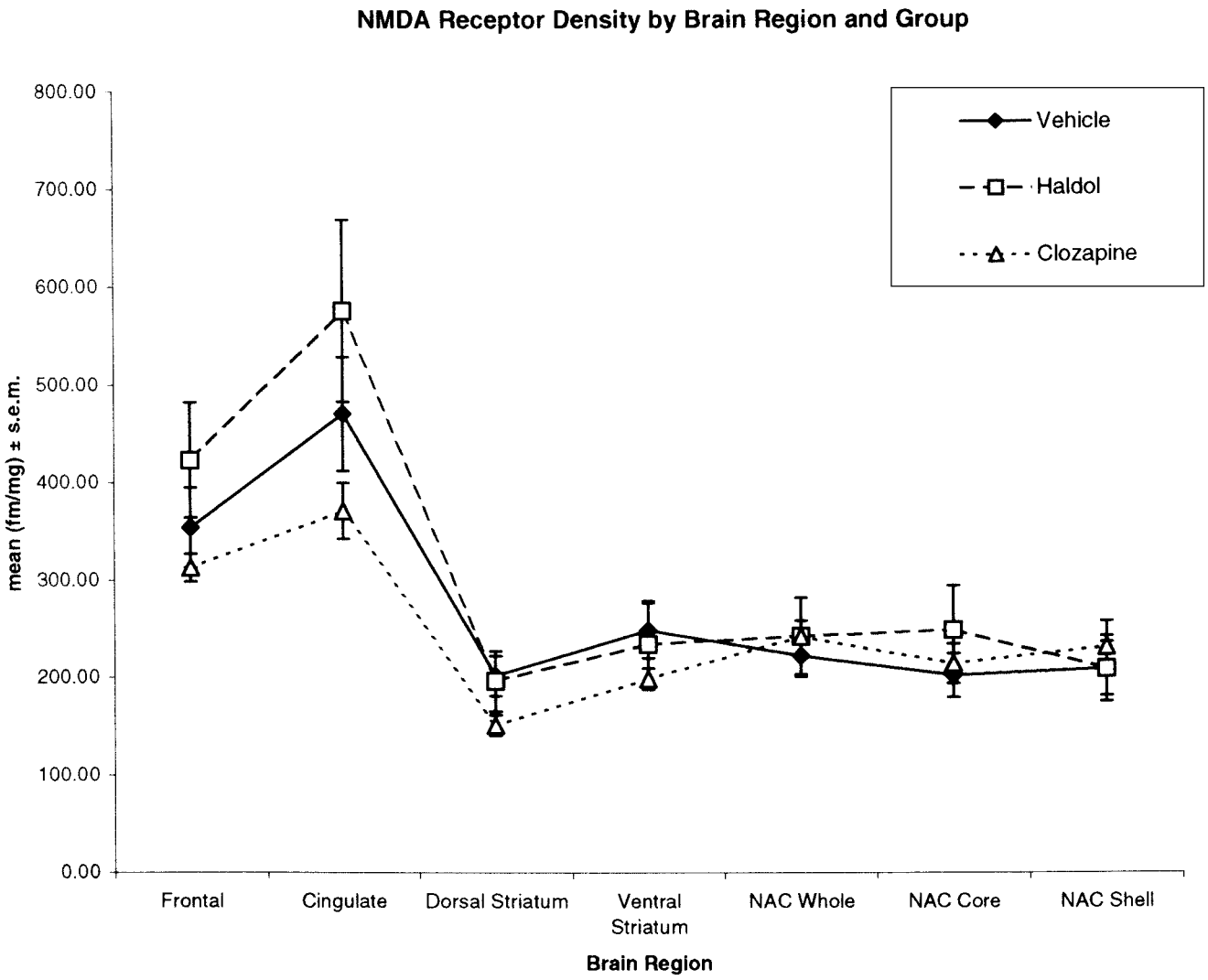

Fig. 5. Graphic representation of the binding density of NMDA receptors across selected brain regions following CLZ, HDL, and vehicle treatment. The group by region interaction is reflected in the relatively high density of NMDA receptors in the cortical regions following CLZ treatment.

induced reductions in NMDA receptor density seem somewhat counter-intuitive given the clinical data regarding NMDA receptor antagonism and psychosis.

Neuroleptics may alter the extracellular levels of glutamate, thereby secondarily altering post-synaptic receptor density. Yamamoto and Cooperman (1994) found 21 days of treatment with CLZ (20 mg/kg/day i.p.) did not produce changes in any brain region while HDL (0.5 mg/kg/day i.p.) increased extracellular glutamate in the caudate and nucleus accumbens (Yamamoto and Cooperman, 1994). In another experiment, Yamamoto and colleagues did not find a change in striatal glutamate levels after 21 days of treatment with CLZ (20 mg/kg/day i.p.), but a five fold increase after 21 days of treatment with HDL (0.5 mg/kg/day i.p.) (Yamamoto et al., 1994). In earlier studies, Bardgett et al. (1993) found no differences in glutamate levels in the striatum after 28 days of treatment with HDL (0.18 mg/kg/day s.c.) or CLZ (19.4 mg/kg/day s.c.). However, extracellular glutamate levels may not accurately reflect the amount of glutamate released from axon terminals since changes may reflect alterations in metabolism or GABA-mediated transmission rather than selective changes in the neurotransmitter pool (Deutch et al., 1989).

Neuroleptics might effect glutamate neurotransmission through indirect mechanisms. Dopaminergic nigrostriatal fibers may mediate a presynaptic inhibitory influence via D_2 receptors located on glutamatergic corticostriatal terminals in the striatum (Kim et al., 1980; Kornhuber and Kornhuber, 1986). Thus, a hyperdopaminergic state such as schizophrenia might lead to decreased levels of glutamate release in the striatum. In such a case, neuroleptic-induced D_2 receptor block-

TABLE I. Receptor binding results (mean ± s.e.m. fm/mg) for MK-801, kainic acid, CNQX, and d-aspartic acid for the frontal cortex, cingulate gyrus, dorsal striatum, ventral striatum, nucleus accumbens-total, nucleus accumbens-core, and nucleus accumbens-shell

Ligand	Group	Frontal cortex	Cingulate gyrus	Dorsal striatum	Ventral striatum	N accumbens—total	N. accumbens—core	N. accumbens—shell
MK-801	Normal	354.18 ± 41	470.43 ± 58	202.06 ± 21	248.57 ± 28	222.34 ± 21	202.14 ± 23	209.00 ± 27
	HDL	423.18 ± 59	575.77 ± 93	196.59 ± 31	234.71 ± 44	242.80 ± 40	248.79 ± 45	208.88 ± 33
	CLZ	312.87 ± 14	371.07 ± 29	151.26 ± 11	198.90 ± 11	241.98 ± 17	214.41 ± 20	230.97 ± 27
Kainic Acid	Normal	131.15 ± 3	147.86 ± 4	171.74 ± 4	199.25 ± 4	151.90 ± 6	160.77 ± 6	134.79 ± 6
	HDL	140.05 ± 7	149.59 ± 7	173.16 ± 9	201.24 ± 9	152.54 ± 13	163.32 ± 14	137.09 ± 6
	CLZ	128.21 ± 5	152.21 ± 7	169.91 ± 6	203.27 ± 7	146.13 ± 6	157.93 ± 4	131.02 ± 6
CNQX	Normal	644.24 ± 44	678.71 ± 45	406.94 ± 24	504.65 ± 35	454.67 ± 31	513.88 ± 45	401.95 ± 41
	HDL	721.60 ± 72	747.66 ± 108	348.53 ± 35	421.78 ± 41	386.10 ± 44	409.78 ± 95	244.03 ± 65
	CLZ	831.67 ± 41*	959.74 ± 74*	499.46 ± 29**	625.38 ± 47**	605.07 ± 74**	652.72 ± 62***	422.83 ± 72
D-aspartate	Normal	853.24 ± 51	868.63 ± 55	707.67 ± 43	758.45 ± 48	693.67 ± 50	752.17 ± 47	657.33 ± 47
	HDL	818.21 ± 47	811.06 ± 60	679.87 ± 34	725.18 ± 44	628.71 ± 62	711.38 ± 63	603.36 ± 64
	CLZ	699.68 ± 55	741.94 ± 67	626.90 ± 45	683.99 ± 46	625.76 ± 41	723.45 ± 57	577.22 ± 43

*Significant ($p < .05$) difference when compared to control group.
**Significant ($p < .05$) difference when compared to HDL and control groups.
***Significant ($p < .05$) difference when compared to HDL group.

ade might decrease dopamine, restore glutamate and thus produce antipsychotic effects. In normal rats, neuroleptics may decrease striatal dopamine release, resulting in relatively excessive amounts of glutamate release. In turn, this may cause a down-regulation in some post-synaptic glutamate receptors or changes in glutamate receptor subunit composition. The cortico-thalamocortical negative feedback loop also may play a role in neuroleptic-induced glutamate receptor density changes (Carlsson and Carlsson, 1990). In this model, corticostriatal excitatory glutamatergic and nigrostriatal inhibitory dopaminergic systems independently modulate GABA-mediated inhibition of the excitatory thalamocortical pathway. Increased dopamine might decrease the inhibitory GABA-mediated signal from the basal ganglia to the thalamus, and putatively decreased glutamate release could lead to further disinhibition of the thalamocortical signals, in turn eliciting the clinical symptoms of hyperarousal, disturbed perception, and psychotic behavior (Wachtel and Turski, 1990). D_2 blockade would ameliorate symptoms in such a model by attenuating the DA mediated inhibition of GABA transmission from the basal ganglia to the thalamus. Our experiment found negligible changes in glutamate receptor density following HDL treatment, potentially supporting a more independent relationship between glutamate and dopamine as in the cortico-thalamocortical loop, and less of a reciprocal, presynaptically mediated relationship as posited by the initial hypotheses.

CLZ, an atypical neuroleptic, has a pharmacological profile that includes relatively low D_2 affinity (Farde et al., 1988), and high affinity for D_4 receptors (Van Tol, 1991). It also has potent effects on several 5-HT receptor subtypes including 5-HT_{2a}, 5-HT_{2c}, and 5-HT_3 (Breier, 1995), and antagonism of the histaminergic, cholinergic, and noradrenergic systems (Breier et al., 1994; McMillen and Shore, 1978; Meltzer, 1991; Peroutka et al., 1977; Peroutka and Synder, 1980). CLZ's proposed mechanism of action may involve its effects on the relative balance between 5-HT and DA systems (Breier, 1995; Meltzer et al., 1989). However, this profile of 5-HT_2/D_2 antagonism may not explain all of CLZ's effects. In a study of non-human primates, Casey and colleagues found that the addition of a wide range of 5-HT_2 antagonists to typical neuroleptic treatment did not have a potent anti-parkinsonian effect (Casey et al., 1993). Similarly, depletion of central 5-HT did not alter CLZ catalepsy nor did it change the profile of supersensitivity to the DA agonist apomorphine that emerges after chronic CLZ treatment (Jaskiw, personal communication). These results suggest that 5-HT_2 antagonism may not be the sole main mechanism of action for atypical neuroleptics, and the effects on AMPA receptor-mediated neurotransmission may be pertinent.

CLZ may exert some of its unique therapeutic effects through alterations in glutamate neurotransmission. Electrophysiological studies by Lidsky and colleagues show that CLZ has glutamate antagonistic properties which potentially occur at therapeutic levels (Lidsky et al., 1993). Glutamate receptor antagonism may occur primarily through direct antagonism at the NMDA receptor or secondarily through CLZ's antidopaminergic effects. In rat studies, NMDA receptor antagonism reduced catalepsy induced by typical neuroleptics (Elliott et al., 1990; Yoshida et al., 1991), suggesting that CLZ's potential NMDA antagonism may play a role in preventing extrapyramidal side effects. Glutamate has also been shown to increase the release of DA in the rat striatum (Roberts, 1978) and increase burst firing in the ventral tegmental area (Charlety et al., 1991). Thus, glutamate antagonism could potentially decrease DA levels and contribute to the antipsychotic profiles of CLZ.

We found minimal effects of HDL or CLZ treatment on NMDA receptor density. This might suggest that as assayed, the NMDA receptors are relatively insensitive to antipsychotic drugs and are not likely to be critically involved in either typical or atypical antipsychotic drug

effects. It is also possible that given the widespread distribution of glutamate and, presumably, glutamatergic receptors; HDL and CLZ only affect certain populations of glutamate receptors. Recent studies suggest that HDL may bind to a site on the NR1A/NR2B subtype of the NMDA receptor (Coughenour and Cordon, 1997). Regions with high density of NMDA receptors of the NR1A/NR2B subtype might be effected differentially by treatment with HDL. Additional investigations need to be performed with CLZ. In addition, regional selectivity could be secondary to the type and amount of dopaminergic innervation of a region. This putative selectivity is noted with significant increases in AMPA receptors in the prefrontal and cingulate cortices. Fitzgerald and colleagues found similar molecular results and proposed a hypothesis of increased AMPA receptor subunits on prefrontal cortex interneurons, potentially increasing GABA-mediated inhibition of glutamatergic output to the subcortical dopaminergic systems (Fitzgerald et al., 1995).

In conclusion, CLZ increased the density of AMPA receptors significantly in the frontal and anterior cingulate cortices compared with normal controls. In the dorsal and ventral striatum, and nucleus accumbens as a whole, CLZ-treated rats had a higher AMPA receptor density compared to both the HDL- and vehicle-treated groups. In the core of the nucleus accumbens, CLZ-treated rats had a higher density of AMPA receptors compared to the HDL group. There was a group by region interaction for NMDA receptor density, primarily reflecting the tendency of HDL-treated rats to have high receptor densities in the frontal and anterior cingulate cortices. Kainate receptors and glutamate reuptake site densities did not differ significantly when HDL and CLZ groups were compared to controls. These results support the notion that alterations in glutamate neurotransmission play a role in the unique therapeutic efficacy of CLZ.

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