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Short communication

Chronic treatment with typical and atypical antipsychotics increases the AMPA-preferring form of AMPA receptor in rat brain

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Abstract

We assessed the effects of chronic (21 day) administration of antipsychotic drugs on the density of the α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptor in rat brain. We used two typical antipsychotic drugs, haloperidol and pimozide, and two atypical antipsychotic drugs, risperidone and clozapine. Antipsychotic drugs as a group significantly elevated the density of the AMPA receptor measured with an AMPA receptor agonist ([^{3}H]AMPA), but not with an AMPA receptor antagonist, 6-cyano-7-nitro-quinoxaline-2,3-dione ([^{3}H]CNQX). In all regions studied, the magnitude of the increase seen with chronic typical antipsychotic drugs was significantly greater than that seen with chronic atypical antipsychotic drugs. In frontal cortex and striatum, typical antipsychotics but not atypical antipsychotics elevated AMPA receptor binding over control. These findings suggest that antipsychotic drugs alter the agonist affinity of the AMPA receptor without altering the number of AMPA receptors. Typical antipsychotic drugs may be more potent in this effect than atypical antipsychotic drugs, especially in critical corticostriatal circuits.

Keywords: Autoradiography; [^{3}H]AMPA (α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid); [^{3}H]CNQX (6-cyano-7-nitro-quinoxaline-2,3-dione); Antipsychotic drug

1. Introduction

Evidence suggests that interactions between dopamine and glutamate may be significant in understanding schizophrenia and both the therapeutic action and side effects of antipsychotic drugs (Iversen, 1995). Altered gene expression of one subtype of glutamate receptor, the α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptor, has recently been implicated in the pathophysiology of schizophrenia (Harrison et al., 1991; Eastwood et al., 1995) and antipsychotic drug mechanism of action (Fitzgerald et al., 1995; Eastwood et al., 1996).

Many theories have been advanced regarding the mechanism of action of antipsychotic drugs with most centering on dopaminergic mechanisms due to their high affinity for dopamine D_2-like receptors. However, the long held concept that antipsychotic drug efficacy is due solely to blockade of dopamine D_2-like receptors has been challenged with the introduction of atypical antipsychotic agents such as clozapine that are highly effective in treating refractory forms of schizophrenia (i.e., cases with prominent negative symptoms), cause less extrapyramidal side effects and are less potent than classical antipsychotic drugs in postsynaptic blockade of dopamine receptors (Meltzer, 1989). Clozapine and other atypical antipsychotic drugs have been shown to have modulatory actions on serotonergic, adrenergic, cholinergic (Meltzer, 1992) and more recently glutamatergic systems (Yamamoto and Cooperman, 1994). Therefore, mechanisms other than or in addition to dopamine receptor blockade may account for both the advantages of atypical agents in treating refractory schizophrenia and their decreased potential for extrapyramidal side effects.

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While chronic administration of antipsychotic drugs has been shown to increase AMPA receptor subunit gene expression (Fitzgerald et al., 1995), neither haloperidol nor clozapine had an effect on AMPA receptor binding as measured by tritiated 6-cyano-7-nitroquinoxaline-2,3-dione ([^{3}H]CNQX), an AMPA receptor antagonist (Tarazi et al., 1995). It is unknown if agonist binding to the AMPA receptor is altered following treatment with antipsychotic drugs as [^{3}H]CNQX binds with equal affinity to two states of the AMPA receptor which have different affinities for the agonist [^{3}H]AMPA (Nielsen et al., 1990). Also, [^{3}H]CNQX and [^{3}H]AMPA binding have different patterns of developmental regulation (Massicotte et al., 1992), show differing responses to the in vitro application of thiocyanate (Nielsen et al., 1990) and differing responses to phospholipases (Massicotte et al., 1992). Therefore we chose to study the binding of both [^{3}H]AMPA and [^{3}H]CNQX to the AMPA receptor following chronic treatment with typical and atypical antipsychotic drugs.

2. Materials and methods

2.1. Drug treatment

Male Sprague-Dawley rats (180–250 g at purchase, Charles River Breeding Laboratories, Wilmington, MA, USA; $n = 41$) were individually housed and maintained on a 12 h light/dark cycle. Animals were maintained at approximately 300 g during the period of injection via daily calorie restriction. Two typical antipsychotics, haloperidol (0.5 mg/kg), pimozide (0.5 mg/kg), and two atypical antipsychotics, clozapine (20 mg/kg), risperidone (1.0 mg/kg), or saline vehicle (1 ml/kg) were administered intraperitoneally for 21 days. These dosage regimens were based on the following: (1) previous studies comparing neurochemical and receptor binding changes for haloperidol and clozapine (Stockmeier et al., 1993; Yamamoto and Cooperman, 1994); (2) pimozide and haloperidol have an identical in vitro K_i (nM) for the dopamine D_2-like receptor; and (3) recent in vitro work suggests that at this dose range for haloperidol, risperidone must be at least twice the haloperidol dose to reach similar dopamine D_2-like dopamine receptor occupancy (Alain Schotte, personal communication). There were eight animals in each drug treated group and nine in the saline treated group. All drug solutions were prepared daily and adjusted to pH 5.0–5.5 with 1 M NaOH. Rats were weighed daily and injected between 08:00 and 10:00 h. After 21 days of treatment, all rats weighed between 290 and 320 g.

2.2. Tissue preparation and binding assay

Twenty-four hours after the last injection, the animals were killed by rapid decapitation, their brains removed, frozen by immersion in isopentane at −30°C for 10–15 s followed by several minutes in powdered dry ice. 10 μm sagittal sections were cut on a cryostat microtome with the brain mounted in Lipshaw embedding matrix. Sections were thaw-mounted onto gelatin-coated slides. The slides were dehydrated at 25°C for several minutes and then frozen at −30°C for less than 48 h prior to assay.

Detailed descriptions of AMPA receptor autoradiography have been published using [^{3}H]AMPA (Nielsen et al., 1988) and [^{3}H]CNQX (Nielsen et al., 1990). These studies have shown that [^{3}H]AMPA labels two populations of sites with different affinities: a high affinity site ($K_d \approx$ 10 nM) and a low affinity site ($K_d \approx 500$ nM). By using a low nanomolar concentration of [^{3}H]AMPA, and a low micromolar concentration of KSCN our binding assay reflects binding to only the high affinity state (McCoy and Richfield, 1996; Standley et al., 1995). Briefly, slides were warmed to 4°C and prewashed in incubation buffer at 4°C for 30 min and dried under a stream of room temperature air. Sections were then placed in incubation buffer containing a single concentration of radioactive ligand, 5 nM [^{3}H]AMPA or 30 nM [^{3}H]CNQX. Nonspecific binding was assessed with the addition of 10 μM AMPA or 25 μM CNQX, respectively. For [^{3}H]AMPA, the tissue was incubated for 60 min in 25 mM Tris-HCl (pH 7.2) with 2.5 mM $CaCl_2$ and for [^{3}H]CNQX, the tissue was incubated for 45 min in 50 mM Tris-HCl (pH 7.2). Both incubation buffers contained 30 mM of the chaotropic ion thiocyanate as potassium thiocyanate (KSCN). After the incubation, sections were rinsed quickly (3–5 s) in 4°C buffer. For the [^{3}H]AMPA assay, this rinse was followed by a quick dip (1–2 s) in chilled acetone. Sections were blown dry with warm air. For the both assays, nonspecific binding represented less than 10% of total binding.

2.3. Autoradiography and densitometry

Dried slides were placed in an X-ray cassette with ^{14}C plastic standards previously calibrated with ^{3}H brain paste sections (Richfield, 1991) and exposed to Amersham Hyperfilm at 25°C for 12–14 days. All quantitative binding data were determined directly from film densities using a video based image analysis system (Imaging Research, St Catherines, Ontario, Canada). For the following brain regions, measurements were taken from two adjacent saggital sections per animal: medial frontal cortex (FC); stratum radiatum of CA1 (CA1); stratum moleculare of dentate gyrus (DG); dorsal striatum (DS); ventral striatum (VS) and cerebellum (CB). Specific binding was calculated as the difference between total binding and nonspecific binding.

2.4. Data analysis

To control for multiple comparisons, we first analyzed a single binding value (an arithmetic average of the six regions) for the five treatment groups (haloperidol, pimozide, clozapine, risperidone and saline) using one-way

analysis of variance (ANOVA). By using post-hoc contrasts and setting a P value of < 0.05, we determined whether antipsychotic drugs as a group were different from saline and whether typical agents were different from atypical agents. Statistical comparison of regional effects were determined using one-way ANOVA with Tukey's test for post-hoc comparisons using the SPSS 6.1 statistical package for Macintosh (SPSS, Chicago, IL, USA).

2.5. Materials

[^{3}H]AMPA (53.0 Ci/mmol) and [^{3}H]CNQX (14.03 Ci/mmol) were obtained from Dupont/New England Nuclear (Boston, MA, USA). Unlabeled AMPA and CNQX were purchased from Research Biochemicals (Natick, MA, USA). Haloperidol (R.W. Johnson Pharmaceutical Research Institute), pimozide and risperidone (Janssen Research Foundation) and clozapine (Sandoz Pharmaceuticals) were gifts from the respective companies. All other chemicals were purchased from Sigma (St. Louis, MO, USA).

3. Results

3.1. Effect of chronic antipsychotic drugs on the AMPA receptor measured with [^{3}H]AMPA

All of the antipsychotic drugs examined elevated the six region average density of [^{3}H]AMPA binding (Fig. 1A). When the drug treated groups were considered together this was significantly higher than the saline treated group ($F(4,36) = 25.67$, $P < 0.001$; Fig. 1A). We used one-way ANOVA to compare the six region average of [^{3}H]AMPA binding for the four drug treated groups and the one saline treated group. Post-hoc contrasts revealed that antipsychotic drugs considered together, typical antipsychotics as a group, or atypical antipsychotics as a group, each resulted in higher [^{3}H]AMPA binding compared to saline (Fig. 1A). The typical antipsychotics, haloperidol and pimozide (65 and 58% higher than saline treatment, respectively) resulted in significantly higher ($P < 0.001$) binding than the atypical antipsychotics, clozapine and risperidone (25 and 10% higher than saline treatment, respectively).

3.2. Effect of antipsychotic drugs on the AMPA receptor measured with [^{3}H]CNQX

Since our [^{3}H]AMPA binding assay labels only the high affinity state of the AMPA receptor, we next examined both high and low affinity AMPA receptor states using [^{3}H]CNQX. Following chronic antipsychotic treatment, the overall ANOVA for [^{3}H]CNQX binding showed no difference between antipsychotic drug treated and saline treated groups ($F(4,36) = 0.82$, $P = 0.53$). Although not significantly different from the saline group, [^{3}H]CNQX binding values in the drug treated groups were all slightly higher than saline treated animals (Fig. 1B).

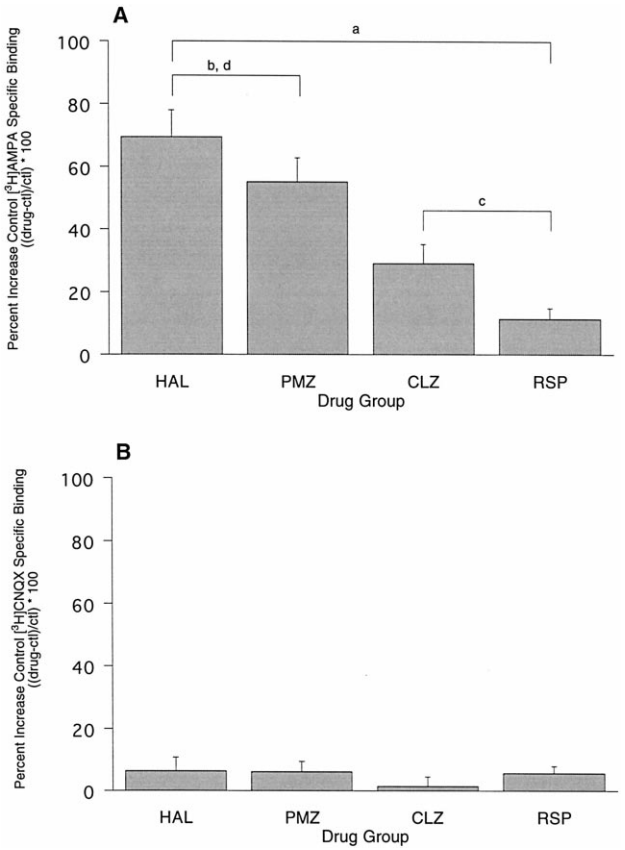

Fig. 1. Effect of chronic saline versus antipsychotic drug treatment on [^{3}H]AMPA (A) and [^{3}H]CNQX (B) binding. The antipsychotic drug group showed increased [^{3}H]AMPA binding compared to controls whereas [^{3}H]CNQX binding was unchanged. The values represent a six region average mean of tritiated ligand binding density and are expressed as percentage of saline treated animals. HAL, haloperidol; PMZ, pimozide; CLZ, clozapine; RSP, risperidone. [a] $P < 0.001$ (HAL, PMZ, CLZ, RSP versus saline). [b] $P < 0.001$ (HAL, PMZ versus saline). [c] $P = 0.002$ (CLZ, RSP versus saline). [d] $P < 0.001$ (HAL, PMZ versus CLZ, RSP).

3.3. Effect of typical and atypical antipsychotic drugs on regional [^{3}H]AMPA binding

In all regions studied, haloperidol and pimozide treatment caused significant increases in [^{3}H]AMPA binding compared to saline treated animals (Table 1). Clozapine treatment resulted in significantly elevated [^{3}H]AMPA binding only in the dentate gyrus and cerebellum, whereas risperidone treatment did not significantly change binding in any region. Specific regional effects of antipsychotic drugs on [^{3}H]AMPA binding were assessed using individ-

Table 1
Antipsychotic drug effects on [^{3}H]AMPA receptor binding in rat brain

	Control	Haloperidol	Pimozide	Risperidone	Clozapine
Cortex	312 ± 17	524 ± 36 [a]	442 ± 16 [a]	354 ± 17	386 ± 21
Striatum					
dorsal	287 ± 14	468 ± 43 [a]	406 ± 23 [a]	284 ± 12	364 ± 21
ventral	334 ± 11	505 ± 40 [a]	454 ± 26 [a]	338 ± 16	397 ± 19
Hippocampus					
CA1	789 ± 48	1289 ± 47 [a]	1209 ± 67 [a]	862 ± 21	978 ± 38
dentate gyrus	679 ± 38	1234 ± 40 [a]	1157 ± 59 [a]	818 ± 37	915 ± 32 [a]
Cerebellum	177 ± 11	322 ± 19 [a]	332 ± 15 [a]	212 ± 15	254 ± 15 [a]

Antipsychotic drugs were injected i.p. for 21 days. AMPA receptors were labeled with [^{3}H]AMPA and data (fmol/mg protein) are expressed as means ± S.E.M. for 8–9 animals per drug group. Individual one-way ANOVAs were conducted for each region with Tukey's post-hoc testing to control for multiple comparisons ([a] $P < 0.05$, versus saline). Differences for pimozide are similar to haloperidol and there are no significant regional differences for risperidone.

ual one-way ANOVAs for each region and differences between each treatment group were determined by Tukey's post-hoc testing with overall significance level set at $P = 0.05$ (Table 1).

4. Discussion

The major finding of the present study is that chronic antipsychotic drug treatment increased [^{3}H]AMPA binding but not [^{3}H]CNQX binding. This suggests the effect of antipsychotic treatment is a change in the affinity state of the AMPA receptor. The finding complements two recent studies showing increased AMPA receptor gene expression (GluR1 and GluR2) following chronic antipsychotic treatment (Fitzgerald et al., 1995; Eastwood et al., 1996). Also, the lack of change in [^{3}H]CNQX binding is in agreement with a previous preliminary study using haloperidol and clozapine (Tarazi et al., 1995). There is evidence in the literature that AMPA receptors desensitize in the continued presence of agonist and therefore the alteration in high affinity AMPA binding may represent binding to the desensitized receptor (Hall et al., 1993; Arai et al., 1995). Therefore, the functional effect on the receptor (i.e., excitation or inhibition) of long-term administration of antipsychotic drugs is unclear.

Our results are consistent with findings reported by Yamamoto and Cooperman (1994), who demonstrated an increase in extracellular glutamate in the caudate nucleus following chronic haloperidol but not clozapine treatment. The authors suggest the effect may be due to a disinhibition of corticostriatal glutamatergic transmission via antagonism of the dopamine D_2-like receptor. If the increase in the high affinity state of the AMPA receptor in cortex and striatum produced by the typical antipsychotic drugs in the present study signifies inhibition of the excitatory corticostriatal pathway then this may be related to the extrapyramidal side effects associated with these agents. However, any strong conclusions about class differences must be tempered by the fact that the present study compared single drug doses (see Section 2). The selection of comparable doses for various antipsychotic drugs is crucial to attribute differential actions to distinct pharmacologic properties and yet no methodological approach to this selection process is completely satisfactory.

The interpretation of the present findings is complicated by the fact that few studies have reported an in vivo change in AMPA receptor properties. An exception is a recent study describing differential patterns of development for the high and low affinity states of the AMPA receptor and receptor subunits in the rat hippocampus (Standley et al., 1995). The authors report that the developmental pattern of [^{3}H]CNQX binding is remarkably similar to that of the low affinity [^{3}H]AMPA binding and that neither measure correlates with AMPA receptor subunit expression. Conversely they found a positive correlation between flop-subunit expression and high affinity AMPA receptors (Standley et al., 1995). This might suggest that the flop subunit imposes the affinity state of the native receptors for AMPA. Antipsychotic drugs may therefore regulate the expression of the flop subunit or as one study has suggested they may regulate subunit (flip:flop) ratio (Eastwood et al., 1994). Alternatively, chronic antipsychotic treatment may alter AMPA affinity states by post-translational mechanisms.

In summary, this study suggests chronic treatment with typical antipsychotic drugs affects the high affinity state of the AMPA receptor more than atypical antipsychotic drugs. The three conceptual areas that have been described to distinguish typical and atypical antipsychotic drug action include a dose-response separation, anatomic specificity of pharmacologic actions and selective neurotransmitter receptor interactions (for review, see Kinon and Lieberman, 1996). This study adds an interaction with AMPA receptors to the potential profile of neurotransmitter receptor interactions that characterize differences between typical and atypical antipsychotic drugs. It remains to be shown if this difference in receptor binding is causally related to the differences in side effect liability and/or efficacy in these two antipsychotic drug groups.

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