

The effects of antipsychotic drugs on GABA_A receptor binding depend on period of drug treatment and binding site examined

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Abstract

Changes in GABA_A receptors are observed in schizophrenia, with benzodiazepine-sensitive GABA_A receptor subtypes being affected differently to other subtypes. However, long-term antipsychotic drug use in schizophrenia may underlie these changes. To test this, we examined the effects of administering a typical (haloperidol) and an atypical (olanzapine) antipsychotic drug on the GABA_A receptor agonist (orthosteric) and benzodiazepine (allosteric) binding sites in rat prefrontal cortex. As antipsychotic drugs have delayed maximal therapeutic effects we also examined different drug treatment periods. Male SD rats received a sucrose solution containing either haloperidol (1.5 mg/kg), olanzapine (6.5 mg/kg) or no drug daily for either 7, 14 or 28 days. Sections of rat brain were then labelled with [³H]muscimol, which labels the total population of GABA_A receptors, or the benzodiazepine site ligand [³H]flunitrazepam in separate saturation binding experiments using quantitative receptor autoradiography. [³H]Muscimol binding was enhanced in the prefrontal cortex after 7 days but no differences were observed after longer periods of drug administration. In contrast there was a delayed increase in density of benzodiazepine-sensitive GABA_A receptors in the PFC, suggesting that antipsychotic drugs have different effects on different GABA_A receptor subtypes. These changes in the properties of GABA_A receptor binding following antipsychotic drug administration are not consistent with those observed in schizophrenia and suggest a 'reshuffling' in GABA_A receptor subtypes over time.

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1. Introduction

Post-mortem studies of the brains of people with schizophrenia suggest that GABA_A receptors are affected in a subtype-selective fashion. Each GABA_A receptor is made up of 5 protein subunits but different GABA_A

receptor subtypes vary in their subunit combinations (there are 16 identified subunits) (Chebib and Johnston, 2000). In the pre-frontal cortex (PFC) of people with schizophrenia, the total population of GABA_A receptors (labelled by [³H]muscimol) is increased (Benes et al., 1996b; Dean et al., 1999; Hanada et al., 1987), yet there is a reduction in GABA_A receptor subtypes with benzodiazepine binding sites (labelled by [³H]flunitrazepam) (Pandey et al., 1997). Thus, different GABA_A receptor subtypes appear to be affected differently in schizophrenia, suggesting that certain subtypes may represent important therapeutic targets.

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The potentially confounding effects of pre-mortem antipsychotic drug treatment on studies of the schizophrenic brain make understanding the effects of antipsychotic drug administration on GABA_A receptors essential. Animal models of antipsychotic drug dosing have suggested that following at least 28 days of drug administration to rats, [³H]muscimol binding decreases in the hippocampus and temporal regions (Farnbach-Pralong et al., 1998; Zink et al., 2004), but increases in the anterior cingulate cortex (Zink et al., 2004). Few studies have examined [³H]flunitrazepam binding following antipsychotic drug administration and the existing studies, which found no change in binding to striatal (Rupniak et al., 1987) or cortical (Gavish et al., 1988) membrane preparations, have only used short drug administration periods (up to 21 days) and low drug doses. Thus, the aim of the present study was to determine the effects of antipsychotic drug treatment on both [³H]muscimol and [³H]flunitrazepam binding in the prefrontal cortex (PFC), a region of the brain strongly implicated in schizophrenia. Additionally, as the effects of antipsychotic drugs evolve over a time course of up to 28 days before they reach full therapeutic efficacy (Kapur et al., 2005; Leucht et al., 2005) we have also examined how GABA_A receptor binding is affected over this 28 day period.

2. Methods

2.1. Chemicals

[³H]Muscimol and [³H]flunitrazepam were purchased from NEN Perkin-Elmer (Ontario, Canada). Hydrochloric acid for pH adjustments was purchased from APS Finechem (Seven Hills, Australia). Orally administered olanzapine was obtained from the Royal Prince Alfred Hospital pharmacy (Eli Lilly Pharmaceuticals, West Ryde, Australia). Tissue-Tek-OCT embedding compound was purchased from Sakura Finetechnical (Tokyo, Japan.). All other chemicals were obtained from Sigma-Aldrich (St. Louis, MO, USA).

Kodak Biomax MS film, autoradiography cassettes and tritium microscale standards (2.0–110.0 nCi/mg and 0.1–15.4 nCi/mg) were purchased from Amersham (Castle Hill, NSW, Australia). Phenisol developer and Hypam Rapid Fixer were obtained from Ilford (Mt Waverley, NSW, Australia).

2.2. Antipsychotic drug administration

Male Sprague-Dawley rats aged 8 weeks on arrival (initial weight 250–300 g, Laboratory Animal Services, Perth, Australia) were housed in groups of three per cage

under a 12 h/12 h light/dark cycle and permitted food and water ad libitum. Animals were housed under these conditions for a minimum of one week and were then handled for an additional week to habituate to experimenters prior to intervention.

Rats were randomly assigned to drug (vehicle, haloperidol, olanzapine) and time (7, 14 or 28 day) treatment groups ($n=6$ per group). Drugs were administered daily in drinking water to account for the difference between human and rat drug half-life (Kapur et al., 2003). Control rats received the vehicle solution of 8% sucrose at pH 6. Animals were weighed three times a week and these weights were used to calculate the doses of either 1.5 mg/kg haloperidol or 7.5 mg/kg olanzapine to be dissolved in the drinking solution. Drug doses were selected that had been shown to produce plasma concentrations following oral administration in rats equivalent to those therapeutically effective in humans (Volavka et al., 1992). Water consumed per cage was measured daily and mean drug intake was found to average 1.5 mg/kg/day in the haloperidol group and 6.5 mg/kg/day in the olanzapine group. All animal experiments were approved by the Animal Ethics Committee of the University of Sydney.

2.3. Tissue acquisition and preparation

Following 7, 14 and 28 days of treatment, 6 animals from each drug treatment group were sacrificed between 12 and 3 pm. Carbon dioxide gas was used to anaesthetise rats prior to decapitation. Brains were removed from the cranium over ice then immediately immersed in liquid isopentane on dry ice (−30 °C) for 15 s to ensure rapid freezing. Tissue was stored at −70 °C until sectioning. Coronal sections were cut at 12 μm thickness in a cryostat (Damon/IEC Division, Nedham Heights, MA, USA) maintained at −14 °C then thaw-mounted onto slides pre-treated with 2% silane in acetone. Three sections were mounted per slide and slides were stored for a maximum of 12 days at −70 °C prior to receptor binding assays.

2.4. Receptor binding assays

Brain sections were thawed for 20 min at room temperature then washed twice for 15 min in 50 mM Tris–HCl buffer (pH 7.4) to remove endogenous ligands. Sections were then incubated at 0 °C in 50 mM Tris–HCl (pH 7.4) containing either 50 nM [³H]muscimol for 40 min or 10 nM [³H]flunitrazepam for 60 min as previous experiments had shown these concentrations produced saturable binding (Palacios et al., 1981;

Table 1

Differences in GABA_A receptor binding relative to controls following different periods of antipsychotic drug administration

		7 days		14 days		28 days	
		Haloperidol	Olanzapine	Haloperidol	Olanzapine	Haloperidol	Olanzapine
[³ H]Muscimol	% change (SEM)	+67(9)%*	+69(7)% *	+7(7)%	+9(5)%	-15(9)%	-18(5)%
	<i>n</i>	5	6	6	6	5	6
[³ H]Flunitrazepam	% change (SEM)	-5(1)%	-7(1)%	+9(6)%	+5(2)%	+11(2)%**	+10(2)%*
	<i>n</i>	5	6	6	6	6	6

Abbreviations: % change (% change relative to vehicle-treated rats), SEM (standard error of mean). The levels of significance relative to controls are indicated as follows $p < 0.001$ (**), $p < 0.05$ (*).

Young and Kuhar, 1979). Using additional sections, non-specific binding of [³H]muscimol was determined by adding 100 μ M bicuculline, a selective GABA_A receptor antagonist, and non-specific binding of [³H]flunitrazepam was determined by adding 3 μ M diazepam. Following incubation with the radioligand, sections were then rapidly washed in four separate flasks of ice cold buffer to terminate binding. All sections were dried and stored at 4 °C overnight then placed in autoradiography cassettes with two tritium microscale standards (0.1–15.4 nCi/mg and 2.0–110.0 nCi/mg) and exposed to Kodak Biomax-MS film at -20 °C for 2 or 4 weeks. Films were developed for 5 min in Ilford Phenisol then fixed for 7 min in Ilford Hypam Rapid Fixer.

2.5. Image analysis

Films were scanned using a BIO-RAD densitometry scanner (GS-690 Imaging Densitometer, Faculty of Pharmacy, University of Sydney) connected to a PowerMac 8600/200. Images were analysed using Image Quant v1.1 software (Molecular Dynamics, ITC-Academic Computing Health Science, University of Virginia, USA) to determine average optical density (sum of pixel values/number of pixels) in sections. For all brain sections, four optical density measurements were made per area of interest, two in each hemisphere (no differences were apparent between hemispheres). These hemispheric values were averaged to give a mean optical density value per region, per section, with two sections per animal. Brain regions were defined by the experimenter circling a region of interest on the autoradiograph following examination of cresyl violet stained slides and reference to the rat brain atlas (Paxinos and Watson, 1997).

Optical density measurements were converted to concentration of radioactivity per weight of tissue (nCi/mg) using the [³H]microscales. Specific binding was ascertained by subtracting non-specific binding from total binding.

2.6. Statistical analysis

Statistical analyses were performed using SPSS 11.0 (SPSS, Inc., Chicago, Ill., USA). Differences between drug treatment groups in the maximal binding of each radioligand for each drug administration period were assessed using a between subjects one-way ANOVA. Where the overall one-way ANOVA was significant, post-hoc contrast analysis using the Tukey HSD test was employed to determine which groups were statistically different. To examine the effects over time, between-subjects two-way ANOVA's were conducted in SuperANOVA 1.1 (Abacus Concepts, Berkeley, Ca., USA) using Scheffe's post-hoc tests to determine the source of significant main effects, and means comparison contrasts to examine the drug \times time interaction.

3. Results

Table 1 provides results from bicuculline-sensitive [³H]muscimol binding and diazepam-sensitive [³H]flunitrazepam binding experiments in rat PFC. Following 7 days of antipsychotic drug administration, increased [³H]muscimol binding site density was observed in the PFC of both haloperidol ($q=4.8$, $p < 0.05$) and olanzapine treated rats ($q=3.9$, $p < 0.05$) compared with controls ($p=0.0094$). No significant differences in [³H]muscimol binding were observed after 14 or 28 days of drug administration. Significant decreases in [³H]muscimol binding occurred over time in the PFC of haloperidol ($p=0.0001$) and olanzapine treated groups ($p=0.0001$).

[³H]flunitrazepam binding was only affected after 28 days of drug administration where both haloperidol ($q=4.9$, $p < 0.05$) and olanzapine ($q=4.2$, $p < 0.05$) treatment increased [³H]flunitrazepam binding site density relative to vehicle-treated controls ($p=0.007$). Over time [³H]flunitrazepam binding increased in all treatment groups at 28 days relative to 7 days ($p=0.0001$).

4. Discussion

Results showed that the effects of antipsychotic drugs on the total GABA_A receptor density vary according to the period of antipsychotic drug administration. Treatment with haloperidol and olanzapine initially increased the total density of GABA_A receptors relative to controls in the PFC, but after longer treatment periods no difference was observed. Consistent with the latter finding another study also found total GABA_A receptor density was unchanged in the PFC of animals treated long-term (6 months) with haloperidol or clozapine (Zink et al., 2004).

Interestingly, the GABA_A receptor pattern mapped over antipsychotic drug administration period correlates with that of synaptic monoamine levels which also increase initially but return to baseline following about three weeks (O'Donnell and Grace, 1996). Synaptic monoamine levels presumably re-adjust to the high affinity antagonist action of typical and atypical antipsychotic drugs at dopamine receptors. Thus, the change in GABA_A receptor expression over the initial treatment period observed in this study, suggests that slow re-adjustments occur in the GABAergic system in response to antipsychotic drug administration.

In contrast to the results for [³H]muscimol binding, there were no effects on benzodiazepine-sensitive GABA_A receptor binding density following shorter treatment periods in the present study. Previous studies have similarly observed that short treatment periods of 6 (Fuchs et al., 1986) or 21 days with haloperidol (Gavish et al., 1988) and 14 days with olanzapine (Wong et al., 1996) failed to produce a change in benzodiazepine receptor expression in cortical membrane preparations.

Of greatest interest from our [³H]flunitrazepam binding experiments was the finding that the density of benzodiazepine-sensitive GABA_A receptors was increased in the PFC of both haloperidol and olanzapine treated rats, but only after 28 days of drug administration. This small yet significant increase indicates that antipsychotic drugs have delayed effects on benzodiazepine-sensitive GABA_A receptors in the PFC, which may be of relevance to the delayed clinical effects of these drugs. Furthermore, it appears that benzodiazepine receptors are differentially regulated from other GABA_A receptors following antipsychotic treatment. Increased benzodiazepine site density in the PFC without alterations in the total population of GABA_A receptors following 28 days treatment suggests that antipsychotic drug administration results in a 'reshuffling' of GABA_A receptor subtypes.

Altered GABA_A receptor densities observed in post-mortem schizophrenic brain are inconsistent with the

effects of prolonged antipsychotic drug administration on GABA_A receptors observed here in healthy rats. Studies using post-mortem tissue from people with schizophrenia have found increased GABA_A receptors (Benes et al., 1996a,b) but reduced or unchanged expression of benzodiazepine-sensitive GABA_A receptors in the PFC and hippocampus (Benes et al., 1996a,b; Owen et al., 1981; Pandey et al., 1997; Squires et al., 1993). Our results indicate that prolonged antipsychotic drug administration affects the PFC density of benzodiazepine-sensitive GABA_A receptors, but this change is in the opposite direction to that observed in schizophrenia. Although changes observed in schizophrenic brain may be due to a number of factors, including the disease state, our findings support the notion that changes in GABA_A receptor binding density are at least not due to antipsychotic drug treatment. A longer treatment period of greater than 28 days as well as drug withdrawal prior to sacrifice (Huffman and Ticku, 1983; See et al., 1990) may be required in a future study to confirm that the changes we observed here prevail.

In conclusion, GABA_A receptor density in the PFC varied according to the period of antipsychotic drug administration and the GABA_A receptor subtype that was examined. We observed initial increases in the total GABA_A receptor population that disappeared over time. However, after 28 days, antipsychotic drug administration appeared to have induced a 're-shuffling' of GABA_A receptor subtypes given that there was an increased density of benzodiazepine-sensitive GABA_A receptors, despite there being no net change in the total population of GABA_A receptors. Such changes in GABA_A receptors in the brains of healthy rats receiving antipsychotic drugs are not consistent with those observed in post-mortem studies of schizophrenia, suggesting that the disease pathophysiology alters GABA_A receptors in a manner that may be compensated for by antipsychotic drugs.

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