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Clozapine and olanzapine treatment decreases rat cortical and limbic $GABA_A$ receptors

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Abstract

The density of $GABA_A$ receptors in the hippocampus and the temporal cortex from rats treated for 28 days with either haloperidol, chlorpromazine, clozapine or olanzapine was measured. Compared to haloperidol (0.01 and 0.1 mg kg^{-1} day^{-1}) and chlorpromazine (0.1 and 1 mg kg^{-1} day^{-1}), clozapine and olanzapine (0.1 and 1 mg kg^{-1} day^{-1}) markedly decreased the density of $GABA_A$ receptors in these two brain regions. These data suggest that modulation of GABAergic transmission could be an important action of some antipsychotic drugs.

Keywords: Antipsychotic; GABA (γ-aminobutyric acid); Schizophrenia

The action of antipsychotic drugs was initially thought to predominantly involve antagonism of dopamine D_2-like receptors (Carlsson, 1978). It has now been shown that drugs such as clozapine and olanzapine antagonize a range of neurotransmitter receptors (Gerlach and Peacock, 1995), making the relationship between the pharmacological properties of antipsychotic drugs and their clinical action less clear.

Monoamine and cholinergic projections synapse onto GABAergic interneurons in cortico-limbic brain regions, and these neurons offer a potential locus where the diverse sites of antipsychotic drug action may converge. Indeed, chronic administration of haloperidol increases GABA in axosomatic terminals surrounding pyramidal cell bodies in the rat medial prefrontal cortex, an effect which would likely be associated with an increased GABA release (Vincent et al., 1994). Increased GABA has been shown to down-regulate postsynaptic $GABA_A$ receptors (Mhatre and Ticku, 1994) and therefore, it could be postulated that antipsychotic drug treatment should result in reduced levels of $GABA_A$ receptors.

To determine whether antipsychotic drug treatment reduces the level of $GABA_A$ receptors, 9 groups of 5 rats were treated for 28 days with either haloperidol (0.01 or 0.1 mg kg^{-1} day^{-1}), chlorpromazine, clozapine or olanzapine (all at 0.1 or 1 mg kg^{-1} day^{-1}), or vehicle (ethanol, less than 0.1%) in drinking water. After 2 days without treatment, the animals were killed by cervical dislocation, their brains removed and frozen at −70°C. Coronal sections (20 μm) were cut at Bregma −5.80 mm (Paxinos and Watson, 1986) and thaw mounted on gelatin coated slides. The density of $GABA_A$ receptors was measured in the hippocampus and the temporal cortex using quantitative autoradiography, and taken as the difference between the binding of [^{3}H]muscimol (100 nM) in the absence or presence of the $GABA_A$ receptor antagonist SR95531 (10 μM). Drugs were incubated with tissue in 50 mM Tris–citrate buffer, pH 7.1, for 1 h at 4°C. Sections were carefully washed prior to and after incubation with the radioligand. Autoradiographic images were generated by exposing dried sections and Amersham [^{3}H]microscales to Amersham ^{3}H-sensitive Hyperfilm, and the images were analysed using the MCID image analysis system (Imaging Research, Ontario, Canada).

Rats treated with haloperidol (0.1 mg kg^{-1} day^{-1} only) and chlorpromazine showed a small but significant decrease in the density of $GABA_A$ receptors in the hippocampus and the temporal cortex (Fig. 1). In the temporal cortex, the decrease in $GABA_A$ receptors was also significant in rats treated with haloperidol at 0.01 mg kg^{-1}

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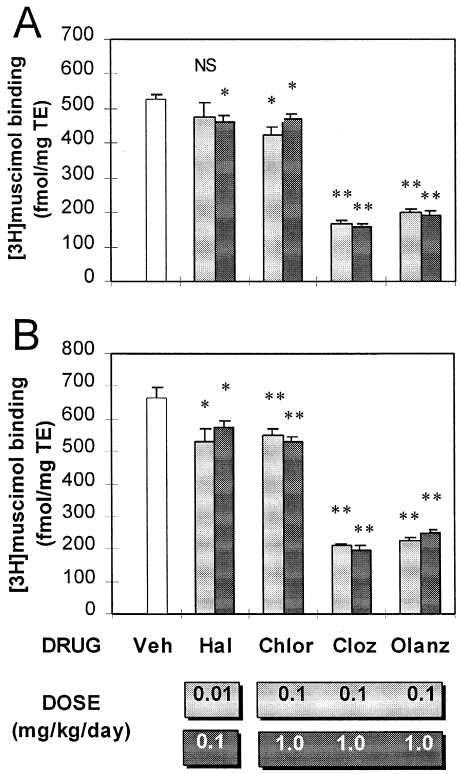

Fig. 1. The density (mean ± S.E.M.) of $GABA_A$ receptors in the hippocampus (areas CA1 + CA2 + CA3) (A) and temporal cortex (areas Te1 + Te3) (B) from adult male Sprague–Dawley rats treated for 28 days with antipsychotic drugs. Statistical differences between treatment groups and vehicle groups were analysed using the Mann–Whitney test (Prism 2.00, GraphPad Software). NS: not significant. $*$: $P < 0.05$. $**$: $P < 0.01$. Veh: vehicle. Hal: haloperidol. Chlor: chlorpromazine. Cloz: clozapine. Olanz: olanzapine.

day^{-1}. In rats treated with clozapine and olanzapine, there was a more marked reduction in the density of the $GABA_A$ receptor in both regions (Fig. 1). Notably, the reduction appeared maximal with 0.1 mg kg^{-1} day^{-1} of clozapine or olanzapine, as a ten fold increase in dosage had no further effect on $GABA_A$ receptor density (Fig. 1).

Clozapine and olanzapine have been shown to have a low affinity for the $GABA_A$ receptor ($K_i > 10$ μM) (Bymaster et al., 1996). It has been shown that in clozapine treated rats, the concentration of drug in brain tissue rises to about 2 μM per mg/kg dose, with no accumulation after repeated daily dosing (Baldessarini et al., 1993). Together, these data make it unlikely that the decrease in $GABA_A$ receptors observed after treatment with clozapine and olanzapine is a $GABA_A$ receptor mediated or an effect of residual drug. Thus, the decrease in $GABA_A$ receptors is likely to be due to distal effects of clozapine and olanzapine, possibly involving increased GABA release following the blockade of multiple neurotransmitter receptors on GABAergic interneurons.

It has been shown that there is a decrease in the density of GABAergic interneurons in cortico-limbic regions, as well as an increase in the density of postsynaptic $GABA_A$ receptors in the same brain regions from subjects with schizophrenia (Benes, 1995). Together these findings suggest that there may be a decrease in GABAergic activity in schizophrenia. Our data would therefore support the hypothesis that antipsychotic drugs, such as clozapine and olanzapine, would assist in substantially reversing an under-active GABAergic system in schizophrenia and hence exert some of their therapeutic effects. The neuropharmacological mechanisms by which antipsychotic drugs affect GABAergic neurons need to be investigated to further explore this hypothesis.

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