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SPECIAL REPORT

Interaction of clozapine with the histamine H_3 receptor in rat brain

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We examined possible interactions between neuroleptics and the histamine H_3 receptor and found an interaction of clozapine with this receptor. In competition binding experiments, using the H_3 antagonist, [^{125}I]-iodophenpropit, we observed a K_i of 236 ± 87 nM. Functionally, clozapine was studied on the H_3-mediated inhibition of [^{3}H]-5-hydroxytryptamine ([^{3}H]-5-HT) release from rat brain cortex slices. Clozapine acts as an antagonist with an apparent K_B value of 79.5 nM.

Keywords: Histamine H_3 receptor; clozapine; neuroleptic; rat brain slices; [^{125}I]-iodophenpropit; [^{3}H]-5-HT release

Introduction Recent studies suggest that histamine may be involved in schizophrenia. In treatment-resistant patients, the mean level of *tele*-methylhistamine (t-MH) in cerebrospinal fluid, an index of histaminergic activity, was 2.6 fold higher than in controls (Prell *et al.*, 1995). Cerebrospinal fluid levels of t-MH correlated positively with those of other neurotransmitters metabolites, and with severity of schizophrenic symptoms (Prell *et al.*, 1995). Consonant with increased histaminergic activity are a reduction in densities of H_1 receptors in *postmortem* frontal cortex (Nakai *et al.*, 1991) and the reported therapeutic actions of famotidine, an H_2 antagonist in schizophrenic patients (Kaminski *et al.*, 1990; Deutsch *et al.*, 1993). Evidence for elevated histaminergic activity suggests possible functional anomalies in H_3 receptors. H_3 autoreceptors regulate release of histamine as well as other neurotransmitters (H_3 heteroreceptors), that may be involved in schizophrenia (e.g. 5-hydroxytryptamine (5-HT) and dopamine) (Fink *et al.*, 1990). Therefore, we examined the effects of neuroleptics on H_3 receptors, giving particular attention to clozapine.

Methods *Binding assays* Competition binding experiments were performed according to the method fully described by Jansen *et al.* (1994).

Functional assays (Based on Van der Werf *et al.*, 1987). [^{3}H]-5-hydroxytryptamine (5 μCi, 11 Ci mmol^{-1}) in 2 ml Krebs Ringer buffer was used to preload the rat neocortex slices. Loaded slices were perfused at 0.3 ml min^{-1}. Six 10 min fractions were collected, tritium overflow was evoked by electrical stimulation (rectangular pulses, 30 mA and 2 ms, 1 Hz) during the second fraction. Lastly, the slices were perfused with 0.1 N HCl to extract the remaining tritium in the tissue.

Results *Binding studies* Displacement of 0.25 nM [^{125}I]-iodophenpropit binding by clozapine (Figure 1a) fitted best to a one site model ($P<0.05$) using the non-linear curve fitting programme LIGAND, resulting in a K_i value of 236 ± 87 nM ($n = 6$, mean ± s.e.mean). Fitting data to a sigmoidal curve yielded a slope equal to 0.80 ± 0.05. Other neuroleptics studied showed relatively low affinity for [^{125}I]-iodophenpropit binding (Table 1).

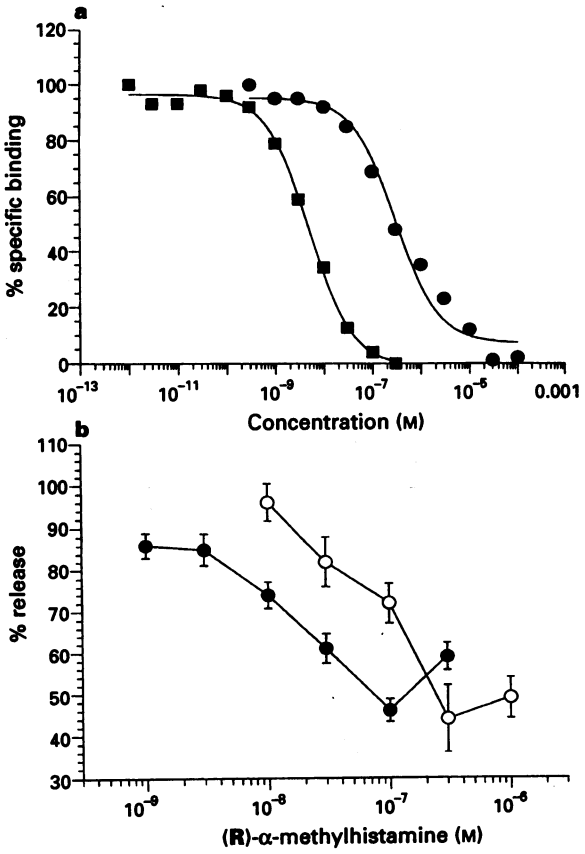

Figure 1 (a) Inhibition of [^{125}I]-iodophenpropit binding (0.25 nM) by the H_3 receptor antagonist, thioperamide (■) and clozapine (●). Data are expressed as % specific binding, determined using 0.3 μM thioperamide and represented 55%-65% of the total binding. The curves show one site fittings of a single representative experiment ($n = 6$) with triplicate determinations. (b) Inhibition by (R)-α-methylhistamine of the electrically stimulated [^{3}H]-5-HT release from rat neocortex slices in the absence (●) and presence (○) of 1 μM clozapine. Each point represents mean ± s.e.mean of three or four different experiments performed in duplicate. The EC_{50} values are determined from the mean values according to the equation $E = 100 - (100 - E_{max})/(1 + (EC_{50}/[A])$ where E represents the effect observed at any agonist concentration of [A], EC_{50} represents the agonist concentration that produces half-maximal effect, and E_{max} the maximal effect. The dissociation constant (K_B) was calculated from the equation $[A']/[A] = 1 + [B]/K_B$, where [A] is the concentration of the agonist that produces half-maximal effect, [A'] is the concentration of the agonist that produces half-maximal effect in the presence of the antagonist concentration [B].

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Table 1 Affinity of H_3 receptor agonists and antagonists and of several neuroleptics [RBI] for [^{125}I]-iodophenpropit binding sites to rat cortex compared with their functional potency (apparent values of K_B and K_i are shown, based on competitive antagonism)

Ligand	*Inhibition of [^{3}H]-5-HT release*	*[^{125}I]-iodophenpropit binding*
Histamine	EC_{50} = 48.2 nM (3)	K_{iH} = 38 ± 10 nM (4)[a]
(R)-α-methylhistamine	EC_{50} = 6.0 nM (4)	K_{iH} = 3.5 ± 1.2 nM (4)[a]
Thioperamide	K_B = 1.1 nM (3)	K_i = 4.3 ± 1.6 nM (7)[a]
Impromidine	K_B = 19.5 nM (3)	K_i = 51 ± 9 nM (3)[a]
Clozapine	K_B = 79.5 nM (4)	K_i = 236 ± 87 (6)
Chlorpromazine	ND	IC_{50} > 10 μM (2)
Haloperidol	ND	IC_{50} > 10 μM (2)
Spiperone	ND	IC_{50} > 10 μM (2)

For the binding studies data shown are mean ± s.e.mean. The values in parentheses represent the number of independent experiments. Functional data represent the value obtained from the fitting of mean data (3–4 experiments) as described in detail in the legend of Figure 1b.
ND: Not determined. [a]Jansen *et al.*, 1994.

Functional studies The electrically evoked tritium overflow was inhibited in a concentration-dependent manner by histamine and the H_3 agonist, (R)-α-methylhistamine (Table 1, Figure 1b). H_3-antagonists inhibited the (R)-α-methylhistamine effect with potencies comparable to their affinity for the [^{125}I]-iodophenpropit binding sites (Table 1). Clozapine (1 μM) shifted the (R)-α-methylhistamine concentration-response curve to the right (Figure 1b) yielding an apparent K_B value of 79.5 nM. Clozapine, itself, did not affect the stimulated (104 ± 1%; n = 3, mean ± s.e.mean) or the basal release of [^{3}H]-5-HT (100 ± 2%; n = 3, mean ± s.e.mean).

Discussion Clozapine, an atypical neuroleptic, has therapeutic efficacy in the treatment of drug-resistant schizophrenia (Baldessarini & Frankenburg, 1991). High affinity interactions of clozapine with 5-hydroxytryptamine 5-HT_2 and 5-HT_3 receptors, α_1- and α_2-adrenoceptors, histamine H_1, dopamine D_1 and D_4, and muscarinic receptors have been reported (Baldessarini & Frankenburg, 1991). Yet, in comparison to the typical neuroleptics, clozapine shows lower *in vitro* affinity for striatal dopamine D_2 receptors.

In the present work, we observed an intriguing interaction of clozapine with the histamine H_3 receptor, where clozapine acts as an H_3 antagonist. Other neuroleptics showed low affinity for the H_3 receptor.

The interaction of the non-imidazole compound, clozapine with the H_3 receptor is striking, since the imidazole moiety was thought to be required for high affinity H_3 binding.

In man, plasma concentrations of clozapine associated with clinical responses are approximately 0.6–1.2 μM (Baldessarini & Frankenburg, 1991). In rat, with doses higher than 5 mg kg^{-1} and within the therapeutic range in man, clozapine levels in the brain averaged 29 fold higher than corresponding serum drug levels (Balderassini *et al.*, 1993). Assuming total serum levels of 0.9 μM and 95% protein bound, total brain levels of 1.1 μM can be obtained. If the K_i value for clozapine at the rat H_3 receptor is similar for its human counterpart, brain clozapine concentrations might be high enough for a functional interaction with the H_3 receptor under clinical conditions. In conclusion: clozapine, showed a higher affinity for the histamine H_3 receptor than several other neuroleptics. Functionally, it revealed an antagonistic behaviour.

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