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The role of α_2-adrenoceptor antagonism in the anti-cataleptic properties of the atypical neuroleptic agent, clozapine, in the rat

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1 The mechanism underlying the anticataleptic properties of the atypical neuroleptic agent, clozapine, has been investigated in the rat.

2 The close structural analogues of clozapine, loxapine (0.1 mg kg^{-1} s.c.) and iso-clozapine (1 and 3 mg kg^{-1} s.c.) induced catalepsy in rats. In contrast, clozapine and the regio-isomer of loxapine, iso-loxapine (up to 10 mg kg^{-1} s.c.) did not produce catalepsy, but at a dose of 1 mg kg^{-1} significantly inhibited catalepsy induced by loxapine (0.3 mg kg^{-1} s.c.).

3 Radioligand binding assays showed that cataleptogenic potential was most clearly predicted by the D_2/5-HT_{1A}, D_2/5-$HT_{1B/1D}$ and D_2/α_2-receptor affinity (K_D) ratios: i.e. 30–100-fold higher ratios were calculated for loxapine and iso-clozapine, whereas the ratios were less than 1 for clozapine and iso-loxapine. The ratios of affinities for D_2 to 5-HT_{2A}, 5-HT_{2C} or D_1 did not reflect the grouping of cataleptic and non-cataleptic compounds.

4 Co-treatment with the α_2-adrenoceptor antagonists, yohimbine (1–10 mg kg^{-1} s.c.), RX 821002 (1–10 mg kg^{-1} s.c.) and MK-912 (0.3 and 1 mg kg^{-1} s.c.) dose-dependently inhibited the cataleptic response to loxapine (0.3 mg kg^{-1}). Yohimbine (1–10 mg kg^{-1} s.c.) also dose-dependently inhibited the cateleptic response to haloperidol (0.3 mg kg^{-1} s.c.). The α_2-adrenoceptor antagonists had no effect *per se*.

5 Neither yohimbine (10 mg kg^{-1}) nor RX821002 (3 mg kg^{-1}) altered the cataleptic response to the D_1 receptor antagonist, SCH 23390 (1 mg kg^{-1} s.c.), while, like clozapine, both compounds abolished the response to the 5-HT_{2A} receptor antagonist, MDL 100,151 (3 mg kg^{-1} s.c.).

6 The present data strongly implicate α_2-adrenoceptor blockade in the anticataleptic properties of clozapine and suggest that its lack of extrapyramidal side effects in the clinic may also be a consequence of this property.

Keywords: Catalepsy; yohimbine; RX 821002; clozapine; loxapine; neuroleptic drugs; α_2-adrenoceptor antagonists

Introduction

Clozapine is distinguished from most other neuroleptics by its failure to induce catalepsy in rats and by its low liability to induce extrapyramidal side effects (EPS) in man (Baldessarini & Frankenburg, 1991). Both catalepsy and EPS are thought to reflect blockade of dopamine D_2 receptors in the basal ganglia. Since clozapine has appreciable affinity for D_2 receptors (Meltzer *et al*., 1989), we have reasoned that clozapine must be able to inhibit its own expression of these behaviours, since it is able to block the expression of catalepsy induced by structurally related neuroleptics (Kalkman *et al*., 1997). However, the mechanism by which these effects are achieved is unknown. Interestingly, clozapine is being used increasingly to treat psychosis and dyskinesia in patients with Parkinson's disease (Factor & Friedman, 1997; Durif *et al*., 1997) and its efficacy here may well reflect the anticataleptic properties seen in the rat.

Clozapine is notable for its significant affinity for a wide range of monoamine neurotransmitter receptors and many hypotheses based on its separate or multiple pharmacological properties have been generated in an attempt to explain its atypicality (atypical being defined as having neuroleptic activity in the absence of EPS). The classical approach has been to compare the radioligand binding profile or the functional responses of the 'typical' or EPS-inducing neuroleptic drugs (compounds often with a predominantly high affinity for D_2 receptors, such as haloperidol) with the polyvalent profile of clozapine (Chiodo & Bunney, 1983; Robertson & Fibiger, 1992; Seeman *et al*., 1997a). Differences in responses, affinity, or ratios of affinities found in this way have then used to speculate on the basis of the atypical nature of clozapine's neuroleptic activity. The disadvantage of this approach is that whilst many differences have been found, there has been no objective way of recognizing those that might be essential to the atypical nature of the drug. We have attempted, therefore, a different approach.

It has been noted by Schmutz & Eichenberger (1982) that the position of the chlorine substituent in the clozapine structure is important with respect to catalepsy. The movement of the chlorine from position 8 to 2 gave a regio-isomer (iso-clozapine, Figure 1) that behaved as a typical neuroleptic. Also loxapine, known to induce catalepsy, has the chlorine substituent in the 2-position. Importantly though, the loxapine analogue with chlorine in the position equivalent to clozapine (iso-loxapine) was free from catalepsy (Schmutz & Eichenberger, 1982). This dichotomy between clozapine and iso-loxapine on the one hand and iso-clozapine and loxapine on the other, has been studied further in our laboratory. Comparison of the radioligand binding profiles of the two pairs of compounds led to the recognition of a possible role of α_2-adrenoceptor blockade in suppressing dopamine D_2 receptor-mediated catalepsy. Evidence consistent with this hypothesis was then obtained by examining the anticataleptic properties of selective α-$_2$-adrenoceptor antagonists.

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Methods

Animals

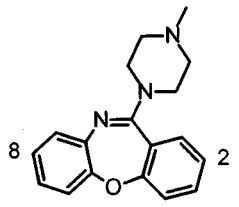
Male Wistar rats (220–250 g) were housed in groups of five with food and water freely available under a 12 h light/dark cycle (lights on at 07 00 h). The ambient temperature was held at 22±2°C with a relative humidity of 50%.

Experimental procedure

Catalepsy was measured at $t=0$ h, 0.5 h, 1 h and then at hourly intervals up to 5 h in a quiet, dimly lit laboratory. Rats were placed with their forepaws on a wooden block (7 cm high) and the time spent in that position without a deliberate move to step down, up to a maximum of 45 s, was measured. Three consecutive attempts to place the rat onto the wooden block were made at each time point with the longest latency to step down taken as the reading. Data are expressed as means±s.e.mean. Each experimental group generally consisted of 10 animals. The experiments were performed strictly according to a protocol accepted by the Basel Stadt Cantonal Veterinary Service.

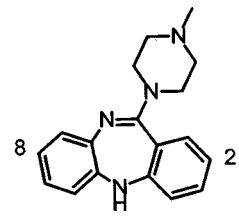
Statistics

The Student *t*-test with Bonferroni correction for multiple comparisons was used to compare groups.



Loxapine: 2 - Cl, 8 - H

iso-Loxapine: 8 - Cl, 2 - H



Clozapine: 8 - Cl, 2 - H

iso-Clozapine: 2 - Cl, 8 - H

Figure 1 Chemical structure of loxapine, iso-loxapine (top), and clozapine and iso-clozapine (bottom).

Drugs

Clozapine, iso-clozapine, iso-loxapine, MK-912 HCl ((−)-1′,3′-dimethylspiro (1,3,4,5′,6,6′,7,12b-octohydro-2H-benzo[b]-furo [2,3-a] quinolizine) -2,4′-pyrimidin -2′- one) and MDL 100,151* (±)-α-(2,3-dimethoxyphenyl)-1-[2-(4-fluorophenyl) ethyl] - 4 - piperidinemethanol; *the active (+)-enantiomer known as MDL 100,907) were synthesized at Novartis Pharma Ltd. Loxapine succinate, yohimbine. HCl, 2-methoxy-idazoxan HCl (RX 821002) and SCH 23390 HCl were purchased from RBI. MDL 100,151, loxapine, iso-loxapine, iso-clozapine and clozapine were dissolved in a small volume of 0.2 ml acetic acid (10%) and further diluted with physiological saline. MK-912, yohimbine and RX 821002 were dissolved in warm H_2O and further diluted in saline. All compounds were administered subcutaneously in the back of the neck.

Radioligand binding

Radioligand binding was performed according to published procedures as referenced in Table 1.

Results

The cataleptic response to loxepine, iso-loxapine, clozapine and iso-clozapine

Loxapine dose-dependently increased the time the animal held its position on the wooden block with a minimum effective dose of 0.1 mg kg^{-1} (Figure 2). The maximum response (i.e. a latency of 45 s developed during the first 120 min after injection of 0.3 mg kg^{-1} and was maintained over the following 3 h.

The regio-isomer of clozapine, iso-clozapine also induced a marked cataleptic response in the rat at 1 and 3 mg kg^{-1}. In contrast, iso-loxapine up to a dose of 10 mg kg^{-1} did not significantly increase the catalepsy score above the value for vehicle-treated controls (Figure 3) but blocked the catalepsy induced by 0.3 mg kg^{-1} loxapine (Figure 4).

Affinity for monoamine receptor subtypes

Clozapine, loxapine and their regio-isomers all had appreciable affinities for dopamine, 5-HT and α-adrenergic receptors (Table 2). Clozapine had the highest affinity for the 5-HT_{2C} receptor ($pK_D=7.93$) and lowest for dopamine D_1($pK_D=6.29$) and 5-$HT_{1B/1D}$ ($pK_D=6.17$) receptors. Loxapine had highest affinity for the 5-HT_{2A} receptor ($pK_D=8.05$) and lowest for the 5-HT_{1A} subtype ($pK_D=5.5$). In grouping the compounds into non-cataleptogenic (clozapine with iso-loxapine) and cateleptogenic (loxapine with

Table 1 Details of the methodology used for the determination of the affinity for various monoamine receptor recognition sites

Receptor	*Ligand*	*Tissue*	*Non-specific binding*	*References*
D_1	[^{3}H]-SCH 23390	calf caudate	10 μM SCH 23390	Markstein *et al*., 1996
D_2	[^{3}H]-spiperone	calf caudate	10 μM spiperone	Markstein *et al*., 1996
5-HT_{1A}	[^{3}H]-8-OH-DPAT	pig cerebral cortex	10 μM 5-HT	Hoyer *et al*., 1985
5-$HT_{1B/1D}$	[^{125}I]-GTI	calf caudate	10 μM 5-HT	Bruinvels *et al*., 1992
5-HT_{2A}	[^{3}H]-ketanserin	rat cerebral cortex	50 μM mianserin	Hoyer *et al*., 1985
5-HT_{2C}	[^{3}H]-mesulergine	recombinant human	10 μM 5-HT	Hoyer *et al*., 1985
α_1	[^{125}I]-BE 2254	rat cerebral cortex	10 μM phentolamine	Engel & Hoyer, 1981
α_2	[^{3}H]-idazoxan	rat cerebral cortex	10 μM yohimbine	Markstein *et al*., 1996

iso-clozapine), it is clear that, with the exception of dopamine D_1 receptors for clozapine and iso-loxapine, there was little difference in the pK_D values between clozapine and iso-loxapine or between loxapine and iso-clozapine. However, both loxapine and iso-clozapine had approximately 10-fold lower affinity for 5-HT_{1A} receptors and α_2-adrenoceptors than clozapine and iso-loxapine whilst the pK_D values for all other receptor subtypes determined differed by not more than 5-fold across all four compounds.

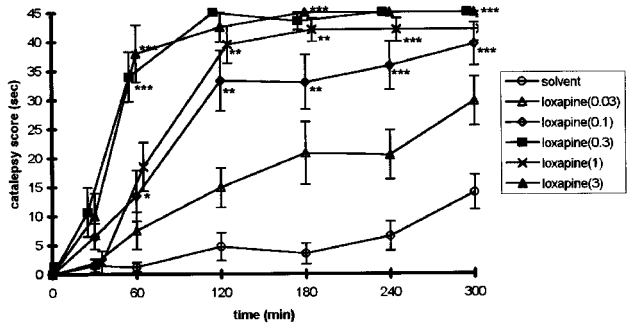


Figure 2 The cataleptic effect of the D_2 receptor antagonist loxapine (0.03–3 mg kg^{-1}, administered s.c. at $t=0$) determined at 60 min intervals over a 300 min period. Data represent mean values ± s.e.mean of groups of 10 rats. Loxapine induced significant catalepsy at 0.1–3 mg kg^{-1} (*t*-test with Bonferroni correction for multiple comparisons; $*P<0.05$, $**P<0.01$, $***P<0.001$).

On the assumption that catalepsy in the rat reflects dopamine D_2 receptor blockade, and that the non-cataleptogenic compounds suppress the cataleptic consequences of their own interaction with D_2 receptors via one of the receptor subtypes here considered, the differences in pK_D values for D_2 and other monoamine receptors have been calculated (Table 3). Differences of more than one log unit were found for both cataleptogenic compounds when comparing D_2 with 5-HT_{1A}, 5-$HT_{1B/1D}$ and α_2-adrenergic receptors. Thus, 5-HT_{1A}, 5-$HT_{1B/1D}$ and/or α_2-adrenergic receptors are potential candidates for a role in the anticataleptic effects of clozapine and iso-loxapine whilst it seems unlikely that 5-HT_{2A}, 5-HT_{2C}, α_1-adrenergic or D_1 receptors are involved.

Antagonism of loxapine-induced catalepsy by α_2-adrenoceptor antagonists

None of the three structurally different α_2-adrenoceptor antagonists, yohimbine (0.3–10 mg kg^{-1}), RX 821002 (1–10 mg kg^{-1}) and MK-912 (0.3–1 mg kg^{-1}) increased the catalepsy score of otherwise untreated animals when given at doses previously shown to antagonize α_2-adrenoceptors in the rat (see, for example, Figure 7 for MK-912; Figure 8 for yohimbine; Figure 9 for RX 821002). However, when each of the compounds, was given over this same dose range concomitantly with loxapine (0.3 mg kg^{-1}), its subsequent cataleptic response was dose-dependently inhibited (Figure 5, 6 and 7). Yohimbine was also found to suppress the cataleptic response to haloperidol (Figure 8).

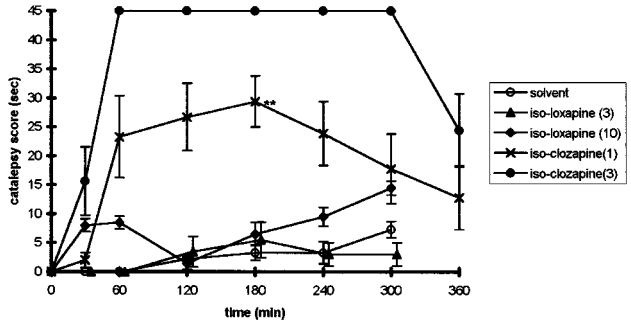


Figure 3 The cateleptic effect, determined at 60 min intervals over 300 min or 360 min period, of iso-clozapine (1 and 3 mg kg^{-1}, s.c.) and the absence of a cateleptic response to iso-loxapine (1–10 mg kg^{-1}, s.c.). Data represent mean values ± s.e.mean of groups of 10 rats. Iso-clozapine (1 mg kg^{-1}) induced significant catalepsy at $t=120$ min ($**P<0.01$; *t*-test with Bonferroni correction). Scores for iso-clozapine (3 mg kg^{-1}) at times 60–300 min were 45 ± 0 s.

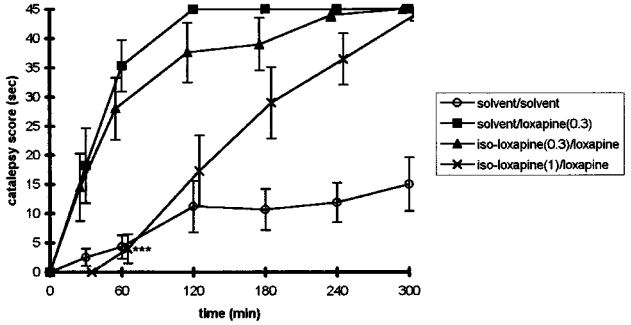


Figure 4 Inhibition of loxapine (0.3 mg kg^{-1})-induced catalepsy by iso-loxapine (0.3 or 1 mg kg^{-1}). Both compounds or respective solvent were injected s.c. at $t=0$ min. Catalepsy was measured at 60 min intervals during a 300 min period. Iso-loxapine inhibited loxapine-induced catalepsy at $t=60$ ($***P<0.001$; *t*-test with Bonferroni correction). Data represent mean values ± s.e.mean of groups of 10 rats.

Table 2 The affinity of clozapine, loxapine and their regio-isomers for various monoamine receptor subtypes. The data shown are the mean pK_D values (± s.e.mean) of three independent measurements. Values in parenthesis indicate the respective differences from the pK_D values for clozapine

	Non-cataleptogenic		*Cataleptogenic*	
Receptor	*Clozapine*	*Iso-loxapine*	*Loxapine*	*Iso-clozapine*
D_1	6.29 ± 0.15	7.35 ± 0.05 (1.06)	7.01 ± 0.14 (0.72)	7.21 ± 0.25 (0.92)
D_2	6.90 ± 0.09	7.19 ± 0.06 (0.29)	7.82 ± 0.06 (0.92)	7.20 ± 0.22 (0.3)
5-HT_{1A}	6.70 ± 0.05	6.98 ± 0.10 (0.28)	5.50 ± 0.22 (−1.2)	5.45 ± 0.19 (−1.25)
5-$HT_{1B/1D}$	6.17 ± 0.12	6.62 ± 0.24 (0.45)	5.74 ± 0.13 (−0.43)	5.92 ± 0.22 (−0.25)
5-HT_{2A}	7.51 ± 0.03	7.66 ± 0.12 (0.15)	8.05 ± 0.15 (0.54)	7.84 ± 0.10 (0.33)
5-HT_{2C}	7.93 ± 0.08	7.96 ± 0.12 (0.03)	7.55 ± 0.22 (−0.38)	7.78 ± 0.14 (−0.15)
α_1	7.44 ± 0.21	7.84 ± 0.15 (0.40)	7.19 ± 0.07 (−0.25)	6.97 ± 0.06 (−0.47)
α_2	6.76 ± 0.11	6.78 ± 0.11 (0.02)	6.02 ± 0.11 (−0.74)	5.65 ± 0.14 (−1.11)

Effects of α_2-adrenoceptor antagonists on catalepsy induced by SCH 23390 and MDL 100,151

Since clozapine has been found to be unable to block catalepsy induced by the dopamine D_1 receptor agonist, SCH 23390 (Kalkman *et al*., 1997) and yet to inhibit the catalepsy induced by the 5-HT_{2A} receptor antagonist, MDL 100,151 (Kalkman *et al*., 1998), the effects of α_2-adrenoceptor blockade on SCH 23390- and MDL 100,151-induced catalepsy were tested. Neither yohimbine (10 mg kg^{-1}) nor RX821002 (3 mg kg^{-1}) affected the cataleptic response to the D_1 receptor antagonist, SCH 23390 (Figure 9), whilst both compounds abolished the response to the 5-HT_{2A} receptor antagonist, MDL 100,151 (Figure 10).

Table 3 The logarithmic differences between the pK_D values of clozapine, loxapine and their regio-isomers for dopamine D_2 and their pK_D values for other receptor binding sites

	Non-cataleptogenic		*Cateleptogenic*	
Receptor	*Clozapine*	*Iso-loxapine*	*Loxapine*	*Iso-clozapine*
D_1	0.61	−0.16	0.81	−0.01
5-HT_{1A}	0.20	0.21	2.32	1.75
5-$HT_{1B/1D}$	0.73	0.57	2.08	1.28
5-HT_{2A}	−0.61	−0.47	−0.23	−0.64
5-HT_{2C}	−1.03	−0.77	0.27	−0.58
α_1	−0.54	−0.65	0.63	0.23
α_2	0.14	0.41	1.80	1.55

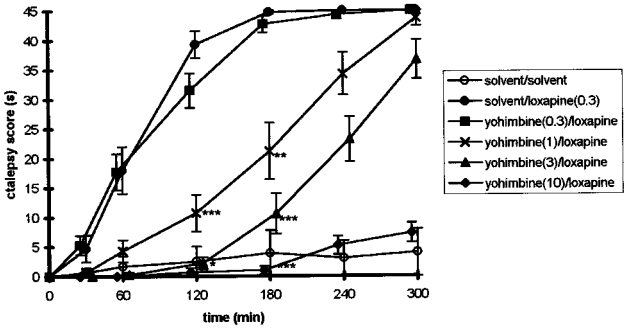


Figure 5 Inhibition of loxapine (0.3 mg kg^{-1})-induced catalepsy by yohimbine. Both compounds were injected s.c. at $t=0$ min. Catalepsy were measured at 60 min intervals during a 300 min period. Data represent mean values ± s.e.mean of groups of ten rats. Significant inhibition of the effect of loxapine was observed after treatment with yohimbine 1, 3 and 10 mg kg^{-1} (** indicates $P<0.01$; ***$P<0.001$; *t*-test with Bonferroni correction).

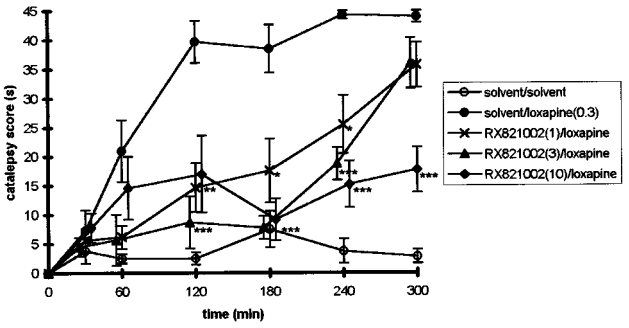


Figure 6 Inhibition of loxapine (0.3 mg kg^{-1})-induced catalepsy by RX821002. Both compounds were injected sc. at $t=0$ min. Catalepsy was measured at 60 min intervals during a 300 min period. Data represent mean values ± s.e.mean of groups of ten rats. Significant inhibition of the effect of loxapine was observed after treatment with all doses of RX821002 (* indicates $P<0.05$; **$P<0.01$; ***$P<0.001$; *t*-test with Bonferroni correction. RX821002 given alone did not induce catalepsy (see Figure 9).

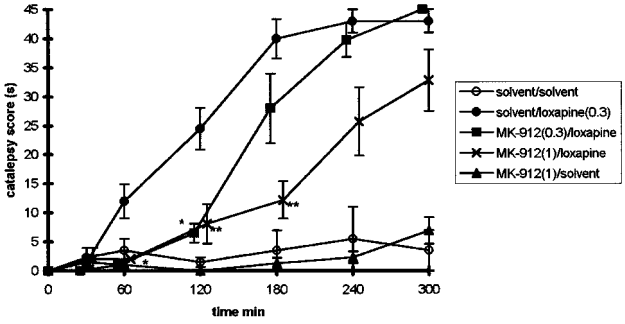


Figure 7 Inhibition of loxapine (0.3 mg kg^{-1})-induced catalepsy by MK-912 (0.3 and 1 mg kg^{-1}). Both compounds were injected s.c. at $t=0$ min. Catalepsy was measured at 60 min intervals during a 300 min period. Data represent mean values ± s.e.mean of groups of ten rats. Significant inhibition of the effect of loxapine was observed after treatment with two doses of MK-912 (*indicates $P<0.05$; **$P<0.01$; *t*-test with Bonferroni correction). MK-912 (1 mg kg^{-1}) given alone did not induce catalepsy.

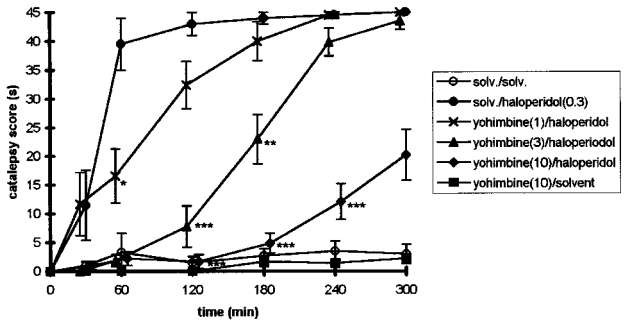


Figure 8 Inhibition of haloperidol (0.3 mg kg^{-1})-induced catalepsy by yohimbine (0.3 – 10 mg kg^{-1}). Both compounds were injected s.c. at $t=0$ min. Catalepsy was measured at 60 min intervals during a 300 min period. Data represent mean values ± s.e.mean of groups of ten rats. Significant inhibition of the effect of haloperidol was observed after treatment with yohimbine 1, 3 and 10 mg kg^{-1} (* indicates $P<0.05$; **$P<0.01$; ***$P<0.001$; *t*-test with Bonferroni correction). Yohimbine (10 mg kg^{-1}) given alone did not induce catalepsy.

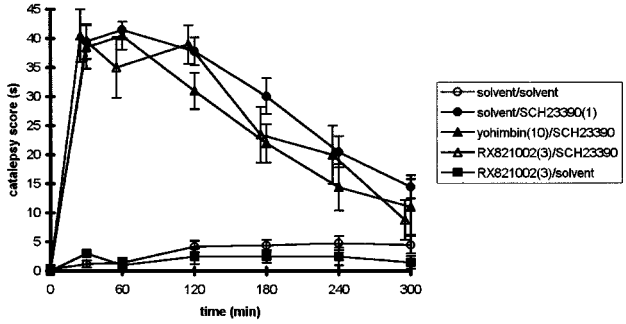


Figure 9 Failure of yohimbine (10 mg kg^{-1}) or RX 821002 (3 mg kg^{-1}) to inhibit SCH 23390 (1 mg kg^{-1})-induced catalepsy. Combinations of compounds were injected s.c. at $t=0$ min. Catalepsy was measured at 60 min intervals during a 300 min period. Data represent mean values ± s.e.mean of groups of ten rats, except in the group that received SCH-23390 alone where $n=20$.

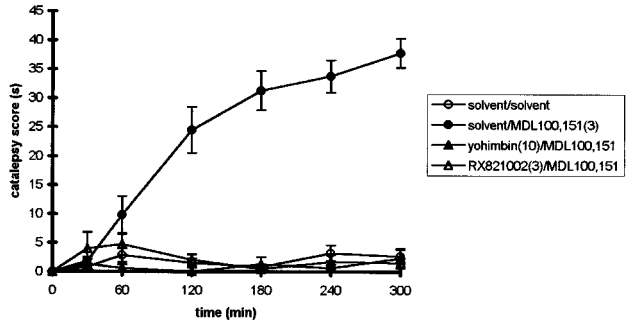


Figure 10 Inhibition of MDL 100,151 (3 mg kg^{-1})-induced catalepsy by yohimbine (10 mg kg^{-1}) or RX 821002 (3 mg kg^{-1}). Combinations of compounds were injected s.c. at $t=0$ min. Catalepsy was measured at 60 min intervals during a 300 min period. Data represent mean values$\pm$s.e.mean of groups of ten rats, except the group receiving MDL 100, 151 only, where the group size was $n=20$.

Discussion

Consistent with previous findings (Schmutz & Eichenberger, 1982; Sorensen *et al*., 1993; Hoffman & Donovan, 1995; Kalkman *et al*., 1997), loxapine and iso-clozapine, but not clozapine or iso-loxapine induced catalepsy in the rat. In fact, both clozapine (Kalkman *et al*., 1997) and iso-loxapine (present results) inhibited the cataleptic response to the typical neuroleptic, loxapine.

The separation between the cataleptogenic and non-cataleptogenic analogues of clozapine and loxapine coincides with the positioning of the chlorine substituent in the tricyclic dibenzazepine structure at C8 (Figure 1). The importance of the halogen substituent in position 8 is further supported by the atypical nature of the neuroleptics, fluperlapine (fluorine in position 8) and the thiophene-compound, NT 104-252 (chlorine in the equivalent position). This latter compound (47 *in* Schmutz & Eichenberger, 1982) was found to have 'antipsychotic activity in man without appreciable extrapyramidal side effects' (Hunziker *et al*., 1981). Unlike NT 104-252, olanzapine carries its substituent in the thiophene ring, which is comparable to the position of chlorine in loxapine and iso-clozapine. Similar to loxapine and iso-clozapine, but in contrast to clozapine, olanzapine causes catalepsy in rats (Moore *et al*., 1993; Hoffman & Donovan, 1995; Kalkman *et al*., 1997), which can be antagonized by clozapine (Kalkman *et al*., 1997).

Many theories have been put forward to explain the low cataleptogenic and EPS liability of clozapine in rats and humans respectively, based on its affinity for dopamine D_2 relative to other receptors. Most notable are the suggestions that its atypical profile is due to relatively high affinity for dopamine D_1 receptors (Josselyn *et al*., 1997) or 5-HT_{2A} receptors (Meltzer *et al*., 1989). In a comparison of the binding profile of twenty neuroleptic compounds, including clozapine and fluperlapine, Meltzer *et al*. (1989) concluded that high D_1 receptor affinity was not involved. This conclusion is supported by the present work in which similar D_2/D_1 ratios were found for clozapine and loxapine despite their differences in cataleptogenic activity.

By the same argument, the present results do not support a role for 5-HT_{2A} blockade in the suppression of catalepsy, as proposed by Meltzer *et al*. (1989) and Leysen *et al*. (1993), who found that clozapine displays high affinity to 5-HT_{2A} receptors and only moderate affinity to D_2 receptors; the 5-HT_{2A}/D_2 hypothesis. Seeman *et al*. (1997b) investigated the D_2, D_4 and 5-HT_{2A} affinities of clozapine, iso-clozapine, loxapine and iso-loxapine as well as other neuroleptics. Consistent with the present findings, these authors noticed that cataleptic and non-cataleptic drugs have similar 5-HT_{2A}/D_2 ratios. Similar conclusions have been made by Nutt (1994) and Kapur (1996). It is also clear that selective 5-HT_{2A} receptor antagonists can induce catalepsy in the rat that is sensitive to blockade by clozapine (Kalkman *et al*., 1998).

By contrast, both non-cataleptogenic compounds showed much greater affinity for 5-HT_1 receptors than the cataleptogenic derivatives and 5-HT_{1A} ligands with low intrinsic activity, such as buspirone, gepirone, BMY-7378 or NAN-190, have been found to inhibit haloperidol- or fluphenazine-induced catalepsy in rats (Elliott *et al*., 1990; McMillan *et al*., 1988). Clozapine can also act as a partial agonist at 5-HT_{1A} receptors (Newman-Tancredi *et al*., 1996) but its anticataleptic effect, in contrast to that of the 5-HT_{1A} receptor agonist, 8-OH-DPAT, could not be blocked by the 5-HT_{1A} receptor antagonist, WAY 100,635 (Bartoszyk *et al*., 1996). Conversely, the low EPS liability of clozapine is unlikely to be caused by 5-HT_{1A} receptor blockade since the 5-HT_{1A} receptor antagonist, WAY 100,635 itself induced a cataleptic response, albeit at high doses (Kalkman *et al*., 1998).

Clozapine and iso-loxapine, in contrast to loxapine and iso-clozapine, displayed relatively high affinity for 5-$HT_{1B/1D}$ receptors. Whilst it is unknown whether clozapine acts as a 5-$HT_{1B/1D}$ receptor agonist, it seems unlikely that 5-$HT_{1B/1D}$ receptor blockade is involved in clozapine's atypical profile, since the selective 5-$HT_{1B/1D}$ receptor antagonist, GR127,935 did not affect loxapine-induced catalepsy (Kalkman *et al*., 1998).

α_1-Adrenoceptor blockade has also been suggested to account for the atypical nature of clozapine (Baldessarini *et al*., 1992; Prinssen *et al*., 1994). However, unpublished data from our laboratory indicate that the α_1-adrenoceptor antagonists, prazosin, WB 4101 and BE 2254, if anything, potentiate rather than inhibit D_2-receptor-mediated catalepsy.

Cholinergic receptor antagonists have been used extensively to control the extrapyramidal side effects of neuroleptic drugs and it has been hypothesized that the high muscarinic receptor affinity of clozapine could explain its low potential to induce catalepsy in rats. However, clozapine, iso-clozapine (Schmutz & Eichenberger, 1982) and olanzapine (Moore *et al*., 1993) have similar high affinity for muscarinic receptors, yet only the latter two compounds are cataleptogenic. It seems unlikely, therefore, that cholinergic mechanisms have a major influence on the anticataleptic properties of clozapine.

Since the two non-cataleptogenic drugs, clozapine and iso-loxapine had considerable affinity for the α_2-adrenoceptor binding site compared to loxapine and iso-clozapine, the effects of α_2-adrenoceptor blockade on catalepsy induced by loxapine were investigated. The α_2-adrenoceptor antagonists, yohimbine, RX 821002 and MK-912 all suppressed in a dose-related fashion the cataleptic effect of loxapine. Yohimbine and RX 821002 (not shown) also inhibited haloperidol-induced catalepsy, but, like clozapine, failed to inhibit catalepsy induced by the D_1 receptor antagonist, SCH 23390.

It is notable that, like clozapine (Kalkman *et al*., 1997) each of the α_2-adrenoceptor antagonists significantly reduced loxapine-induced catalepsy for only about a 2 h period; thereafter catalepsy became increasingly severe. The reasons for this may be pharmacokinetic, reflecting a shorter half-life and lower C_{max} of the compounds in brain compared to loxapine. Further studies are required, however, to determine the significance of these temporal phenomena.

Three different subtypes of α_2-adrenoceptors have been characterized by molecular cloning and given the appellation, α_{2A}, α_{2B} and α_{2C} (for reviews see: Bylund *et al*., 1994; Kendall, 1996). The rat homologue of the human α_{2A} receptor has a somewhat different binding profile and is, for historical reasons, often referred to as α_{2D} (Kendall, 1996). In mammalian species presynaptic inhibitory α_2-auto- and heteroreceptors belong almost exclusively to the $\alpha_{2A/2D}$ class (Trendelenburg *et al*., 1997). It is possible that $\alpha_{2A/D}$-adrenoceptor blockade could increase extracellular dopamine and/or 5-HT availability and so suppress the neuroleptic-induced catalepsy. Whether this involves interaction with D_1 or 5-HT_{1A} receptors is less clear, since although 5-HT_{1A}, D_1 and/or D_2 receptor stimulation is known to block the cataleptic response to SCH 23390 (Wadenberg, 1992; Verma & Kulkarni, 1992), the α_2-adrenoceptor antagonists did not.

As with clozapine (Kalkman *et al*., 1997; Bartoszyk *et al*., 1996), the α_2-adrenoceptor antagonists, yohimbine and RX821002 did, however, inhibit the cataleptic response to D_2 (haloperidol) and 5-HT_{2A} (MDL 100,151) receptor blockade, so clozapine could be inhibiting its own expression of catalepsy via an interaction with α_2-adrenoceptors. The effect of clozapine on plasma noradrenaline levels in man (Breier *et al*., 1994) is a clear indication that clozapine blocks α_2-adrenoceptors at therapeutic doses.

The hypothesis that α_2-adrenoceptor blockade could contribute to neuroleptic atypicallity is not new. Nutt (1994) noted that atypical neuroleptics had a much greater affinity for α_2-adrenoceptors than older drugs. Anderson (1985) reported that reserpine-induced rigidity is blocked by yohimbine, phentolamine and imiloxan in rats, whilst Gomez-Mancilla & Bedard (1993) found that yohimbine reduced dyskinetic movements in MPTP-treated monkeys.

With the presently available pharmacological tools it will be difficult to determine which of the α_2-adrenoceptor subtypes is involved in the anticataleptic activity of yohimbine and clozapine, but both the α_{2B} and α_{2C} receptor subtypes are interesting candidates. In the rat brain, α_{2B}-adrenoceptors are exclusively localised in the thalamus (Scheinin *et al*., 1994), an area which is involved in psychotic processes in man (Buchsbaum, 1995), and in sensorimotor gating and catalepsy in rats (Young *et al*., 1995). On the other hand, α_{2C} receptors are found in high density in the caudate nucleus of many species, including man (Ordway *et al*., 1993; Perälä *et al*., 1992) and α_{2C}-adrenoceptor 'knock-out' mice display altered dopamine turnover in the brain (Sallinen *et al*., 1997). Interestingly, clozapine displays somewhat higher affinity for the human α_{2B} and α_{2C} receptor subtypes (pK_D values of 7.7 and 8.0, respectively) than for human α_{2A} receptors ($pK_D = 7.3$; Schotte *et al*., 1996).

In conclusion, the present data strongly implicate α_2-adrenoceptor blockade as a primary principle in the anti-cataleptic properties of clozapine. The possibility that this is also the fundamental reason for the lack of extrapyramidal side effects seen with the drug in the clinic deserves further consideration.

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References

ANDERSON, R.J. (1985). Modification of resperine induced rigidity by dopaminergic and alpha-adrenergic drugs. *Acta Neurol. Scand.,* **72,** 584 – 589.

BALDESSARINI, R.J. & FRANKENBURG, F.R. (1991). Clozapine, a novel antipsychotic agent. *N. Engl. J. Med.,* **324,** 746 – 754.

BALDESSARINI, R.J., HUSTON-LYONS, D., CAMPBELL, A., MARSH, E. & COHEN, B.M. (1992). Do central antiadrenergic actions contribute to the atypical properties of clozapine? *Br. J. Psychiat.,* **160,** 12 – 16.

BARTOSZYK, G.D., ROOS, C. & ZIEGLER, H. (1996). 5-HT_{1A} receptors are not involved in clozapine's lack of cataleptogenic potential. *Neuropharmacol.,* **11,** 1645 – 1646.

BREIER, A., BUCHANAN, R.W., WALTRIP, R.W., LISTWAK, S., HOLMES, C. & GOLDSTEIN, D.S. (1994). The effect of clozapine on plasma norepinephrine: relationship to clinical efficacy. *Neuropsychopharmacol.,* **10,** 1 – 7.

BRUINVELS, A.T., LERY, H., NOZULAK, J., PALACIOS, J.M. & HOYER, D. (1992). 5-HT_{1D} binding sites in various species: similar pharmacological profile in calf, guinea-pig, dog, monkey and human brain membranes. *Naunyn Schmiedeberg's Arch. Pharmacol.,* **346,** 243 – 248.

BUCHSBAUM, M.S. (1995). Charting the circuits. *Nature,* **378,** 128 – 129.

BYLUND, D.B., EIKENBERG, D.C., HIEBLE, J., LANGER, S.Z., LEFKOWITZ, R.J., MINNEMAN, K.P., MOLINOFF, P.B., RUFFOLO, JR. R.R. & TRENDELENBURG, U. (1994). International Union of Pharmacology nomenclature of adrenoceptors. *Pharmacol. Rev.,* **46,** 121 – 136.

CHIODO, L.A. & BUNNEY, B.S. (1983). Typical and atypical neuroleptics: Differential effects of chronic administration on the activity of A9 and A10 midbrain dopaminergic neurons. *Neurosci.,* **3,** 1607 – 1619.

DURIF, F., VIDAILHET, M., ASSAL, F., ROCHE, C., BONNET, A.M. & AGID, Y. (1997). Low-dose of clozapine improves dyskinesia in Parkinson's disease. *Neurol.,* **48,** 658 – 662.

ELLIOTT, P.J., WALSH, D.M., CLOSE, S.P., HIGGINS, G.A. & HAYES, A.G. (1990). Behavioural effects of serotonin antagonists in the rat and marmoset. *Neuropharmacol.,* **29,** 949 – 956.

ENGEL, G. & HOYER, D. (1981). ^{125}I-BE 2254, a new high affinity radioligand for α_1-adrenoceptors, *Eur. J. Pharmacol.,* **73,** 221 – 224.

FACTOR, S.A. & FRIEDMAN, J.H. (1997). The emerging role of clozapine in the treatment of movement disorders. *Movement Disorders,* **12,** 483 – 496.

GOMEZ-MANCILLA, B. & BEDARD, P.J. (1993). Effect of nondopaminergic drugs on L-dopa-induced dyskinesias in MPTP-treated monkeys. *Clin. Neuropharmacol.,* **16,** 418 – 427.

HOFFMAN, D.C. & DONOVAN, H. (1995). Catalepsy as a rodent model for detecting antipsychotic drugs with extrapyramidal side effect liability. *Psychopharmacol.,* **120,** 128 – 133.

HOYER, D., ENGEL, G. & KALKMAN, H.O. (1985). Molecular pharmacology of 5-HT_1 and 5-HT_2 recognition sites in rat and pig membranes: radioligand binding studies with [^{3}H]5-HT, [^{3}H]8-OH-DPAT, (−)[^{125}I]iodocyanopindolol, [^{3}H]mesulergine [^{3}H]ketanserin. *Eur. J. Pharmacol.,* **118,** 13 – 23.

HUNZIKER, F., FISCHER, R., KIPFER, P., BUERKI, H.R., EICHENBERGER, E. & WHITE, T.G. (1981). Seven-membered heterocycles. 28. Neuroleptic piperazinyl derivatives of 10H-thieno[3,2-c][1]benzazepines and 4H-thieno[2,3-c][1]benzazepines. *Eur. J. Med. Chem.,* **16,** 391 – 398.

JOSSELYN, S.A., MILLER, R. & BENINGER, R.J. (1997). Behavioral effects of clozapine and dopamine receptor subtypes. *Neuroscience and Biobehav. Rev.,* **21,** 531 – 558.

KALKMAN, H.O., NEUMANN, V. & TRICKLEBANK, M.D. (1997). Clozapine inhibits catalepsy induced by olanzapine and loxapine, but prolongs catalepsy induced by SCH 23390 in rats. *Naunyn-Schmiedeberg's Arch. Pharmacol.,* **355,** 361 – 364.

KALKMAN, H.O., NEUMANN, V., NOZULAK, J. & TRICKLEBANK, M.D. (1998). Cataleptogenic effect of subtype selective 5-HT receptor antagonists in the rat. *Eur. J. Pharmacol.,* **343,** 201 – 207.

KAPUR, S. (1996). 5-HT_2 antagonism and EPS benefits: is there a causal connection? *Psychopharmacol.,* **124,** 35 – 39.

KENDALL, D.A. (1996). Classification of α_2-adrenoceptors. *J. Psychopharmacol.,* **10 (suppl.),** 2 – 5.

LEYSEN, J.E., JANSSEN, P.M.J., SCHOTTE, A., LUYTEN, W.H.M.L. & MEGENS, A.A.H.P. (1993). Interaction of antipsychotic drugs with neurotransmitter receptor sites in vitro and in vivo in relation to pharmacological and clinical effects: role of $5HT_2$ receptors. *Psychopharmacol.,* **112,** S40 – S54.

MARKSTEIN, R., GULL, P., RUEDEBERG, C., URWYLER, S., JATON, A.L., MCALLISTER, K., DIXON, K. & HOYER, D. (1996). SDZ PSD 958, a novel D-1 receptor antagonist with potential limbic selectivity. *J. Neural Transmission,* **103,** 261 – 276.

MCMILLAN, B.A., SCOTT, S.M. & DAVANZO, E.A. (1988). Reversal of neuroleptic-induced catalepsy by novel aryl-piperazine anxiolytic drugs. *J. Pharm. Pharmacol.,* **40,** 885 – 887.

MELTZER, H.Y., MATSUBARA, S. & LEE, J.C. (1989). Classification of typical and atypical antipsychotic drugs on the basis of dopamine D-1, D-2 and serotonin$_2$ pK_i values. *J. Pharmacol. Exp. Ther.,* **251,** 238 – 246.

MOORE, N.A., CALLIGARO, D.O., WONG, D.T., BYMASTER, F. & TYE, N.C. (1993). The pharmacology of olanzapine and other new antipsychotic agents. *Curr. Opin. Invest. Drugs,* **2,** 281 – 293.

NEWMAN-TANCREDI, A., CHAPUT, C., VERRIELE, L. & MILLAN, M.J. (1996). Clozapine is a partial agonist at clones, human serotonin 5-HT_{1A} receptors. *Neuropharmacol.,* **35,** 119 – 121.

NUTT, D.J. (1994). Putting the 'A' in atypical: does α_2-adrenoceptor antagonism account for the therapeutic advantage of new antipsychotics? *J. Psychopharmacol.,* **8,** 193 – 195.

ORDWAY, G.A., JACONETTA, S.M. & HALARIS, A.E. (1993). Characterization of subtypes of alpha$_2$ adrenoceptors in the human brain. *J. Pharmacol. Exp. Ther.,* **264,** 967 – 976.

PERÄLÄ, M., HIRVONEN, H., KALIMO, H., ALA-UOTILA, S., REGAN, J.W., ÅKERMAN, K.E.O. & SCHEININ, M. (1992). Differential expression of two α_2-adrenergic receptor subtype mRNAs in human tissue. *Molec. Brain Res.,* **16,** 57 – 63.

PRINSSEN, E.P.M., ELLENBROEK, B.A. & COOLS, A.R. (1994). Combined antagonism of adrenoceptors and dopamine and 5-HT receptors underlies the atypical profile of clozapine. *Eur. J. Pharmacol.,* **262,** 167 – 170.

ROBERTSON, G.S. & FIBIGER, H.C. (1992). Neuroleptics increase *C-Fos* expression in the forebrain: contrasting effects of haloperidol and clozapine. *Neurosci.,* **46,** 315 – 328.

SALLINEN, J., LINK, R.E., HAAPALINNA, A., VIITIMAA, T., KALATUNGU, M., SJÖHOLM, B., MACDONALD, E., PELTHUIKKO, M., LEINO, T., BARSH, G.S., KOBILKA, B.K. & SCHEININ, M. (1997). Genetic alteration of α_{2C}-adrenoceptor expression in mice: influence on locomotor, hypothermic, and neurochemical effects of dexmedetomidine, a subtype-nonselective α_2-adrenoceptor agonist. *Mol. Pharmacol.,* **51,** 36 – 46.

SCHEININ, M., LOMASNEY, J.W., HAYDEN-HIXSON, D.M., SCHAMBRA, U.B., CARON, M.G., LEFKOWITZ, R.J. & FREMEAU, JR. R.T. (1994). Distribution of α_2-adrenergic receptor subtype gene expression in the rat brain. *Mol. Brain. Res.,* **21,** 133 – 149.

SCHMUTZ, J. & EICHENBERGER, E. (1982). *In Chronicles of drug discovery*. ed. Bindra, J.S. & Hednicer, D. pp. 39 – 59. Chichester: John Wiley & Sons.

SCHOTTE, A., JANSSEN, P.F.M., GOMMEREN, W., LUYTEN, W.H.M.L., VAN GOMPEL, P., LESAGE, A.S., DE LOORE, K. & LEYSEN, J.E. (1996). Risperidone compared with new and reference antipsychotic drugs: in vitro and in vivo receptor binding. *Psychopharmacol.,* **124,** 57 – 73.

SEEMAN, P., CORBETT, R. & VAN TOL, H.H.M. (1997a). Atypical neuroleptics have low affinity for dopamine D_2 receptors or are selective for D_4 receptors. *Neuropsychopharmacol.,* **16,** 93 – 110.

SEEMAN, P., TALLARICO, T., CORBETT, R., VAN TOL, H.H.M. & KAMBOJ, R.K. (1997b). Role of dopamine D_2, D_4 and serotonin$_{2A}$ receptors in antipsychotic and anticataleptic action. *J. Psychopharmacol.,* **11,** 15 – 17.

SORENSEN, S.M., KEHNE, J.H., FADAYEL, G.M., HUMPHREYS, T.M., KETTELER, H.J., SULLIVAN, C.K., TAYLOR, V.L. & SCHMIDT, C.J. (1993). Characterization of the 5-HT_2 receptor antagonist MDL 100907 as a putative atypical antipsychotic: behavioral electrophysiological and neurochemical studies. *J. Pharmacol. Exp. Ther.,* **266,** 684 – 691.

TRENDELENBURG, A.-U., SUTEJ, I., WAHL, C.A., MOLDERINGS, G.J., RUMP, L.C. & STARKE, K. (1997). A re-investigation of questionable subclassifications of presynaptic α_2-autoreceptors: rat vena cava, rat atria, human kidney and guinea-pig urethra. *Naunyn-Schmiedeberg's Arch. Pharmacol.,* **356,** 721 – 737.

VERMA, A. & KULKARNI, S.K. (1992). D_1/D_2 dopamine and N-methyl-D-aspartate (NMDA) receptor participation in experimental catalepsy in rats. *Psychopharmacol.,* **109,** 477 – 483.

WADENBERG, M.L. (1992). Antagonism by 8-OH-DPAT, but not ritanserin, of catalepsy induced by SCH 23390 in the rat. *J. Neural. Transm.,* **89,** 49 – 59.

YOUNG, K.A., RANDALL, P.K. & WILCOX, R.E. (1995). Startle and sensorimotor correlates of ventral thalamic dopamine and GABA in rodents. *Neuroreport,* **6,** 2495 – 2499.

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