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European Journal of Pharmacology 282 (1995) 137–144

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Why does clozapine stimulate the motor activity of reserpine-pretreated rats when combined with a dopamine D_1 receptor agonist?

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Received 9 January 1995; revised 11 May 1995; accepted 16 May 1995

Abstract

The aim of the present experiments was to investigate the locomotor stimulant effects of the atypical antipsychotic agent, clozapine, in rats depleted of their dopamine by reserpine and α-methyl-*p*-tyrosine pretreatment. Clozapine itself induced a slight but never significant stimulation of locomotor activity which was enhanced by the addition of the selective dopamine D_1 receptor agonist, SKF38393 (2,3,4,5-tetrahydro-7,8-dihydroxy-1-phenyl-1*H*-3-benzazepine), but not by the selective dopamine D_2 receptor agonist, quinpirole. The stimulation produced by clozapine plus SKF38393 was blocked by the selective dopamine D_1 receptor antagonist, SCH23390 (7-chloro-8-hydroxy-3-methyl-1-phenyl-2,3,4,5-tetrahydro-1*H*-3-benzapine hydrochloride), while the selective dopamine D_2 receptor antagonist, haloperidol, was ineffective. A combination of SCH23390 and haloperidol blocked the clozapine plus SKF38393-induced locomotion. Unlike clozapine, neither the selective 5-HT_2 receptor antagonist, ritanserin, nor the dopamine D_2 receptor antagonists, haloperidol and remoxipride, caused locomotor activation when given alone or in combination with SKF38393. The indirectly acting sympathomimetic amine, *d*-amphetamine, was inactive in the monoamine-depleted rats, indicating that no dopamine was available for release by *d*-amphetamine. The muscarinic receptor antagonist, scopolamine, alone did not alter locomotion, but produced marked stimulation when combined with SKF38393 but not with quinpirole. This stimulation was not affected by haloperidol. However, the scopolamine plus SKF38393-induced stimulation was partially blocked by SCH23390 or by a combination of haloperidol and SCH23390. The data indicate that clozapine, in rats depleted of their dopamine stores, exhibits properties consistent with those of a dopamine receptor agonist. The pharmacology of this behavioural stimulation was similar but not identical to that seen with the muscarinic receptor antagonist, scopolamine. The behavioural effects of clozapine reported here might be explained by a dual effect: antagonism of muscarinic receptors and agonist-like activity at dopamine receptors.

Keywords: Dopamine receptor agonist; Clozapine; Dopamine D_1 receptor; Dopamine D_2 receptor; Quinpirole; Antipsychotic, atypical; Scopolamine; SKF38393; (Rat)

1. Introduction

Clozapine is an atypical antipsychotic agent that is efficacious against the negative symptoms of chronic schizophrenia and is effective in some therapy-resistant patients, i.e. patients who are resistant to traditional neuroleptics such as haloperidol. Furthermore, clozapine has a low potential to induce extrapyramidal side effects. Many hypotheses have been proposed in an attempt to explain the unique clinical profile of clozapine.

Clozapine has a high affinity for 5-HT_2 receptors and several authors have suggested that this affinity may contribute both to its clinical efficacy and to its low incidence of extrapyramidal side effects (Meltzer and Gudelsky, 1992). Another influential hypothesis is based on the affinity of clozapine for the dopamine D_4 receptor, with K_i values varying between 9 nM and 29 nM (Van Tol et al., 1991, 1992; Shaikh et al., 1993; Lahti et al., 1993). With the distribution of dopamine D_4 receptors being predominantly limbic, it was suggested that antagonism of this receptor may explain the antipsychotic effect of clozapine. Another clue may be found in the interesting binding characteristics of clozapine to dopamine D_2-like receptors. Clozapine binds to cloned human dopamine D_{2B} receptors (short

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Elsevier Science B.V.
SSDI 0014-2999(95)00308-8

isoform) with an affinity (K_i = 35 nM, Malmberg et al., 1993) comparable to that reported for dopamine D_4 receptors. Furthermore, its binding to both dopamine D_{2A} (long isoform) and dopamine D_{2B} receptors is sensitive to sodium ions, with their omission causing a 3-fold increase in the affinity of clozapine for these receptors (Malmberg et al., 1993). The affinity of clozapine for dopamine D_4 receptors is similarly affected by sodium (Van Tol et al., 1992). Interestingly, a decreased affinity in the presence of sodium has previously been reported for dopamine D_2 receptor agonists (Neve et al., 1989). It was also reported that the displacement by clozapine of [^{3}H]raclopride binding to dopamine D_{2B} receptors was described best by a two-site binding model (both in the presence and absence of sodium) (Malmberg et al., 1993). These studies indicate agonist-like binding properties in vitro. However, clozapine has substantial affinity for many receptors other than 5-HT_2 and dopamine D_2 receptors. Thus, it binds to muscarinic receptors (K_i = 12 nM, Richelson and Nelson, 1984), 5-HT_{1C} receptors (Coward, 1992; Canton et al., 1990, pK_i = 8.07 nM), α_1-adrenoceptors (K_i = 9 nM, Richelson and Nelson, 1984) and dopamine D_1 (K_i = 174 nM, Jackson et al., 1992) receptors. Some of these receptors (muscarinic, for example) can modulate dopamine-mediated functions.

The high affinity of clozapine for muscarinic receptors (Richelson and Nelson, 1984; Bolden et al., 1991) is interesting. A variety of behavioural and biochemical studies have clearly indicated that muscarinic receptors regulate dopamine neurotransmission and vice versa (Jackson, 1974; Arnt et al., 1981; Ögren and Fuxe, 1988; De Boer et al., 1992). It seems likely, for example, that the antimuscarinic properties of clozapine contribute to its weak ability to induce catalepsy in rats (Jackson et al., 1992), dystonia in monkeys sensitised to neuroleptic challenge (Casey, 1991) and extrapyramidal side effects in man (Meltzer and Gudelsky, 1992). Recently, in vitro studies have shown that that clozapine has a high affinity for all muscarinic receptor subtypes (Bolden et al., 1991) and that clozapine may be an antagonist at muscarinic M_1, M_2, M_3 and M_5 receptors but an agonist at M_4 receptors (Zorn et al., 1994).

In the present study, we examined whether it was possible to demonstrate behavioural properties of clozapine in vivo which would be compatible with a direct or indirect stimulation of dopamine receptors. We chose a behavioural model that is documented to involve both dopamine D_1 and D_2 receptors and is modulated by cholinergic systems, i.e., dopamine receptor agonist-induced locomotor stimulation in rats depleted of their stores of dopamine. The granule-depleting agent, reserpine, and the inhibitor of tyrosine hydroxylase, the rate-limiting step in the synthesis of dopamine, α-methyl-*p*-tyrosine, were used. Our results indicate that clozapine when combined with a dopamine D_1 receptor agonist can cause locomotor activation in vivo which may be mediated via an interaction of clozapine with muscarinic receptors and with dopamine receptors.

2. Materials and methods

2.1. Animals

Male Sprague-Dawley rats (B&K Universal, Sollentuna, Sweden) weighing between 250 and 350 g were used. They were maintained on a diurnal cycle of 12 h dark/12 h light, with lights on at 06:00 h in the morning, and were kept in the laboratory environment for 5–7 days before use. Food pellets and tap water were available ad libitum, except for the period in the activity cages. The experiments were run during the light phase. Each animal was used only once.

2.2. Apparatus

Seven Plexiglas activity cages from Kungsbacka Mät-Och Reglerteknik, Fjärås, Sweden, with a floor area of 700 × 700 mm, were used. They were housed in sound-proofed ventilated boxes with no lighting. Briefly, two rows of photocells (total 16 photocells, one low row to measure activity at floor level, the other row placed higher to measure rearing) enable a computer-based system to determine the location of the animal at any time. In the present study, we measured a variable called horizontal activity which represents the total number of times photocells in the lower row were broken.

2.3. Drugs

Clozapine (a gift of Sandoz, Basel, Switzerland), haloperidol and reserpine (both from Sigma, St. Louis, MO, USA) and ritanserin (a gift of Janssen Pharmaceutica, Beerse, Belgium) were dissolved in a minimum of glacial acetic acid and diluted to volume with distilled water. SCH23390 (7-chloro-8-hydroxy-3-methyl-1-phenyl-2,3,4,5-tetrahydro-1*H*-3-benzapine hydrochloride, Research Biochemicals, Natick, MA, USA) was dissolved in a few drops of propanediol and diluted to volume with distilled water. Quinpirole hydrochloride (Research Biochemicals, Natick, MA, USA), remoxipride hydrochloride monohydrate tartrate (synthesised in the chemical laboratories of Astra Arcus) and scopolamine hydrochloride (Sigma, St. Louis, MO, USA) were dissolved in saline. α-Methyl-*p*-tyrosine methyl ester hydrochloride (Sigma, USA) was dissolved in distilled water. SKF38393 (2,3,4,5-tetrahydro-7,8-di-

hydroxy-1-phenyl-1*H*-3-benzazepine, Research Biochemicals, Natick, MA, USA) was dissolved in 0.05% ascorbic acid in water. *d*-Amphetamine sulphate (Calaise Chimie, France) was dissolved in saline.

The pH of the final injected solutions was between 4.2 and 5.7. All drugs, except quinpirole and SKF38393 which were injected intraperitoneally, were injected subcutaneously in the neck. The injection volume was 2 ml/kg.

2.4. Experimental methods

In all experiments described in this paper, the rats were pretreated with reserpine (8.2 μmol/kg, 5 mg/kg) and 20 h later with α-methyl-*p*-tyrosine (814 μmol/kg, 200 mg/kg). The dose of SKF38393 used in all experiments was 82 μmol/kg, equivalent to 24 mg/kg. This dose was selected after preliminary experiments with doses of 6 and 24 mg/kg.

In the first study, clozapine, with or without SKF38393, was injected 1 h after the α-methyl-*p*-tyrosine injection. In the second study, rats were injected 1 h after the α-methyl-*p*-tyrosine injection with clozapine and different doses of quinpirole. In the third and fourth studies, we examined the effect of dopamine D_1 and D_2 receptor antagonists (alone and in combination) given 1 h after the α-methyl-*p*-tyrosine on the clozapine and SKF38393-induced locomotor stimulation. Clozapine and SKF38393 in this experiment were administered 30 min after the test antagonists. In the fifth study, we examined the effects of scopolamine. Scopolamine was first administered together with SKF39393 or quinpirole 1 h after the α-methyl-*p*-tyrosine injection. Finally, we examined the behavioural effect of a combination of haloperidol (or remoxipride) and SKF38393 1 h after the α-methyl-*p*-tyrosine injection. The activity in all experiments was measured for 2 h after the last injection.

2.5. Statistics

The data were analysed by appropriate analyses of variance followed by post-hoc comparisons (least-squares differences) of appropriate means. Statistical details are given in the legends to the figures.

3. Results

3.1. Clozapine and SKF38393-induced excitation

The effects of clozapine on locomotor activity are illustrated in Fig. 1 (top graph). Analysis of variance indicated that clozapine itself marginally increased activity. When combined with SKF38393 it significantly increased activity. Post-hoc comparisons of the activity data indicated that all doses of clozapine plus SKF38393 produced significantly more activity than seen in the animals that received SKF38393 alone. The *difference* between the clozapine dose-response curves in the absence and presence of SKF38393 was exam-

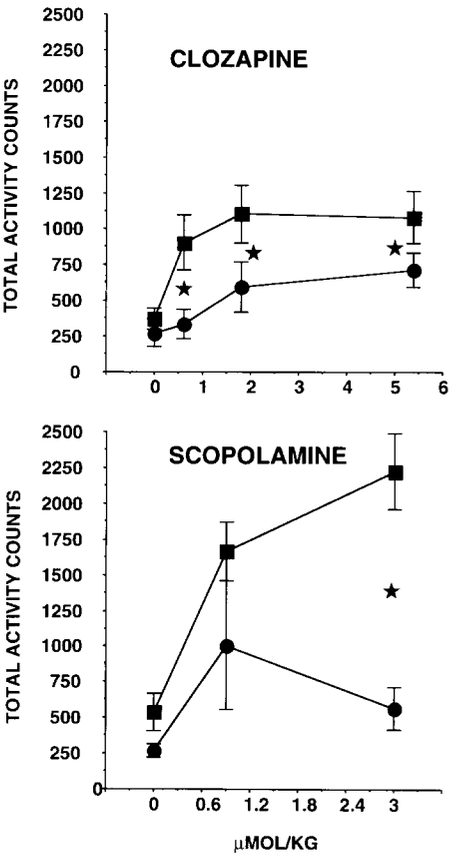

Fig. 1. The effect of clozapine or scopolamine in combination with SKF38393 on locomotor activity was studied in rats pretreated with reserpine (8.2 μmol/kg) and 20 h later with α-methyl-*p*-tyrosine (814 μmol/kg). In each graph, the data illustrate the total number of counts recorded over 2 h with the standard errors of the means. The top graph shows the results of injection of clozapine (0.6, 1.8 and 5.4 μmol/kg, 0.2, 0.6 and 1.8 mg/kg) with (■) or without (●) SKF38393 (82 μmol/kg) on locomotor activity. Analysis of variance (ANOVAR) gave the following results: the effect of clozapine alone on activity ($F(3,24) = 2.69$, $P = 0.0687$); the effect of clozapine and SKF38393 together on activity ($F(3,24) = 4.122$, $P = 0.0172$); the difference between the two dose-response curves (clozapine with or without SKF38393) ($F(1,48) = 6.2$, $P = 0.0012$). The stars indicate a significant post-hoc difference (least-squares means) between the groups that received a particular dose of clozapine with or without SKF38393. The number of animals per group was 7. The bottom graph shows the results of scopolamine (0.9 or 2.9 μmol/kg, 0.3 or 1.0 mg/kg) with (■) or without (●) SKF38393 (82 μmol/kg) on locomotor activity. Scopolamine itself was without significant effect ($F(2,20) = 2.311$, $P = 0.13$) but 2.9 μmol/kg of scopolamine in combination with SKF38393 produced significant stimulation ($F(2,20) = 17.35$, $P = 0.0001$). The difference between the two dose-response curves was significant ($F(1,35) = 4.13$, $P = 0.0245$). The star indicates a significant post-hoc difference between the groups that received 2.9 μmol/kg scopolamine with or without SKF38393. The number of animals per group was 6 or 7.

ined and the individual post-hoc comparisons are given in Fig. 1. SKF38393 significantly increased the stimulant effect of clozapine. SKF38393 alone exerted no significant effect.

Thus, clozapine itself induced a marginal stimulation of activity which was potentiated by addition of SKF38393.

3.2. The effect of clozapine plus quinpirole

Clozapine itself caused an increase in activity but this was not significant (Table 1, in which statistical details are given). Quinpirole alone produced a significant stimulation. However, clozapine neither potentiated nor inhibited the quinpirole-induced activity. The latter result indicates minimal dopamine D_2 receptor antagonism by clozapine at the experimental doses.

3.3. The effect of dopamine receptor antagonists on clozapine plus SKF38393-induced stimulation

The combination of clozapine (5.4 μmol/kg) plus SKF38393 produced a significant increase in activity compared to the control group (Fig. 2, top panel, left-hand pair of columns). This activity was not significantly affected by haloperidol.

The effect of clozapine plus SKF38393 after pretreatment of the rats with different doses of SCH23390 (or vehicle) is illustrated in Fig. 2 (bottom panel). The combination of clozapine and SKF38393 produced a significant increase in activity above that in the control groups which was significantly affected by SCH23390. Post-hoc tests indicated that significant stimulation occurred after all doses of SCH23390 (including the

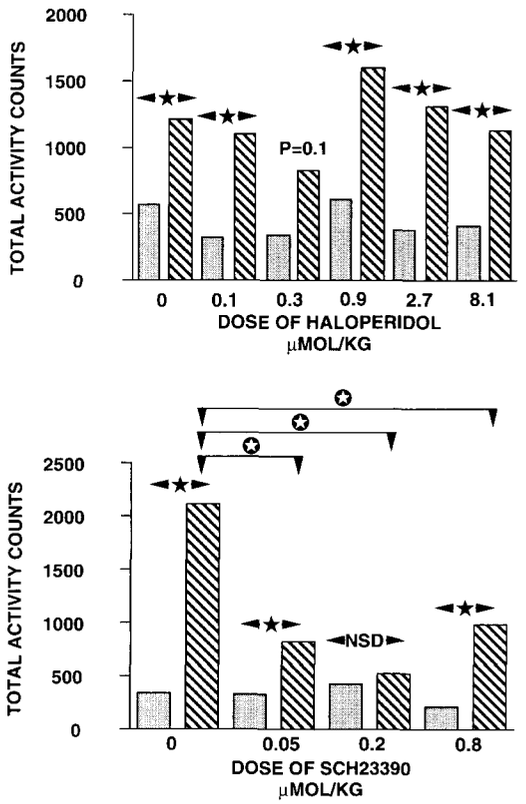

Fig. 2. The effect of dopamine receptor-selective antagonists on the locomotor stimulation produced by clozapine in combination with SKF38393 was studied in rats pretreated with reserpine (8.2 μmol/kg) and 20 h later with α-methyl-*p*-tyrosine (814 μmol/kg). One hour after the α-methyl-*p*-tyrosine pretreatment they received different doses of haloperidol (top graph) or SCH23390 (bottom graph). 30 min after the antagonist injection, clozapine (5.4 μmol/kg) with SKF38393 (82 μmol/kg) (hatched columns), or the clozapine-SKF38393 vehicle alone (shaded columns), was injected. The data illustrate the total number of counts recorded during the 2 h. ANOVAR of the data in the top graph: the effect of clozapine and SKF38393 compared to results for vehicle-treated animals ($F(1,87) = 40.8$, $P = 0.001$) and the effect of haloperidol on the stimulant action ($F(5,87) = 0.414$, $P = 0.8381$). ANOVAR of the data in the bottom graph: the effect of clozapine and SKF38393 compared to results for vehicle-treated animals ($F(1,48) = 34.9$, $P = 0.0001$) and the effect of SCH23390 on this stimulation ($F(3,48) = 6.632$, $P = 0.0008$). The number of animals per group was between 6 and 11. A filled star indicates a significant difference between clozapine-and SKF38393-treated animals. An open star indicates the effect of the antagonist on clozapine-and SKF38393-treated animals. NSD indicates no significant difference.

Table 1
The effect of clozapine and scopolamine alone and in combination with quinirole on locomotor activity in rats

Pretreatment (μmol/kg)			*n*	Activity
Clozapine	Scopolamine	Quinpirole		Mean ± S.E.M.
0	0	0	15	347 ± 67
0	0	3.9	9	2086 ± 377[abc]
0	0	11.7	9	2728 ± 517[abc]
5.4	0	0	9	791 ± 117
0	0.9	0	7	639 ± 157
0	2.9	0	7	515 ± 168
5.4	0	3.9	10	2545 ± 497[bc]
5.4	0	11.7	10	2406 ± 211[bc]
0	0.9	3.9	6	2097 ± 188[bc]
0	0.9	11.7	7	2358 ± 190[bc]
0	2.9	3.9	7	2042 ± 280[bc]
0	2.9	11.7	7	2881 ± 312[bc]

Rats were pretreated with reserpine (8.2 μmol/kg) and 20 h later with α-methyl-*p*-tyrosine (814 μmol/kg) and activity was then measured for 2 h. The activity shown is total activity expressed as means ± S.E.M. *n* is the number of replicates. A significant treatment effect was seen after quinpirole ($F(2,91) = 38.1$, $P < 0.0001$), but not after clozapine ($F(1,91) = 0.733$, $P = 0.39$) nor scopolamine ($F(2,91) = 0.103$, $P = 0.9024$). There was no significant interaction between scopolamine and quinpirole ($F(4,91) = 0.415$, $P = 0.7972$) or between clozapine and quinpirole ($F(2,91) = 1.262$, $P = 0.2879$). [a]The locomotor effect in both quinpirole-challenged groups was significantly higher ($P < 0.05$) than that seen in the group that received no active drug (the first group in the table). [b]The locomotor activity in these groups was significantly higher ($P < 0.05$) than that seen in the group that received no active drug (the first group in the table). [c]There is no significant difference between any of these treatment groups.

highest dose) excepting 0.2 μmol/kg (comparison of the means, least-square means difference, $P = 0.7$) where almost complete blockade was observed.

When the locomotor response of the groups given various doses of SCH23390 plus the clozapine and SKF38393 challenge was compared to that of the group given SCH23390 vehicle and clozapine plus SKF38393, significant blockade was seen with all doses of SCH23390.

3.4. The effect of combining SCH23390 and haloperidol on clozapine and SKF38393-induced stimulation

The effects of combined pretreatment of rats with SCH23390 and haloperidol on clozapine plus SKF38393-induced stimulation are illustrated in Fig. 3 (top panel).

Clozapine plus SKF38393 induced a significant overall increase in activity compared to that in animals challenged with the vehicle ($P < 0.0001$). Neither haloperidol nor SCH23390 alone completely blocked the behavioural stimulation. However, the two antagonists in combination were effective.

3.5. The effect of scopolamine on locomotor activity

Scopolamine alone produced no significant change in locomotion (see Fig. 1, bottom graph). When combined with SKF38393, it produced a significant locomotor stimulation. Scopolamine (2.9 μmol/kg) plus SKF38393 produced significantly more activity than scopolamine alone, with the difference being not as marked with the lower dose of scopolamine. SKF38393 alone was without significant effect.

When scopolamine was combined with quinpirole (Table 1), no significant interaction between quinpirole and scopolamine was observed.

Finally, rats were pretreated with haloperidol and/or SCH23390 as in Section 3.4 above and challenged 30 min later with scopolamine (0.9 μmol/kg) plus SKF38393. Scopolamine plus SKF38393 induced a significant stimulation (Fig. 3) which was unaffected by haloperidol. Significant blockade was achieved with SCH23390 but significant activity remained compared to that of animals given the antagonists but no clozapine plus SKF38393. The combination of haloperidol and SCH23390 was no more effective than SCH23390 alone. Thus, total blockade was not achieved.

3.6. The effect of miscellaneous drugs

Using the same pretreatment schedule as for clozapine in Section 3.1 above, haloperidol (doses, 0.1, 0.3 and 0.9 μmol/kg, 0.04, 0.11 and 0.34 mg/kg) was injected either alone or in combination with SKF38393. Haloperidol ($F(3,34) = 0.081$, $P = 0.97$) and SKF38393

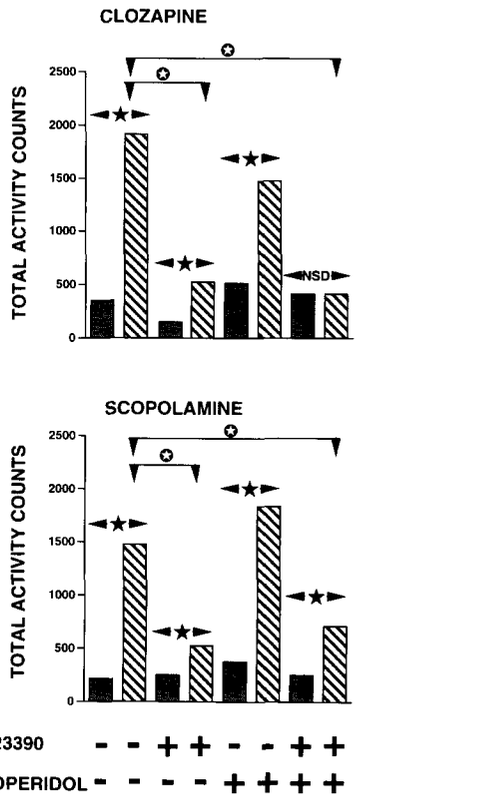

Fig. 3. The effect of selective dopamine antagonists on the locomotor stimulation induced by a combination of clozapine or scopolamine and SKF38393 in rats pretreated with reserpine (8.2 μmol/kg) and 20 h later with α-methyl-*p*-tyrosine (814 μmol/kg). One hour after the α-methyl-*p*-tyrosine pretreatment they also received haloperidol (2.7 μmol/kg) and/or SCH23390 (0.8 μmol/kg). 30 min later, clozapine (5.4 μmol/kg) with SKF38393 (82 μmol/kg) (hatched columns), or clozapine-SKF38393 vehicle alone (shaded columns), was injected (top graph) or with scopolamine (0.9 μmol/kg) with SKF38393, or clozapine-SKF38393 vehicle alone (bottom graph). The data illustrate the total number of counts recorded during this period. A filled star indicates a significant difference between clozapine-and SKF38393-treated animals. An open star indicates the effect of the antagonist on clozapine-and SKF38393-treated animals. NSD indicates no significant difference. The number of animals per group was between 6 and 13.

($F(1,34) = 1.456$, $P = 0.236$) by themselves did not significantly alter activity, and SKF38393 did not alter the response of the animals to haloperidol ($F(3,34) = 0.79$, $P = 0.5061$). In a similar study, remoxipride (2, 7, 21 and 42 μmol/kg, 0.85, 2.98, 8.94 and 17.9 mg/kg) was injected either alone or in combination with SKF38393. Remoxipride was without significant effect on locomotor activity ($F(4,87) = 1.853$, $P = 0.13$) while SKF38393 in this experiment produced a significant stimulation by itself ($P = 0.04$, post-hoc least-squares means). Remoxipride by itself had no significant effect on locomotion and this inactivity was not altered by SKF38393 ($F(4,87) = 0.35$, $P = 0.85$).

d-Amphetamine (5 or 10 μmol/kg, 0.9 or 1.8 mg/kg), administered 1 h after the α-methyl-*p*-tyro-

sine, did not cause any significant change in locomotion ($F(2,27) = 0.408$, $P > 0.6694$). The cumulative 2-h horizontal activities (mean ± S.E.M.) were 327 ± 97, 414 ± 110 and 315 ± 40, for the control, 5 and 10 μmol/kg groups, respectively.

Dopamine-depleted rats were injected with the 5-HT_2 receptor antagonist ritanserin (0, 0.1 or 0.3 mg/kg), with or without SKF38393. No significant effect of ritanserin ($P > 0.05$) or of SKF38393 ($P > 0.05$) was seen. There was no significant ritanserin/SKF38393 interaction ($P > 0.05$).

4. Discussion

Clozapine is an atypical antipsychotic with low extrapyramidal side effect potential and a clinical efficacy superior to that of typical antipsychotics such as haloperidol. Many hypotheses have been proposed to explain its interesting clinical profile but none have been particularly convincing (see Introduction).

In the present study, we have shown that clozapine weakly, but never significantly, stimulates locomotion in rats depleted of their dopamine stores. This weak effect was potentiated by the addition of the selective dopamine D_1 receptor agonist, SKF38393, but not by the selective dopamine D_2 receptor agonist, quinpirole. The locomotion induced by the combination of clozapine plus SKF38393 was resistant to blockade by the selective dopamine D_2 receptor antagonist, haloperidol. In contrast, the dopamine D_1 receptor antagonist, SCH23390, was much more effective and, in some experiments, almost completely blocked locomotor activation. The combination of haloperidol and SCH23390 was completely effective.

In previous studies, it has been noted that both dopamine D_1 receptors (by SKF38393, for example) and dopamine D_2 receptors (by quinpirole, for example) must be stimulated for optimal locomotion to be manifested (Gershanik et al., 1983; Jackson and Hashizume, 1986). Dopamine D_1 or D_2 receptor agonists by themselves are either much less effective or inactive in animals depleted of their dopamine stores. Thus clozapine behaved in the present behavioural studies like a dopamine D_2 receptor agonist. Two other antipsychotics, the typical substance, haloperidol, and the atypical substance, remoxipride, were tested under similar conditions. No stimulation, either with the drug alone or when combined with SKF38393, was observed.

The muscarinic receptor antagonist, scopolamine, like clozapine, induced only moderate locomotor stimulation by itself, but marked excitation when combined with SKF38393. No interaction was seen with quinpirole. The ability of SCH23390 and haloperidol to block the behavioural stimulation induced by scopolamine and clozapine (when these agents were combined with SKF38393) was similar in each case – a dopamine D_2 receptor antagonist was much less effective than a dopamine D_1 receptor antagonist. One difference was that the antagonist combination completely blocked the clozapine plus SKF38393-but not the scopolamine plus SKF38393-induced stimulation. It has been reported previously that muscarinic antagonists do not produce locomotor activation in dopamine-depleted rodents (see, for example, Carlsson et al., 1991), and such was the case in the present studies. It seems reasonable to propose that the behavioural effects of clozapine are at least partly due to its scopolamine-like muscarinic receptor antagonistic properties. However, in the absence of selective agonists and antagonists for the various muscarinic receptor subtypes, it is impossible at the moment to characterise which subtypes play the major role in the effects of clozapine reported here.

The difficulty involved in completely blocking the response induced by clozapine plus SKF38393 with a selective dopamine D_2 receptor antagonist may involve the uncoupling of the dopamine D_1 and D_2 receptors. When uncoupling has occurred, for example after multiple doses of reserpine, stimulation of either dopamine D_1 or D_2 receptors is adequate to elicit stimulation (Arnt, 1985). Furthermore, where both receptors are being stimulated, both dopamine D_1 and D_2 receptors must be antagonised to achieve complete blockade of the behavioural response –neither a selective dopamine D_1 nor a selective D_2 receptor antagonist is sufficient (Arnt, 1985; Needham et al., 1993). Since SCH23390 partially blocked the behavioural response after both clozapine or scopolamine (when combined with SKF38393), it seems that only a partial uncoupling of the dopamine receptors had occurred. An interesting observation was that quinpirole alone, unlike SKF38393, produced significant locomotor stimulation. This may also reflect partial uncoupling of dopamine D_1 and D_2 receptors since only quinpirole was active.

One of the hypotheses underlying the current experiments was the observation that clozapine binds in vitro to various dopamine receptors in a manner similar to that of an agonist. Our data neither confirm nor disprove this hypothesis. Any direct agonist-like effect of clozapine in in vivo behavioural models has probably been submerged by its antimuscarinic effects.

As described above, clozapine has substantial affinity for a variety of receptors that are involved in motor function, and a role for these cannot be easily eliminated. Thus, 5-HT_{1A} receptors are involved in locomotor control. However, the affinity of clozapine for this receptor type is probably too low to play a significant role (about 200 nM, unpublished observation). Clozapine has considerable affinity for 5-HT_2 receptors where it is an antagonist. The role of 5-HT_2 receptors in

regulating dopamine D_2 receptor agonist or antagonist-induced actions is, however, controversial. Thus, ritanserin has been reported to both antagonise and not to antagonise dopamine D_1/D_2 receptor antagonist-induced catalepsy (Wadenberg, 1992 for discussion and references). When ritanserin was tested alone or in combination with SKF38393, no locomotor stimulation was observed, suggesting that 5-HT_2 receptor antagonism per se did not play a role in the observed results.

Both reserpine and clozapine produce profound falls in body temperature (Maj et al., 1974). The influence of this fall in temperature on the present results cannot easily be determined. However, the effect of any fall in temperature was controlled to the extent that all animals were pretreated similarly and all experiments were run in a random manner.

Dopamine uptake inhibitors such as methylphenidate can stimulate locomotion via an increase in synaptic levels of dopamine, resulting in the stimulation of dopamine D_1 and D_2 receptors. We know of no papers that have reported studies on the effect of clozapine on dopamine uptake.

The degree of dopamine depletion was tested functionally by administering *d*-amphetamine. *d*-Amphetamine was inactive, suggesting that very little so-called newly synthesised dopamine was available for release (Ross, 1979; Scheel-Krüger, 1971). Note that the doses of *d*-amphetamine used, 5 and 10 μmol/kg, are higher than those required to stimulate activity in normal rats (unpublished data).

The potential clinical ramifications of the present study are important. If the end result of the systemic injection of clozapine (functioning as an antimuscarinic agent) is that it behaves in a dopamine D_2 receptor agonist-like way, then this property may tend to antagonise both its dopamine D_1 and D_2 receptor blocking properties, thereby producing a net effect resembling that of a weak partial dopamine agonist. We would propose that such a profile could contribute to clozapine's atypical antipsychotic profile in the clinic.

Acknowledgements

The authors would like to thank Dr. Jørn Arnt for most helpful comments and suggestions.

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