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Further evidence for clozapine as a dopamine D_1 receptor agonist

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Abstract

Clozapine (0.625–10.0 mg kg^{-1} s.c.), but not the two major clozapine metabolites, *N*-desmethylclozapine (0.625–10.0 mg kg^{-1} s.c.) or clozapine-*N*-oxide (0.625–10.0 mg kg^{-1} s.c.), caused a dose-dependent decrease in core temperature in the rat. Furthermore, the clozapine-induced hypothermia (2.5 mg kg^{-1}, s.c.) was fully antagonised by pretreatment with the selective dopamine D_1 receptor antagonist (+)-5-(2,3-dihydrobenzofuran-7-yl)-3-methyl-8-nitro-2,3,4,5-tetrahydro-1*H*-3-benzazepine-7-ol, maleate (NNC 687) (4.0 mg kg^{-1} s.c.). NNC 687 by itself (2.0–8.0 mg kg^{-1} s.c.) did not affect core temperature. The present results provide further evidence for the dopamine D_1 receptor agonist properties of clozapine.

Keywords: Clozapine; Thermoregulation; Dopamine; Dopamine receptor; (Rat)

1. Introduction

Clozapine displays affinity for both dopamine D_1 and D_2 receptors, and generally behaves as an antagonist at these dopamine receptor sites (see Meltzer et al., 1989). Thus, clozapine produces an increase in neostriatal dopamine turnover (see e.g. Coward et al., 1989), in all probability due to blockade of dopamine D_2 autoreceptors, and, at least partially, antagonises various aspects of dopamine D_2 receptor agonist-induced behaviours (see e.g. Tamminga and Gerlach, 1987). Furthermore, clozapine has been shown to block adenylyl cyclase-coupled dopamine D_1 receptors (Andersen and Braestrup, 1986), as well as to antagonise various behavioural indices of dopamine D_1 receptor stimulation (e.g. Murray and Waddington, 1990; Chipkin and Latranyi, 1987).

As recently shown, stimulation of either dopamine D_1 or D_2 receptors results in hypothermia in the rat (Salmi et al., 1993). However, it was not possible to antagonise A 68930- or quinpirole-induced hypothermia in the rat by clozapine treatment (1.0 mg kg^{-1}). In contrast, the hypothermia produced by clozapine itself, at a higher dose (2.5 mg kg^{-1}), was fully blocked by pretreatment with the dopamine D_1 receptor antagonist SCH 23390, providing suggestive evidence for dopamine D_1 receptor agonist properties of clozapine (Salmi et al., 1994). Together with the evidence related above, this demonstrates mixed agonist-antagonist properties of clozapine as a dopamine D_1 receptor ligand, presumably due to partial agonist properties of clozapine at this dopamine receptor subtype.

SCH 23390, in addition to dopamine D_1 receptor affinity, has considerable affinity also for 5-$HT_{2A/C}$ receptors (Hyttel, 1983; Bischoff et al., 1986). In order to further examine dopamine D_1 receptor specificity in clozapine-induced hypothermia in the rat, we have in the present study used a new dopamine D_1 receptor antagonist (NNC 687) with low 5-HT_2 receptor affinity (Andersen et al., 1992). In addition, we have examined a possible contribution of the two major clozapine metabolites, *N*-desmethylclozapine and clozapine-*N*-oxide (Baldessarini et al., 1993; Weigmann and Hiemke, 1992), to the hypothermia produced by clozapine in the rat.

2. Materials and methods

2.1. Animals

Adult male Sprague-Dawley rats (280–320 g) (B & K Universal AB, Sollentuna, Sweden) were used. The rats were housed five per cage, under controlled conditions of temperature (21.0 ± 0.4°C), relative humidity (55–65%) and light-dark cycle (12 : 12 h, lights off at 06:00 h). Food (R36, Ewos, Södertälje, Sweden) and tap water were available ad libitum. The rats arrived in the laboratory at least 1 week before being used in the experiments.

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2.2. Drugs

Clozapine, clozapine *N*-oxide, *N*-desmethylclozapine (Sandoz, Basel, Switzerland), and (+)-5-(2,3-dihydrobenzofuran-7-yl)-3-methyl-8-nitro-2,3,4,5-tetrahydro-1*H*-3-benzazepine-7-ol, maleate (NNC 687) (Novo Nordisk AS, Bagsvaerd, Denmark). All drugs were dissolved in a minimal amount of glacial acetic acid and made up to volume in isotonic glucose. The controls were given the solvent vehicle. All injections were made subcutaneously in a volume of 2 ml kg^{-1}.

2.3. Temperature measurements

Temperature was measured in a temperature-controlled room (ambient temperature 21.0 ± 0.4°C). The rats were transferred to the room 1 h before the experiments started and were housed in a ventilated cabinet between temperature measurements. The rats were individually identified, and for each experiment every *n*th rat was assigned to a different treatment or dose group (including controls). The core temperature was recorded by means of a commercially available telethermometer (YSI-2100, Yellow Springs Instruments Co, Yellow Springs, OH, USA) and an accompanying probe (YSI-402). The probe, lubricated with mucilago etalosi AF-68 (ACO Läkemedel AB, Stockholm, Sweden), was inserted rectally (about 90 mm) in gently hand-restrained rats. The telethermometer was connected to an automatic printer device that was activated when the temperature reading had stabilized (±0.1°C) for 10 s (for further details, see Salmi et al., 1994). All experiments were carried out between 09:00 and 14:00 h.

2.4. Statistical analysis

Parametric statistical description and analyses were used throughout. Thus, results are presented as means ± S.D. and were analysed by means of a one-way analyses of variance (ANOVA), followed by appropriate *t*-tests, as given in legends to Figs. 1 and 2 and text (see Winer, 1971).

3. Results

3.1. Effects of clozapine and its major metabolites

Clozapine (0.625–10.0 mg kg^{-1} s.c.) produced a dose-dependent, and statistically significant, decrease in core temperature (Fig. 1, top). The time course showed an onset of action at 30 min for the 2.5 and 10.0 mg kg^{-1} dose, and a duration of up to 4 h for the highest dose. Administration of the metabolites *N*-desmethylclozapine (0.625–10.0 mg kg^{-1} s.c.) and clozapine-*N*-oxide (0.625–10.0 mg kg^{-1} s.c.) did not produce any statistically significant effects on core temperature (Fig. 1, middle and bottom, respectively).

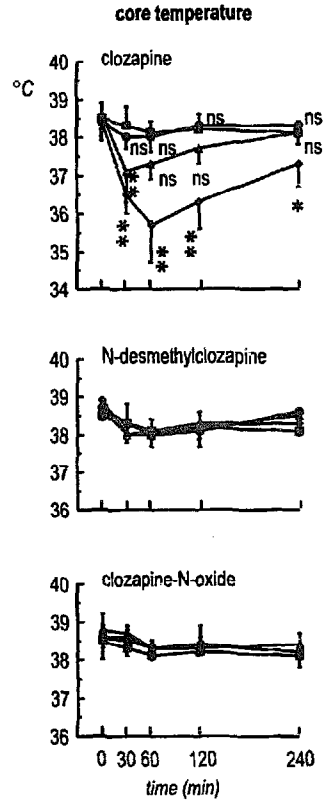

Fig. 1. Effects of clozapine and two of its major metabolites on core temperature in the rat. Clozapine, *N*-desmethylclozapine and clozapine-*N*-oxide were all administered in the dose range 0.625–10.0 mg kg^{-1} s.c. and core temperature was recorded 30–240 min thereafter, as shown in the figure. Results are presented as means ± S.D. based on 5 animals per group. The pre-drug baseline temperature is shown at 0 min in the figure. Statistical analysis was performed by means of a one-way ANOVA at the respective time interval, followed by the Dunnett's *t*-test for comparisons with time-matched vehicle treated controls. Vehicle controls (■); 0.625 mg kg^{-1} (●); 2.5 mg kg^{-1} (▲); 10.0 mg kg^{-1} (◆). ^{ns} $P > 0.05$, * $P < 0.05$, ** $P < 0.01$.

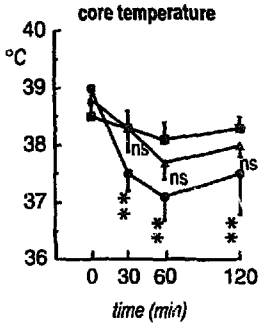

Fig. 2. Antagonism by NNC 687 of clozapine-induced hypothermia in the rat. Clozapine (2.5 mg kg^{-1}) and NNC 687 (4 mg kg^{-1}) were administered s.c., and core temperature was recorded 30, 60 and 120 min thereafter, as shown in the figure. Results are presented as mean values ± S.D., based on 7 animals per group. The pre-drug baseline temperature is shown at 0 min in the figure. Statistical analysis was performed by means of a one-way ANOVA at each time interval, followed by the Dunnett's *t*-test for comparisons with vehicle treated controls. Vehicle controls (■); clozapine (●); NNC 687 + clozapine (▲). ^{ns} $P > 0.05$, ** $P < 0.01$.

Table 1
Effects of NNC 687 on core temperature in the rat

Dose	Time after administration (min)			
(mg kg^{-1})	−10	30	60	120
0	38.4±0.0	38.3±0.3	38.7±0.2	38.1±0.1
2.0	38.7±0.8	38.5±0.2	38.2±0.5	37.5±1.3
4.0	38.3±0.1	38.3±0.3	38.2±0.2	38.0±0.3
8.0	38.7±0.4	38.4±0.2	38.0±0.1	37.8±0.2

NNC 687 was administered s.c. and the core temperature was recorded up to 2 h after administration, as shown in Table 1. Results are presented as means±S.D. based on 3 observations per treatment group. Statistical analysis was performed by means of a one-way ANOVA at the respective time interval. $F(3,8) < 4.07$, $P > 0.05$ in all cases.

3.2. Antagonism by NNC 687 of clozapine-induced hypothermia

The clozapine-induced hypothermia (2.5 mg kg^{-1} s.c.) was fully antagonised by concomitant administration of the selective dopamine D_1 receptor antagonist NNC 687 (4.0 mg kg^{-1} s.c.) (Fig. 2). Thus, there was a statistically significant difference between NNC 687 + clozapine and clozapine-treated animals at 30 and 60 min after injections (Student's *t*-test, $t_{12} = 4.23$, $P < 0.01$ and $t_{12} = 3.11$, $P < 0.01$, respectively), and the core temperature in the NNC 687 + clozapine-treated rats was not statistically significantly different from that of controls at these time intervals. At the 120 min time interval, the difference between NNC 687 + clozapine and clozapine-treated animals did not reach statistical significance. In this experiment, NNC 687 by itself (4.0 mg kg^{-1} s.c.) had no effects on the core temperature (not shown in Fig. 2).

3.3. Effects of NNC 687 on core temperature

The effect of NNC 687 (2.0–8.0 mg kg^{-1} s.c.) on core temperature was examined in a separate experiment. There were no statistically significant effects of NNC 687 on the core temperature 30, 60 and 120 min after administration (Table 1).

4. Discussion

In agreement with data reported in the literature, clozapine produced a robust hypothermia in the rat (Maj et al., 1974; Salmi et al., 1994) that lasted for up to 4 h, depending on the dose. This effect of clozapine, presumably due to stimulation of dopamine D_1 receptors (Salmi et al., 1994), was not seen after administration of its two major metabolites, *N*-desmethylclozapine or clozapine-*N*-oxide. It should be noted that although the metabolites have less affinity for the dopamine D_1 receptor than the parent compound (Kuoppamäki et al., 1993), *N*-desmethyl-clozapine displays a relatively high affinity for 5-HT_{2A} and dopamine D_2 receptors, which in humans could contribute to the drug's antipsychotic efficacy.

The observation that NNC 687 could antagonise the clozapine-induced hypothermia considerably strengthens our previous suggestion that clozapine has dopamine D_1 receptor agonist properties (Salmi et al., 1994). In this latter study, it was found that clozapine-induced hypothermia in the rat was antagonised by pretreatment with the dopamine D_1 receptor antagonist SCH 23390. This compound, however, has significant affinity also for 5-$HT_{2A/C}$ receptors, and the use of NNC 687 as dopamine D_1 receptor antagonist in the present study to a large extent excludes a contribution by 5-HT_2 receptors to the interaction between dopamine and SCH 23390 or NNC 687. It should also be noted that the 5-$HT_{2A/C}$ receptor antagonist ritanserin by itself did not affect core temperature in the rat, nor does this compound antagonise clozapine-induced hypothermia (Salmi et al., 1994). Furthermore, the fact that both SCH 23390 and NNC 687 displays very low affinity for a range of other receptor sites, including α_1-, α_2-, and β-adrenoceptors as well as dopamine D_2, 5-HT_{1A}, $GABA_A$, benzodiazepine and histamine H_1 receptors (Andersen et al., 1992), provides further support for the notion that clozapine behaves as a dopamine D_1 receptor agonist in the present context. Furthermore, it was recently shown that clozapine-induced antisecretory effects in the rat were antagonised by pretreatment with SCH 23390 (Glavin, 1995). This observation provides additional support for clozapine as a dopamine D_1 receptor agonist, since it has previously been shown that dopamine D_1-like receptors are involved in the regulation of gastric acid secretion in the rat (Glavin, 1989).

There are several reports that clozapine can be used to control L-dopa-induced psychoses in Parkinson's disease, without aggravation of the underlying extrapyramidal motor disability (Scholz and Dichgans, 1985; see Safferman et al., 1994). This could possibly be explained by the relatively low affinity of clozapine for dopamine D_2 receptors, linked to extrapyramidal motor functions, and its antipsychotic efficacy being exerted via other dopamine receptor subtypes or non-dopamine receptors. It should be noted, however, that [1] clozapine not only improves L-dopa-induced psychoses but also appears to have antiparkinsonian efficacy (e.g. Casey, 1989), and that [2] dopamine D_1 receptor agonists have been shown to antagonise 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-induced parkinsonism in a primate model (Taylor et al., 1991; Vermeulen et al., 1993) and to improve motor disability in Parkinson's disease (Emre et al., 1992). Results from the present study provide support for the interpretation that the beneficial effects of clozapine in L-dopa-induced psychoses and in Parkinson's disease are related to its intrinsic efficacy at brain dopamine D_1 receptors. Of particular interest in this regard, there is suggestive evidence from a limited blind, cross-over study that the addition of a partial dopamine D_1 receptor agonist to

schizophrenic patients treated with haloperidol not only improves the extrapyramidal side effect profile, but also in fact may result in improved clinical efficacy (Davidson et al., 1990).

Results from the present and previous studies demonstrate the apparent full efficacy of clozapine as an agonist at dopamine D_1 receptors involved in temperature regulation in the rat (Salmi et al., 1993, 1994). In the same experimental model, i.e. rat core temperature, it was found that also the dopamine D_2 receptor antagonist raclopride produced a partial antagonism of clozapine-induced hypothermia. As previously shown, dopamine D_1 and D_2 receptor agonist-induced hypothermia in the rat can be pharmacologically separated (Salmi et al., 1993), and the partial antagonism by raclopride of clozapine-induced hypothermia in fact suggests that clozapine also has weak agonist properties at dopamine D_2 receptors. This notion is in agreement with in vitro and in vivo data reported by Malmberg et al. (1993) and Jackson et al. (1995). Together, these observations indeed suggest that clozapine may have intrinsic efficacy at both dopamine D_1 and D_2 receptors.

Clozapine displays high affinity for the newly cloned dopamine D_4 receptor site (Van Tol et al., 1991; Van Tol et al., 1992), but it is presently not clear to what extent this receptor affinity contributes to the clinical profile of clozapine (see Reynolds and Czudele, 1995). Furthermore, there are at present no selective tools to study the importance of this receptor affinity for the pharmacodynamic profile of clozapine more generally. It is not likely, however, that affinity for dopamine D_4 receptors is of importance for clozapine-induced hypothermia in the present context since, for example, SCH 23390 has negligible affinity for this dopamine receptor site (Van Tol et al., 1991).

In conclusion: The present data provide strong support for the notion that clozapine produces hypothermia in the rat through stimulation of dopamine D_1 receptors, since the selective dopamine D_1 receptor antagonist NNC 687 fully antagonised the clozapine-induced hypothermia. The two major clozapine metabolites, *N*-desmethylclozapine and clozapine-*N*-oxide, do not appear to contribute to the hypothermia produced by systemic clozapine administration. Together with clinical observations on the effects of clozapine, and known dopamine D_1 receptor ligands, in Parkinson's disease and in schizophrenia, the present data provide support for the interpretation that the intrinsic efficacy of clozapine at brain dopamine D_1 receptors may contribute to its atypical profile as an antipsychotic.

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