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Short communication

# Evidence that dopamine $D_3$ receptors participate in clozapine-induced hypothermia

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**Abstract**

In analogy to the dopamine $D_3$ receptor agonist, (+)-7-OH-DPAT (7-hydroxy-2-(di-*n*-propylamino)tetralin) (0.01–0.63 mg/kg s.c.), clozapine dose-dependently (0.63–40.0 mg/kg s.c.) elicited hypothermia in rats. Haloperidol and raclopride, mixed dopamine $D_2/D_3$ receptor antagonists, failed, in contrast, to modify core temperature. Further, they dose-dependently inhibited the action of clozapine with inhibitory $dose_{50}$ values ($ID_{50}$) of 0.3 mg/kg s.c., in each case. The preferential dopamine $D_3$ versus $D_2$ receptor antagonist, (+)-AJ 76 (*cis*-(+)-5-methoxy-1-methyl-2-(*n*-propylamino)tetralin) ($ID_{50} = 2.8$), and the selective dopamine $D_3$ versus $D_2$ receptor antagonist, (±)-S 11566 ((±)-[7-(*N*,*N*-dipropylamino)-5,6,7,8-tetrahydro-naphtho(2,3*b*) dihydro,2,3-furane]) ($ID_{50} = 1.6$) likewise blocked the action of clozapine without reducing core temperature alone. The action of (±)-S 11566 was stereospecific in that its active eutomer, (+)-S 14297 ($ID_{50} = 1.0$), also inhibited the action of clozapine whereas its inactive distomer, (−)-S 17777 ($ID_{50} > 10.0$), was not effective. Antagonist potency for blockade of clozapine-induced hypothermia correlated powerfully both with potency for blockade of (+)-7-OH-DPAT-induced hypothermia ($r = 0.98$) and with affinity at cloned human dopamine $D_3$ receptors transfected into Chinese hamster ovary (CHO) cells ($r = 0.92$). In conclusion, these data suggest that dopamine $D_3$ receptors may be involved in the induction of hypothermia by clozapine in the rat.

*Keywords:* Dopamine $D_3$ receptor; Core temperature; Thermoregulation; Clozapine; Antipsychotic

## 1. Introduction

While a major role of dopamine $D_2$ receptors in the mediation of hypothermia has long been assumed (see Salmi et al., 1994; Vasse et al., 1990), it was recently suggested that activation of the closely related dopamine $D_3$ receptor can induce hypothermia (Millan et al., 1994). Thus, the preferential dopamine $D_3$ versus $D_2$ receptor agonist, (+)-7-OH-DPAT (7-hydroxy-2-(di-*n*-propylamino)tetralin) (Chio et al., 1993; Millan et al., 1994; Sokoloff et al., 1992) elicits a pronounced hypothermia in rats which is antagonised by the mixed dopamine $D_2/D_3$ receptor antagonists, haloperidol and raclopride, the preferential dopamine $D_3$ versus $D_2$ receptor antagonist, (+)-AJ 76 (*cis*-(+)-5-methoxy-1-methyl-2-(*n*-propylamino)tetralin) and the selective dopamine $D_3$ versus $D_2$ antagonist, (±)-S 11566 ((±)-[7-(*N*,*N*-dipropylamino)-5,6,7,8-tetrahydro-naphtho-(2,3*b*)dihydro,2,3-furane]) (Millan et al., 1994; Rivet et al., 1994; Sokoloff et al., 1992). Further, the action of (±)-S 11566 is expressed stereospecifically in that its active eutomer, (+)-S 14297, mimics the effect of racemic (±)-S 11566, whereas its inactive distomer, (−)-S 17777, which possesses only 50-fold lower affinity for dopamine $D_3$ receptors (Millan et al., 1994), is ineffective. In these and other studies (Salmi et al., 1994), the antagonists did not modify core temperature alone. It is, thus, of particular interest that the atypical antipsychotic, clozapine, elicits hypothermia in the rat via a mechanism which remains to be satisfactorily defined (Menon et al., 1990; Salmi et al., 1994). In the light of the above observations, the present study evaluated a possible role of dopamine $D_3$ receptors in the mediation of the clozapine-induced hypothermia in rats. To this end, we determined the affinity of clozapine as compared to that of (+)-7-OH-DPAT at cloned human and rat dopamine $D_3$ and $D_2$ receptors expressed in a Chinese hamster ovary (CHO) cell line,

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and examined the influence of (+)-S 14297 and the other above-mentioned antagonists upon the ability of clozapine to elicit hypothermia in the rat.

## 2. Materials and methods

### 2.1. Binding at cloned rat and human dopamine $D_2$ and $D_3$ receptors

The affinity ($pK_i$) of clozapine at cloned rat or human dopamine $D_3$ or $D_2$ receptors transfected into CHO cells was determined as previously employing [$^{125}$I]iodosulpride (1.0 nM) as the radiolabel and spiperone (10 $\mu$M) to define non-specific binding (Millan et al., 1994; Rivet et al., 1994; Sokoloff et al., 1992).

### 2.2. Influence upon core temperature

This study employed male Wistar rats of 200–220 g maintained for 1 week prior to testing in a temperature (21 ± 1° C) and humidity (60 ± 5%) controlled laboratory with a 12/12 h light/dark cycle: lights on at 7.30 a.m. All experiments were performed in a randomised sequence between 10.00 and 12.00 a.m. Core (rectal) temperature (CT) was determined as previously (Millan et al., 1994) employing a rectal thermisoprobe. Basal CT was determined, rats injected with drug or vehicle and 30 min later, at which time the effect of clozapine and other drugs is well-established (unpublished observation; Salmi et al., 1994), CT was re-determined. The difference to basal values ($\Delta$CT) was calculated. In antagonist studies, basal CT was measured, rats injected with vehicle or antagonist, then 30 min later with vehicle or clozapine; 30 min later CT was re-determined and the difference to basal values calculated ($\Delta$CT).

## 3. Results

### 3.1. Binding to cloned rat and human dopamine $D_2$ and $D_3$ receptors

Clozapine displayed significant affinity at cloned, rat and human dopamine $D_3$ ($pK_i$ = 6.6 and 6.7) and $D_2$ ($pK_i$ = 7.1 and 7.1) receptors. Isotherms were homogeneous and Hill coefficients were 1.02, 1.01, 0.97 and 1.05, respectively. (As explained elsewhere, rat and human dopamine $D_3$ receptors transfected into this population of CHO cells do not display significant coupling to G-proteins and, correspondingly, do not show multiple affinity states. Thus, even for agonists, monophasic displacement curves with Hill coefficients close to unity are seen (Chio et al., 1993; Millan et al., 1994; Sokoloff et al., 1992).) The affinities of other ligands at cloned rat dopamine $D_3$ versus $D_2$ sites are given elsewhere (Millan et al., 1994) except for raclopride (8.7 versus 8.8) and (±)-S 11566 (6.1 versus 7.3).

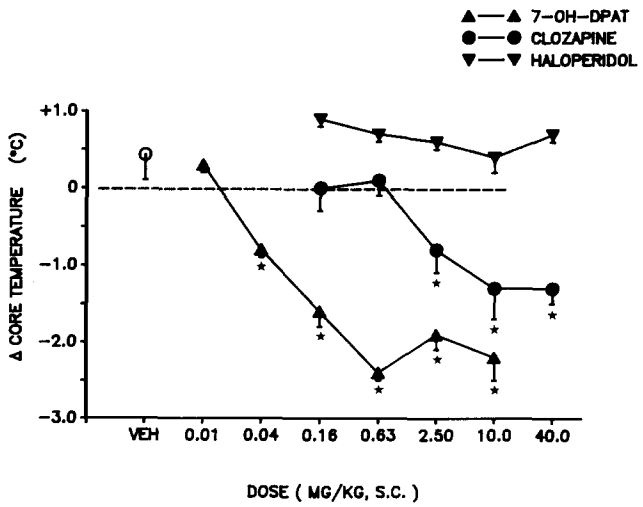


Fig. 1. Influence of clozapine as compared to (+)-7-OH-DPAT upon core temperature in the rat. Data are means ± S.E.M. $n > 6$ per value. ANOVA as follows: (+)-7-OH-DPAT, $F(5,35) = 55.8$, $P < 0.001$; clozapine, $F(5,27) = 3.9$, $P < 0.001$ and haloperidol, $F(4,30) = 0.3$, $P > 0.05$. Asterisks indicate significance of differences to corresponding vehicle values in Dunnett's test; $^*P < 0.05$.

### 3.2. Induction of hypothermia

Clozapine, but not haloperidol, mimicked (+)-7-OH-DPAT in eliciting hypothermia (Fig. 1). Haloperidol, raclopride and (+)-AJ 76 inhibited the action of clozapine (Fig. 2). Similarly, (±)-S 11566 and (+)-S 14297 dose-dependently inhibited clozapine-induced hypothermia, whereas (−)-S 17777 was ineffective (Fig. 2). Antagonist potency for inhibiting clozapine correlated more powerfully with affinity (Millan et al., 1994; see Results) at cloned rat dopamine $D_3$ (0.92, $P < 0.01$) than $D_2$ (0.83, $P < 0.05$) receptors and correlated very highly (0.98, $P < 0.01$) with potency in blocking hypothermia provoked by (+)-7-OH-DPAT (Millan et al., 1994; Fig. 2). Domperidone, a dopamine $D_2/D_3$ antagonist which does not cross the blood-brain barrier, did not modify the action of clozapine: vehicle ($n = 5$) = −1.5 ± 0.2 versus domperidone (0.16 mg/kg s.c., $n = 5$) = −1.6 ± 0.1. This indicates that clozapine acts centrally. The dopamine $D_1$ receptor antagonists, *R*(+)-7-chloro-8-hydroxy-1-phenyl-2,3,4,5-tetrahydro-1*H*-3-benzazepine hydrochloride (SCH 23390) and (−)-*trans*-6,7,7*a*,8,9,13*b*-hexahydro-3-chloro-2-hydroxyl-*N*-methyl-5*H*-benzo[*d*]-naphtho-[2,1-benazazepine] (SCH 39166), each at a dose of 0.63 mg/kg s.c., significantly attenuated, but did *not* abolish, the action of clozapine; vehicle + clozapine = −1.4 ± 0.2 vs. SCH 23390 + clozapine = −0.4 ± 0.2 and SCH 39166 + clozapine = −0.3 ± 0.1; $P < 0.05$ in each case. Simi-

larly, they reduced the action of (+)-7-OH-DPAT; vehicle + (+)-7-OH-DPAT = $-1.5 \pm 0.1$ vs. SCH 23390) +(+)-7-OH-DPAT = $-0.3 \pm 0.1$ and vs. SCH 39166 + (+)-7-OH-DPAT = $-0.7 \pm 0.2$; $P < 0.05$ in each case. None of the dopaminergic antagonists significantly affected CT alone (not shown). Antagonists at other, non-dopaminergic receptor types were inactive (legend to Fig. 2).

## 4. Discussion

In corroboration of the findings of Sokoloff et al. (1992), clozapine displayed substantial affinity at recombinant, human dopamine $D_3$ and $D_2$ receptors. Further, in an extension of these data, it is shown herein that clozapine similarly displays marked affinity at recombinant, rat dopamine $D_3$ and $D_2$ sites (see Results). Like clozapine and haloperidol (Rivet et al., 1994), raclopride manifested similar affinity at cloned, rat dopamine $D_3$ and $D_2$ sites (see Results), whereas (+)-AJ 76 showed a mild (2-fold) preference for dopamine $D_3$ sites (Rivet et al., 1994). Notably, the novel naphthofurane, (±)-S 11566 (see Results), as well as its active eutomer, (+)-S 14297 (Rivet et al., 1994), revealed a marked (25-fold) selectivity for cloned, rat dopamine $D_3$ versus $D_2$ receptors with its distomer, (−)-S 17777, displaying only weak affinity (Rivet et al., 1994).

In line with the results of Salmi et al. (1994), clozapine elicited hypothermia. In this respect, it mimicked the action of the preferential agonist at dopamine $D_3$ versus $D_2$ receptors, (+)-7-OH-DPAT, and, in analogy (Millan et al., 1994), the present data suggest that activation of central dopamine $D_3$ receptors contributes to the action of clozapine. First, the selective dopamine $D_3$ receptor antagonist (±)-S 11566 dose-dependently blocked the action of clozapine, an action mimicked by its active eutomer, (+)-S 14297, whereas its inactive distomer, (−)-S 17777, was ineffective. Second, the action of clozapine was likewise antagonised by other antagonists with marked affinity at dopamine $D_3$ receptors, haloperidol, raclopride and (+)-AJ 76, and, although the difference was only slight, their potency correlated more tightly with affinity at cloned dopamine $D_3$ than $D_2$ receptors. Third, consistent with a common dopamine $D_3$ receptor-mediated mechanism underlying their actions, there was a pronounced

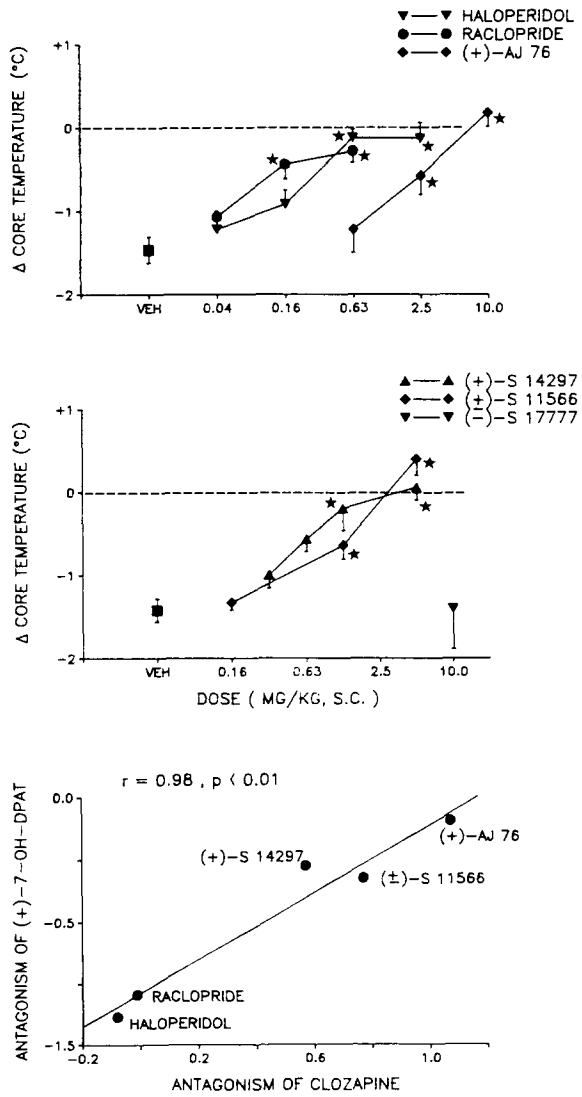


Fig. 2. Influence of antagonists at dopamine $D_3$ and/or dopamine $D_2$ receptors upon the induction of hypothermia by clozapine. Upper and middle panels: Data are means ± S.E.M. $n > 6$ per value. ANOVA as follows: haloperidol, $F(4,37) = 10.7$, $P < 0.001$; raclopride, $F(3,28) = 8.1$, $P < 0.01$; (+)-AJ 76, $F(3,24) = 12.0$, $P < 0.001$; (±)-S 11566, $F(3,25) = 18.7$, $P < 0.001$ and (+)-S 14267, $F(4,28) = 11.5$, $P < 0.001$. Asterisks indicate significance of differences to corresponding vehicle values in Dunnett's test; $^*P < 0.05$. For (−)-S 17777, there was no significant difference to corresponding vehicle values in Student's two-tailed $t$-test ($P > 0.05$). Inhibitory $dose_{50}$ values (95% confidence limits) in mg/kg s.c. were as follows: Haloperidol, 0.31 (0.08–1.21); raclopride, 0.34 (0.10–1.09); (+)-AJ 76, 2.77 (1.01–7.58); (±)-S 11566, 1.60 (0.55–4.65) and (+)-S 14297, 1.01 (0.34–2.98). Lower panel: Correlation analysis (Pearson product-moment) between antagonist potency (log μmol/kg s.c.) in blocking clozapine- as compared to (+)-7-OH-DPAT-induced hypothermia (data from Millan et al., 1994). Antagonists at various other receptor types failed to modify the action of clozapine. For the following (doses in mg/kg s.c./receptor targetted), data are means ± S.E.M. and $n \geq 5$. Vehicle + clozapine (10.0) = $-1.3 \pm 0.1$ versus (−)-alprenolol (10.0)/serotonin$_{1A/1B}$, β-adrenergic + clozapine = $-1.0 \pm 0.3$; 4-(benzodioxan-5-yl)1-(indan-2-yl)piperazine (S 15535) (2.5)/serotonin$_{1A}$ + clozapine = $-1.4 \pm 0.2$; ritanserin (10.0)/serotonin$_{2A/2C}$ + clozapine = $-1.7 \pm 0.2$; prazosin (0.63)/$\alpha_1$-adrenergic + clozapine = $-1.6 \pm 0.2$; idazoxan (2.5)/$\alpha_2$-adrenergic + clozapine = $-1.6 \pm 0.2$; mepyramine (2.5)/histamine$_1$ + clozapine = $-1.2 \pm 0.2$ and scopolamine (2.5)/muscarinic + clozapine = $-1.3 \pm 0.2$: $F(7,49) = 0.9$, $P > 0.05$.

correlation for antagonist potency in blocking the hypothermia elicited by clozapine and (+)-7-OH-DPAT (Fig. 2). In line with this argument, we have recently found that clozapine (5.0 mg/kg s.c.) generalizes to a discriminative stimulus elicited by (+)-7-OH-DPAT (0.31 mg/kg s.c.) in the rat (R. Schreiber, unpublished observation).

Despite the significant affinity of clozapine for cloned dopamine $D_1$, $D_4$ and $D_5$ sites (Van Tol et al., 1991), these are unlikely to be involved in the interactions described herein. Thus, the affinity of (+)-S 14297, raclopride and (+)-7-OH-DPAT for dopamine $D_1$, $D_4$ and $D_5$ sites is, in each case, very low ($pK_i$ values < 6.0) (A. Newman-Tancredi, unpublished observation; Sokoloff et al., 1992). Further, (+)-S 14297 and raclopride do not display significant occupation of, or functional actions at, central dopamine $D_1$ receptors in vivo (V. Audinot, unpublished observation; Leysen et al., 1992). It is interesting, thus, that, in analogy to the recent work of Salmi et al. (1994), the dopamine $D_1$ antagonist, SCH 23390, inhibited the action of clozapine. Further, we show herein that an even more selective dopamine $D_1$ antagonist, SCH 39166, likewise inhibits the action of clozapine and, also, that both SCH 23390 and SCH 39166 inhibit the action of (+)-7-OH-DPAT (see Results). However, active doses of SCH 23390 and SCH 39166 required are high as compared to those needed to block central dopamine $D_1$ sites in ex vivo binding and functional studies (Arnt, 1987; Leysen et al., 1992; McQuade et al., 1990) and their effects are only sub-maximal. Further, there exist conflicting data as to whether selective activation of dopamine $D_1$ receptors can elicit hypothermia (Arnt, 1987; Salmi et al., 1994; Vasse et al., 1990). Most importantly, clozapine behaves as an *antagonist* at dopamine $D_1$ receptors in vitro in blocking dopamine-stimulated adenyl cyclase activity at striatal dopamine $D_1$ receptors and, in vivo, in several functional models (Andersen and Braestrup, 1986; Arnt, 1987). Nevertheless, there is an extensive body of data indicating that functionally intact dopamine $D_1$ receptors may be necessary for the full expression of effects mediated by dopamine $D_2$ receptors (Waddington, 1989). It is, thus, possible that SCH 23390 and SCH 39166 interfere with a comparable, permissive role of dopamine $D_1$ receptors in the expression of dopamine $D_3$ receptor-mediated hypothermia (Waddington, 1989). The significance of these observations will be of interest to examine further.

Clozapine displays interactions at many receptor types and a modulatory role of $\alpha_2$-adrenoceptors has been proposed to be involved in its hypothermic action in the mouse (Menon et al., 1990). However, a previous study (Salmi et al., 1994) provided no evidence for a role of $\alpha_2$-adrenoceptors in rats, and, herein, antagonists at adrenoceptors as well as at serotoninergic and other receptor types failed to significantly inhibit clozapine-induced hypothermia (legend to Fig. 2).

One simple interpretation of the present data would be that clozapine elicits hypothermia via a (possibly, partial) agonist action at (a) population(s) of central dopamine $D_3$ receptors mediating hypothermia. Nevertheless, direct evidence for an agonist interaction of clozapine at dopamine $D_3$ sites in terms of coupling to intracellular transduction mechanisms has not, to our knowledge, been documented. Further, there are several alternative interpretations. First, clozapine can enhance the activity of central dopaminergic pathways (Karoum and Egan, 1992) and it is possible that clozapine elicits the release of dopamine onto (non-$D_3$) dopamine receptors mediating hypothermia. Second, it is possible that an action of clozapine at an, as yet unidentified, receptor targetting dopaminergic neurones represents its primary mechanism of action: that is, dopamine $D_3$ receptors (downstream) are only *indirectly* activated by clozapine. Third, dopamine $D_3$ receptors may play a 'permissive' role in the induction of clozapine-induced hypothermia which is expressed via a non-$D_3$ receptor-mediated mechanism. These possibilities will require additional study.

In conclusion, the present study demonstrates that the hypothermia elicited by clozapine is modulated similarly to that evoked by (+)-7-OH-DPAT. Further, the data suggest that the ability of clozapine to induce hypothermia depends upon functionally intact dopamine $D_3$ receptors in rats. Although a direct agonist action of clozapine at dopamine $D_3$ sites would be the simplest explanation, it may be premature to conclude in the absence of information at the level of intracellular transduction mechanisms. Interestingly, clinical studies suggest that the hypothermic properties of clozapine may be related to its superior antipsychotic profile as compared to that of haloperidol (Heh et al., 1988). Evidently, it will be of importance to establish how a putative (direct or indirect) clozapine-mediated enhancement of activity at dopamine $D_3$ receptors may contribute to its distinctive therapeutic actions. Irrespective of the underlying mechanisms, the present data reinforce interest in the interrelationship between the mechanism of action of antipsychotic drugs and dopamine $D_3$ receptors.

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