Atypical Antipsychotics and Dopamine D₄ Receptor Agonism: An In Vivo Experimental Study Using Core Temperature Measurements in the Rat

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ABSTRACT

The study objectives were to examine the effects of the atypical antipsychotic drugs olanzapine, risperidone, and quetiapine on core temperature in the rat in relation to such effects produced by clozapine and to compare possible in vivo intrinsic efficacy of olanzapine, risperidone, and quetiapine at dopamine (DA) D₄ receptors with such effects previously shown for clozapine. Core temperature measurements were made in adult male Wistar rats maintained under standard laboratory conditions using a reversed 12-h daylight cycle. Clozapine (0–32 μmol/kg s.c.), olanzapine (0–32 μmol/kg s.c.), and risperidone (0–4 μmol/kg s.c.) all produced a dose-dependent hypothermia. Except for slight nondose-dependent hyperthermia, there were no effects of quetiapine (0–16 μmol/kg s.c. or i.p.) on the core temperature. The hypothermia produced by clozapine, but not that produced by equipotent doses of olanzapine or risperidone, was fully antagonized by pretreatment with the DA D₄ receptor antagonist SCH-23,390 (0.1 μmol/kg s.c.). On the other hand, quinpirole-induced hypothermia (4 μmol/kg s.c.) was partially antagonized by olanzapine (2 μmol/kg s.c.), risperidone (4 μmol/kg s.c.), and quetiapine (16 μmol/kg s.c.) but not by clozapine (1 μmol/kg s.c.). Clozapine preferentially stimulates DA D₄ receptors in comparison with olanzapine and risperidone, whereas olanzapine, risperidone, and quetiapine preferentially block DA D₂ receptors compared with clozapine. It is suggested that stimulation of DA D₄ receptors, presumably in the prefrontal cortex, is a distinguishing feature of clozapine responsible for its favorable profile on cognitive functioning in schizophrenia.

Clozapine displays an atypical profile as an antipsychotic. Thus, in contrast to classic antipsychotics, such as chlorpromazine or haloperidol, clozapine also has efficacy against negative symptoms in schizophrenia and is often effective in therapy-resistant patients. In addition, clozapine does not produce the extrapyramidal and endocrine side effects associated with classic agents. However, agranulocytosis represents an idiosyncratic, and potentially fatal, side effect. This has resulted in an extensive search for new atypical antipsychotics, but it has proved difficult to pinpoint the mechanisms of action for clozapine as a reliable lead for the development of new clozapine-like agents (Owens and Risch, 1998).

All clinically effective antipsychotics, including clozapine, display affinity for the dopamine (DA) D₂ receptor (e.g., Seeman, 1980). At clinically effective doses, however, the occupancy of the DA D₂ receptors is not as prominent for clozapine as it is for classic antipsychotics (Farde et al., 1989, 1992), and other mechanisms must be put forward to explain its properties as an atypical antipsychotic. The most influential suggestion includes a high-affinity ratio between DA D₂/5-hydroxytryptamine (5-HT)₂ receptors (Meltzer et al., 1989). Indeed, some recently developed drugs, including olanzapine, risperidone, and quetiapine, compare favorably with clozapine in this regard (Bymaster et al., 1997; Owens and Risch, 1998).

In addition to the receptor affinities mentioned, clozapine displays affinity for a great number of neurotransmitter receptors in the brain, including 5-HT, norepinephrine, DA, and cholinergic receptor subtypes (Schotte et al., 1996; Zhang and Bymaster, 1999), some of which have been used as exploratory leads for drug development (Kehne et al., 1996; Bristow et al., 1997). In a recent series of experiments, we found DA D₄ receptor agonist properties of clozapine (Salmi et al., 1994; Salmi and Ahlenius, 1996). This observation gains significance as an important property of an atypical antipsychotic on the basis of laboratory data and clinical observations (Ahlenius, 1999). The present study was designed to examine possible DA D₄ receptor agonist properties for olanzapine, risperidone, and quetiapine compared with such properties previously shown for clozapine in this laboratory. Core temperature measurement in rats was used

ABBREVIATIONS: DA, dopamine; 5-HT, 5-hydroxytryptamine.
because this offers an experimental model suitable for the assessment of intrinsic efficacy at DA D1 receptors (Salmi et al., 1993; Salmi, 1998).

**Materials and Methods**

**Animals.** Adult male Wistar rats (260–300 g b.wt.; B&K Universal AB, Sol lentuna, Sweden) were used. The animals were housed three or four per cage under controlled conditions of temperature (21.0 ± 0.4°C), relative humidity (55–65%), and light/dark cycle (12-h, lights off at 6:00 PM; Hillegaart and Ahlenius, 1994). Food (R36; Ewos, Södertälje, Sweden) and tap water were available ad libitum. The rats arrived in the laboratory at least 10 days before the experiments started.

**Drugs.** Clozapine (M, 326.8; Sandoz, Basel, Switzerland), olanzapine (M, 312.4; Eli Lilly, Indianapolis, IN), and quetiapine fumarate (M, 883.1; Zeneca Pharmaceuticals, Cheshire, UK) were dissolved in a minimal amount of N HCl and made up to the volume with physiological saline. Risperidone (M, 410.3; Janssen Pharmaceutica, Beerse, Belgium) was dissolved in a minimal amount of glacial acetic acid and made up to the volume with isotonic glucose. SCH-23,390 (M, 324.3) and quinpirole HCl (M, 255.8; both from Research Biochemicals Inc., Natick, MA) were dissolved in physiological saline. All injections were made s.c. in a volume of 2 ml/kg.

**Core Temperature.** Temperature was measured in a room at ambient temperature of 23.7 ± 1.5°C. Recordings were made by means of a commercially available telethermometer (YSI-2100; Yellow Springs Instruments Co., Yellow Springs, OH) and a probe (YSI-402) lubricated with mucilagous etolasi AF-68 (ACO Läkemedel AB, Stockholm, Sweden). The probe was inserted rectally in the rat (about 90 mm), which was gently restrained with a hand. The telethermometer was connected to an automatic printer device that was activated when the temperature reading had stabilized (±0.1°C) for 15 s (Salmi et al., 1994). On the day preceding the experiments, the rats were individually identified, weighed, and subjected to a temperature reading to habituate the animals to the experimental procedures.

**Experimental Design and Statistics.** The animals served as their own controls in a change-over design (Li, 1964). Thus, for example, the four doses of the compounds presented in Fig. 1 were administered in the following order to successive rats: abcd, beda, cdab, and so on. The animals were observed twice a week, and thus the various treatments were separated by 2 or 3 days. Separate groups of animals (n = 6) were used for each experiment (see Figs. 1–3). Parametric procedures for description and analysis were used throughout. In experiments presented in Fig. 1, a two-way ANOVA for repeated measurements was used (A × B × S design; see Keppel, 1982), whereas the results presented in Figs. 2 and 3 were analyzed by means of a one-way ANOVA for repeated measurements, followed by the Newman-Keuls procedure (see Winer, 1971).

The rats were transferred to the experimentation room 1 h before the experiments started and were housed in a ventilated cabinet between the temperature measurement. The core temperature of the rats was measured at different time intervals, as indicated in the figures. All the experiments were performed between 9:00 AM and 2:00 PM.

**Results**

**Effects of Clozapine, Olanzapine, Risperidone, and Quetiapine on Core Temperature in Rat**

**Clozapine.** Clozapine induced a dose-dependent decrease in core temperature. Thus, there was a statistically significant decrease at the 8 and 32 μmol/kg doses (F1,5 = 27.03, P < .01; and F1,5 = 41.80, P < .01, respectively) in comparison with vehicle-treated controls (Fig. 1, top left). The administration of the lowest dose, 2 μmol/kg, did not result in any significant change in the core temperature (F1,5 = 6.23, P > .05). Elaboration for the time course showed a rapid onset for the highest dose with a maximal effect at 60 min and duration of 2 to 4 h. The effect was generally weaker at the middle dose of clozapine, and there was no clear time course of action.

**Olanzapine.** The administration of olanzapine induced a dose-dependent decrease in core temperature. The effect was statistically significant for doses of 2, 8, and 32 μmol/kg (F1,5 = 13.57, P < .05; F1,5 = 46.13, P < .01; and F1,5 = 56.49, P < .01, respectively) in comparison with vehicle-treated controls (Fig. 1, bottom left). The time course shows a rapid onset of action for the highest dose with a maximal effect at 120 min and a duration of 4 to 8 h. A similar pattern of effect was seen with the lower doses, although the peak effect appeared to be somewhat delayed.

**Quetiapine.** There were no statistically significant effects for the dose factor of quetiapine (F1,5 < 6.61, P > .05 for all

![Fig. 1. Effects of clozapine, quetiapine, olanzapine, and risperidone on core temperature in the rat.](http://aspetjournals.org/)
doses of quetiapine; Fig. 1, top right). For the 4 μmol/kg dose, however, there was a statistically significant interaction between dose and time ($F_{1,26} = 3.36, P < .05$). Inspection of the graph indicates this was due to a slight increase in core temperature at this dose.

**Risperidone.** Risperidone produced a dose-dependent decrease in core temperature, and the effect was statistically significant for the 0.25, 1.0, and 4.0 μmol/kg doses ($F_{1,5} = 13.84, P < .05$; $F_{1,5} = 8.93, P < .05$; and $F_{1,5} = 6.79, P < .05$, respectively) in comparison with vehicle-treated controls (Fig. 1, bottom right). The time course shows a rapid onset for the highest dose with a maximal effect between 60 and 120 min and a duration of 4 to 8 h. A similar pattern, although less pronounced, was seen with the two lower doses.

**Effects of SCH-23,390 on Hypothermia Induced by Clozapine, Olanzapine, or Risperidone in Rat**

As expected, clozapine (8 μmol/kg), olanzapine (16 μmol/kg), and risperidone (4 μmol/kg) produced a statistically significant decrease in core temperature 60 min after injection in comparison with vehicle-treated controls. The decrease in core temperature was similar for all three compounds, and there was no statistically significant difference in effect among clozapine, olanzapine, and risperidone ($F_{2,15} = 0.63, P > .05$). The hypothermia induced by clozapine, but not that induced by risperidone or olanzapine, was antagonized by pretreatment with the DA D1 receptor antagonist SCH-23,390 (0.3 μmol/kg; Fig. 2).

The dose of SCH-23,390 was based on results from previous studies in this laboratory (see Introduction) and on dose-effect relationships for antagonism by SCH-23,390 of clozapine-induced hypothermia (data not shown).

There were no statistically significant effects of SCH-23,390 by itself on the core temperature in the dose range used here (0.04–0.6 μmol/kg; Table 1).

**Effects of Clozapine, Olanzapine, Quetiapine, or Risperidone on Hypothermia Induced by Quinpirole in Rat**

The selective DA D2-like receptor agonist quinpirole (1.6 μmol/kg) produced a statistically significant decrease in core temperature in comparison with vehicle-treated controls. This hypothermia was significantly antagonized by olanzapine (2.0 μmol/kg), risperidone (0.25 μmol/kg), and quetiapine (1.0 μmol/kg) but not by clozapine (2.0 μmol/kg; Fig. 3). As shown in the figure, at these doses, neither clozapine, olanzapine, nor risperidone has any effects alone on core temperature. It should be noted, however, that the antagonism of quinpirole-induced hypothermia was only partial and the core temperature in animals treated with the respective atypical antipsychotic and quinpirole still was significantly decreased in comparison with saline-treated controls.

**Discussion**

Clozapine, olanzapine, and risperidone all produced hypothermia under the present conditions. In relation to clinically effective doses (see, e.g., Owens and Risch, 1998; Parfitt, 1999), the effects of clozapine appear to be more specific than the effects obtained with olanzapine or risperidone, both of which affected the core temperature at relatively high doses. Thus, clozapine decreases core temperature at doses below clinically effective doses, whereas the opposite was true for the other two compounds. Needless to say, such comparisons over species are biased by differences in pharmacokinetics, relative importance of receptor populations, and functional coupling in the central nervous system, precluding more precise comparisons. Quetiapine, on the other hand, did not produce any hypothermia or in fact produced a slight hyperthermia. In a parallel study, quetiapine was administered i.p. Also, in this study, using the same dose range for quetiapine as for the s.c. route, quetiapine was ineffective (data not shown).

Based on the dose-effect relationships for the induction of
were apparently not disclosed for clozapine under the present conditions. Regarding clozapine, however, the intrinsic efficacy at DA D _2_ receptors should be weak, because clozapine is not an agonist at DA D _2_ autoreceptors in reserpine-treated rats (S.A., unpublished observations), which is a sensitive model to detect such properties (Andén, 1980).

Taken together, the present results demonstrate that clozapine displays DA D _1_ receptor agonist properties at doses at which no DA D _2_ receptor antagonism was detectable. Regarding olanzapine, risperidone, and quetiapine, the situation was the opposite; that is, all of these latter compounds displayed DA D _2_ receptor antagonist properties, and there was no evidence for a DA D _1_ receptor-mediated hypothermia, as was found for clozapine. Thus, these results present a double-dissociation for the effects of clozapine at DA D _1_ and D _2_ receptors, in comparison with the other atypical agents examined here. It is also interesting to note that the antisercretery effects of clozapine on gastric secretions are blocked by SCH-23,390 (Gavin, 1995). Furthermore, clozapine-induced c-fos expression in atrial natriuretic factor producing hypothalamic neurons is mimicked by SKF-38,392 and sensitive to blockade of DA D _1_ receptors by SCH-23,390 (Lim et al., 1999). These in vivo observations are in contrast to the effects of clozapine on the DA D _1_-induced increase in formation of cAMP in vitro, in which clozapine behaves as a DA D _1_ receptor agonist (Andersen and Braestrup, 1986).

The in vivo observations that clozapine displays DA D _1_ receptor agonist properties raise two critical questions. First, does clozapine differ in clinical efficacy or side effect profile from the potential atypical agents examined here? Second, is there any supportive evidence that DA D _1_ receptor agonism should be of importance in the clinical efficacy of an atypical antipsychotic drug? Regarding the first question, there are several recent reviews on this topic comparing atypical antipsychotic agents with regard to elevation of plasma prolactin levels (Hamner and Arana, 1998; Petty, 1999), extrapyramidal side effects (Barnes and McPhillips, 1998), and effects on cognitive functions (Meltzer and McGurk, 1999). Although the message from these reviews is that risperidone, olanzapine, and quetiapine rate favorably with classic antipsychotics in clinically significant respects, each of these compounds still differs from clozapine in one or more important ways. With regard to the second question, the favorable profile of clozapine on cognitive functioning in schizophrenia is of particular interest (Meltzer and McGurk, 1999). Thus, there is strong supportive evidence for a role of DA D _1_ receptors in cognitive functions and that a stimulation of these receptors should be beneficial in the treatment of negative symptoms of schizophrenia. 1) DA D _1_ receptors are present in the prefrontal cortex (Mansour and Watson, 1995; Halldin et al., 1998), an area strongly implicated in symptoms of cognitive and emotional flattening in schizophrenia (Ingvar, 1987). 2) Stimulation of DA D _1_ receptors in the prefrontal cortex produces cognition-enhancing effects in primates (Lidow et al., 1998). 3) Nonmedicated schizophrenics have a lower density of DA D _1_ receptors in the prefrontal cortex (Okubo et al., 1997). 4) DA D _1_ receptor antagonists do not possess antipsychotic efficacy, and it appears that schizophrenic patients in fact worsen on treatment with such agents (Farde and Sedvall, 1995).

In addition to these observations, it should be noted that clozapine often is used to treat L-dopa-induced psychosis in hypothermia, as presented in Fig. 1, equipotent doses of clozapine, olanzapine, and risperidone were selected. However, only the clozapine-induced hypothermia could be antagonized by pretreatment with the selective DA D _1_ receptor antagonist SCH-23,390. On the other hand, the hypothermia produced by quinpirole (see, e.g., Salmi et al., 1994) was at least partially antagonized by olanzapine, risperidone, and quetiapine but not by clozapine. Because higher doses of olanzapine or risperidone produce hypothermia, it is difficult to use higher doses to assess the maximal DA D _2_ receptor antagonist properties in the present model. It has also been suggested that clozapine and olanzapine are partial DA D _2_ receptor agonists (Jackson et al., 1998; Ninan and Kulkarni, 1998, 1999). Such properties could explain the partial antagonism by olanzapine of quinpirole-induced hypothermia but...
Parkinson’s disease (Auzou et al., 1996) without worsening extrapyramidal symptoms. The fact that selective DA D1 receptor agonists display antiparkinsonian effects in animal models and in humans (Blanchet et al., 1998; Pearce et al., 1999; Rascol et al., 1999) could in part explain the use of clozapine in this situation. It is also interesting to note that highly selective DA D2 receptor antagonist substituted benzamides, such as sulpiride and remoxipride, display an atypical profile as antipsychotic agents (Tamminga and Gerlach, 1987; Lewander et al., 1990). This is possibly due to the fact that this treatment leaves the DA D1 receptor untouched or even indirectly enhances activity at this receptor. Finally, laboratory observations strongly implicate the DA D1 receptor in mechanisms of motivation and reward (Beninger and Miller, 1998). In the consideration of clozapine as a DA D1 receptor-stimulating agent, rewarding properties could contribute to the activating and socializing effects of clozapine in schizophrenia (Meltzer, 1995).

In a recent study, it was unexpectedly noted that d-amphetamine-induced locomotor stimulation in rats was enhanced by the addition of low, but not high, doses of the DA D2 receptor antagonist raclopride or the DA D1 receptor antagonist SCH-23390 (Salimi et al., 1998). Thus, it appears that at increased DA receptor stimulation, the two DA receptors mutually inhibit each other, in contrast to the enabling effects found in preparations with a decreased DA receptor tonus (as, for example, in reserpine-treated animals; see Waddington, 1989). Inasmuch as positive symptoms of schizophrenia are related to enhanced activation of DA D2 receptors, DA D1 receptor stimulation should be beneficial in two respects. Thus, apart from being favorable in its own right, DA D1 receptor stimulation should antagonize symptoms resulting from excessive activation of DA D2 receptors.

In general support for a role of prefrontal DA in the mechanism of action of atypical antipsychotic agents, it has been shown that clozapine, as well as olanzapine and risperidone, produces a relatively greater increase in DA release in the prefrontal cortex than in the neostriatum of the rat or monkey (Hertel et al., 1996; Li et al., 1996; Kuroki et al., 1999; Youngren et al., 1999). This effect is in all probability mediated via blockade of 5-HT2A receptors, because a similar effect is obtained with the 5-HT2A and 5-HT2A/C receptor antagonists M100907 and ritanserin, respectively (Schmidt and Fadayel, 1995; Pehek, 1996). However, because neither of these latter compounds have proved to be effective in schizophrenia, such an effect by itself may not be sufficient for clinical efficacy, although in combination with effects at other receptors, such as DA D2 or DA D3, for example, this may be an advantage (Wadenberg et al., 1996; Hertel et al., 1999).

Poikilothermia is a well known and hazardous side effect of classic antipsychotic drugs, particularly the phenothiazines (Zelman and Guillman, 1970; Yehuda, 1977). In a more recent report, Heh et al. (1988) hypothesized a specific mechanism for the hypothermia encountered under normal conditions and suggested temperature measurements as a surrogate marker for the time-dependent emergence of clinical efficacy. Interestingly, these investigators also found clozapine more effective than haloperidol in producing hypothermia. Present results suggest the possibility that the association between hypothermia and clinical efficacy reported for clozapine by Heh et al. (1988) may in fact be related to agonist activity at DA D1 receptors. Thus, it is an interesting possibility that temperature measurements could be of use as a surrogate marker for antipsychotic efficacy in a manner specific for clozapine.

Clozapine displays affinity for a range of neurotransmitters, in many cases with unknown intrinsic efficacy. It is not likely that the affinity, and the significant intrinsic efficacy, of clozapine at DA D1 receptors should be the only factor responsible for its properties as an atypical antipsychotic agent, although it could be a distinguishing feature in its mechanism of action. The possible role for adrenergic, muscarinic, and serotoninergic activities, as well as additional mechanisms, of clozapine is extensively reviewed elsewhere (Owens and Risch, 1998). To mention but one example, which is related to work in our laboratory, the affinity and intrinsic efficacy of clozapine at 5-HT1A receptors (Mason and Reynolds, 1992; Rollema et al., 1997) could be one such factor (cf. Ahlenius, 1989).

**Conclusions.** The present results, obtained with the use of core temperature measurements in the rat, demonstrate that clozapine preferentially stimulates DA D1 receptors in comparison with olanzapine, risperidone, and quetiapine. In the same model, olanzapine, risperidone, and quetiapine preferentially block DA D2 receptors in comparison with clozapine. It is suggested that stimulation of DA D1 receptors, presumably in the prefrontal cortex, is responsible for the favorable profile of clozapine on cognitive functionin schizophrenia.

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