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ORIGINAL INVESTIGATION

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Clozapine acts as a 5-HT_2 antagonist by attenuating DOI-induced inhibition of male rat sexual behaviour

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Abstract Evidence has been reported that clozapine may derive part of its therapeutic effects in treatment-resistant schizophrenic patients by interacting with the serotonin system. Among the few behavioural models available to test the hypothesis of an interaction of clozapine with 5-HT_2 receptors, male rat sexual behaviour is particularly useful, since in this behaviour 5-HT_{1A} and 5-HT_2 receptors have opposite functions. Stimulation of 5-HT_{1A} receptors facilitates ejaculatory behaviour and stimulation of 5-HT_2 receptors inhibit ejaculation. In the present study, male rat sexual behaviour was depressed by treatment with DOI (1.0 mg/kg), a selective 5-HT_2 receptor agonist. The depressive effect of DOI was attenuated by the administration of clozapine (0.1–1.0 mg/kg) in doses that by themselves did not significantly affect sexual behaviour. It was concluded that clozapine in the male rat sexual behaviour model may be interpreted as serving as a 5-HT_2 antagonist.

Key words Clozapine · DOI · Serotonin receptors · Male rat sexual behaviour

Introduction

Among the various antipsychotic agents used, clozapine is one of the oldest, yet one of the most potent drugs in treatment-resistant schizophrenic patients (see Meltzer 1989; Stephens 1990). However, the mechanism of action of this drug has remained elusive (Coward 1992). Clozapine has been reported to interact with several different neurotransmitters. Clozapine has a high affinity for 5-HT_2 receptors in vitro (Leysen 1990) and recent studies showed that clozapine has an even higher affinity for 5-HT_{1C} receptors than for 5-HT_2 receptors (Canton et al. 1990). Like conventional neuroleptics, such as chlorpromazine and haloperidol, clozapine lowers the locomotor activity of rats, inhibits conditioned avoidance responding, and produces drowsiness (Coward et al. 1989).

Suggestive evidence that clozapine may serve as a serotonin antagonist was reported by Fink et al. (1984), who found that infusion of clozapine into the median raphe nucleus suppressed the hypermotility induced by LSD following simultaneous injection of this substance into the brain. Further indications that clozapine may interact functionally with the serotonin system, and particularly with 5-$HT_{2/1C}$ receptors, are that clozapine, like the 5-HT_2 antagonists ketanserin, pirenperone and ritanserin, blocks the hyperthermia and the increase in serum corticosterone secretion elicited by MK-212 in a dose dependent manner (Gudelsky et al. 1986; Koenig et al. 1987; Nash et al. 1988).

A major difficulty in analysing the functional importance of the various 5-HT receptors is the scarcity of behavioural models available. A suitable animal model for studying the action of the serotonin system in behaviour is provided by male rat sexual behaviour. A considerable amount of evidence indicates that central 5-HT is involved as a neurotransmitter in the neutral mechanisms controlling this behaviour (Ahlenius and Larsson 1991). It was originally thought that brain 5-HT exclusively had an inhibitory function in this behaviour (Malmnäs and Meyerson 1971). Following the discovery of different subclasses of 5-HT receptors, this view of a general inhibitory function of 5-HT in sexual behaviour was modified (Mendelson and Gorzalka 1985). Specifically, two subclasses of receptors, 5-H_{1A} and 5-HT_2 receptors, appear to be of critical importance, exerting opposite functions in male rat sexual behaviour. Stimulation of the 5-HT_{1A} receptors by the selective 5-HT_{1A} agonist 8-OH-DPAT facilitates

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the expression of this behaviour and this effect is antagonized by (-) pindolol, a 5-HT_{1A} antagonist/beta-adrenergic antagonist (Ahlenius and Larsson 1988). On the other hand, the administration of the 5-HT_{1C}/5-HT_2 agonist, DOI suppressed male rat sexual behaviour, and this effect was blocked by selective 5-HT_2 antagonists, such as amperozide, ritanserine or ketanserin (Klint et al. 1992). To the extent that DOI-induced inhibition of copulation in the male rat can be related to activity at 5-HT_2 receptors, studying the efficacy of agents in reversing this inhibition may provide a behavioural bioassay for 5-HT_2 activity.

The present study was designed to use male rat sexual behaviour as a functional model to explore the effects of an interaction of clozapine with 5-$HT_{2/1C}$ receptors.

Materials and methods

Animals

The animals used in this study were sexually experienced adult male and female rats of the Wistar strain (Mol:Wist) obtained from Möllegaard Breeding Laboratories (Vejle, Denmark). The animals were maintained under standard laboratory conditions (22°C; humidity 50–60%) in cages containing four or five animals. The normal light-dark cycle was reversed (light on between 2200 and 1000 hours). Food (R 3 Ewos, Södertälje) and tap water were available in the home cage ad libitum. The animals arrived at the laboratory 2 weeks before the start of the experiments. The male rats were pre-tested three times before the start of treatments, and these animals which ejaculated in each of these tests were included in the experiments.

Drugs

The following compounds were used: (±)2,5-dimethoxy-4-iodophenyl) 2-aminopropane HCL (DOI) (purchased from Research Biochemicals), and clozapine (generously supplied by Sandoz). DOI was solved in 0.9% saline and injected SC −20 min. Clozapine was dissolved in a few drops of glacial acid with 5.5% glucose added to final volume and injected SC -30 min, both drugs in a volume of 2.0 ml/kg.

Behavioural testing procedure

Male rats were presented with an ovariectomized female brought into estrus by sequential treatment with estradiol benzoate (25 µg/rat SC in propylene glycol at −30 and −54 h) and progesterone (1.0 mg/rat SC in sesame oil at −6 h). Male rats were then used repeatedly with at least 2 days between successive drug treatments. The following components of masculine sexual behaviour were recorded: *Mount* (number of mounts without penile intromission), *Intromission* (number of mounts with penile intromission), *Intromission latency* (time from the presentation of the female to the first intromission, *Ejaculation latency* (time from the first intromission until ejaculation), *Postejaculatory interval* (time from ejaculation until the following intromission). The observations were terminated (a) when no intromission occurred within 15 min after the presentation of the female (these animals were excluded in the data analysis); (b) if the male did not ejaculate within 30 min from the first intromission (these animals were assigned an ejaculation latency of > 30 min in the statistical analysis); (c) at the first intromission following ejaculation; (d) 15 min after ejaculation if no intromission had occurred at this time (these animals were assigned a postejaculatory interval of > 15 min). In order to measure the efficiency of the copulatory attempts, a derived measure was calculated, called copulatory efficiency. This coefficient was calculated in the following way: Copulatory efficiency = Intromissions/Intromissions + Mounts.

Experimental design and statistics

A change-over design was used, in which the animals served as their own controls (Li 1964). Statistical evaluation was done with the Friedman two-way analysis of variance followed by the Wilcoxon matched-pairs, signed-ranks test on the basis of the performances of those animals that showed intromission behaviour. The proportion of responsive animals was analysed by the Cochran Q test and the McNemar test for significance of changes (Siegel 1956).

Results

The administration of clozapine was followed by a reduction of the number of intromissions to ejaculation without any statistically confirmed changes in the ejaculation latency (Table 1).The number of intromissions to ejaculation was already evident at the dosage of 0.1 mg/kg clozapine. Treatment with clozapine only caused a non-significant impairment of the sexual

Table 1 Effects of clozapine on male rat sexual beaviour. Clozapine was administrated SC 30 min before the observations were started. The animals (n = 24) served as their own controls in a change-over design. The results are presented as medians ± semi-interquartile range, based on the performance of the active animals: Statistical analysis was performed by the Friedman two-way analysis of variance followed by the Wilcoxon T test (two-tailed), and the Cohran Q test followed by the McNemar test for the significance of changes (% of males ejaculating). Comparison against saline-injected controls; $P < 0.05$*, $P < 0.01$**, $P < 0.001$***

	Intromission latency (min)	Mounts	Intromissions	Ejaculatory latency (min)	Post-ejaculatory interval (min)	Copulatory efficiency	% of males ejaculating
Saline	0.12 ± 0.14	3 ± 2	9 ± 3	2.70 ± 1.36	4.70 ± 0.68	0.76 ± 0.13	100
Clozapine 0.1 mg/kg	0.18 ± 0.12	3 ± 3	7 ± 3**	3.80 ± 1.36	5.25 ± 0.68	0.74 ± 0.09	100
Clozapine 0.3 mg/kg	0.05 ± 0.06	6 ± 3	8 ± 2	4.26 ± 1.36	5.32 ± 0.87	0.65 ± 0.14	88
Clozapine 1.0 mg/kg	0.36 ± 0.66	3 ± 3	3 ± 3*	3.85 ± 2.02	5.28 ± 0.66	0.62 ± 0.21	8

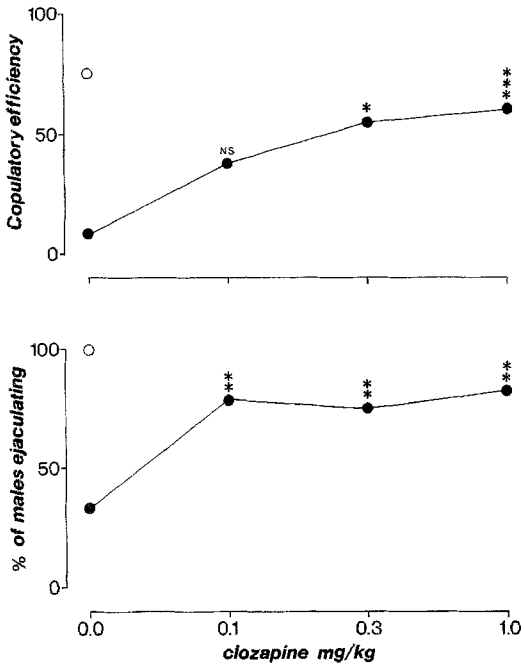

Fig. 1 The antagonist effects of clozapine on DOI-induced depression of sexual behaviour, as evidenced by median copulatory efficiency and the percentage of males ejaculating. *O* denotes saline treated controls. Procedure and statistical evaluation were made according to the legend in Table 1. $P < 0.05$*, $P < 0.01$**, $P < 0.001$***

behaviour as evidenced by the failure of three males to ejaculate after the administration of either 0.3 or 1.0 mg/kg clozapine.

While clozapine by itself in the doses administered had only minor effects on sexual behaviour, the administration of DOI (1 mg/kg) resulted in a marked reduction of sexual activity. Of the 24 participating animals in this study, 11 DOI-treated males showed mounts or intromissions and 8 ejaculated (saline versus DOI $\chi^2 = 14.6$, $P < 0.001$). Animals still ejaculating showed a higher number of mounts and a reduction of the number of intromissions compared to the saline treated males. As a result, the copulatory efficiency was markedly decreased. Furthermore, the intromission latency was significantly prolonged while the ejaculation latency and the postejaculatory intervals showed a tendency only to be prolonged.

Treatment with clozapine antagonized the suppressive influence of DOI. The number of ejaculating animals was increased significantly even in the 0.1 mg/kg group ($\chi^2 = 9.09$, $P < 0.01$) and differed little from that of the saline treated controls ($\chi^2 = 3.20$, NS). An increase of the dosage to 0.3 or 1.0 mg/kg did not essentially alter this picture (DOI versus DOI + 0.3 mg/kg clozapine, $\chi^2 = 6.67$, $P < 0.01$; DOI versus 1.0 mg/kg clozapine, $\chi^2 = 10.28$, $P < 0.01$).

Besides restoring the number of ejaculating animals close to normal levels, the treatment with clozapine altered the behaviour exhibited by the DOI treated animals in several aspects. The number of intromissions preceding ejaculation was significantly increased (from a median of 3 ± 1 to 6 ± 3; $P < 0.001$) and the copulation coefficient was increased compared to the DOI treated males.

Discussion

The suppression of male rat sexual behaviour observed by treatment with DOI confirms previous findings by Foreman et al. (1989) and ourselves (Klint et al. 1992). The prolongation of the intromission and ejaculation latencies, the marked decrease in copulatory efficiency, and the reduction of the number of animals achieving ejaculation following treatment with this compound are all effects testifying to the inhibitory effects upon sexual behaviour. DOI is a selective 5-HT_2 agonist and the suppressive effect of DOI suggests an inhibitory role of 5-HT_2 receptors in male rat sexual behaviour.

The main finding of the present experiments was that treatment with clozapine counteracted the suppressive effect of DOI by increasing the number of ejaculating animals to normal levels and partly normalized the mating pattern. This effect suggests an interaction of clozapine with 5-HT_2 receptors, preventing them from being activated by DOI. Clozapine thereby behaves as other 5-$HT_{2/1C}$ antagonists, including amperozide, ritanserin, ketanserin, mesulergine and pirenperone (Watson and Gorzalka 1991; Klint et al. 1992).

Treament with clozapine only had a weak depressive effect upon sexual behaviour, causing significant changes in several components. These effects were particularly marked at the highest dose levels. It may seem paradoxical that clozapine produces effects similar to those as DOI, but it should be noted that the profile of effects induced by DOI and clozapine were not identical. The copulatory efficiency of the DOI treated animals was greatly reduced compared to the saline treated controls while the clozapine treated animals did not show any such changes in their behaviour. Furthermore, unlike DOI, clozapine did not lower the proportion of ejaculating animals.

Combined treatment of DOI and clozapine did not fully restore normal sexual behaviour. For example, the low copulatory efficiency of the DOI treated animals was significantly elevated by combined treatment with clozapine, although still remaining lowered compared to the saline treated controls. Yet, clozapine behaves as a 5-HT_2 antagonist in preventing DOI from blocking sexual activity. It is therefore concluded that clozapine in the male rat sexual behaviour model acts as a functional 5-HT_2 antagonist.

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