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The serotonin 5-HT_{2A} receptor subtype does not mediate apomorphine-induced aggressive behaviour in male Wistar rats

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Abstract

We have studied the effect of the 5-HT_{2A} receptor antagonists on apomorphine-induced aggressive behaviour in male Wistar rats. In acute behavioural experiments with apomorphine-pretreated (1.0 mg/kg, s.c., once daily, 2 weeks) animals, risperidone (0.5 and 1.0 mg/kg) inhibited aggressive behaviour, but ketanserin and ritanserin (0.5–5.0 mg/kg) had no effect on the latency and intensity of aggressive behaviour. Concomitant risperidone (0.5 mg/kg) and haloperidol (0.03 and 0.3 mg/kg) administration blocked aggressive behaviour completely. In conclusion, our experiments confirm that inhibition of the apomorphine-induced aggressive behaviour is elicited by drugs with dopamine (DA) but not with 5-HT_{2A} antagonistic activity. Moreover, it may be concluded that the serotonin 5-HT_{2A} receptor subtype does not alter the DA-mediated behaviour.

Keywords: Apomorphine-induced aggressive behaviour; Ketanserin; Ritanserin; DOI; Rats

Aggressive behaviour of animals can be influenced by a variety of chemicals acting upon different systems in the central nervous system. There are several data indicating that the serotonergic system is linked to aggressive behaviour in animals [10,12,13,20] and humans (for a review, see Ref. [7]). Serotonin (5-HT) seems to play an important role in the regulation of aggressive behaviour. The action of serotonergic compounds has been studied in various animal models of aggressive behaviour [16,17]. Drugs with 5-HT_1 receptor-agonistic properties have been found to antagonize aggressive behaviour. Buspirone, 5-HT_{1A} receptor partial agonist and anxiolytic, suppresses the dominant behaviour in rats [19] and the territorial aggression in single-housed mice [14]. Specific serotonergic 5-HT_{1A} agonist, 8-OH-DPAT, and selective serotonin 5-HT_1 agonist, eltoprazine, both exert antiaggressive effect in the dominance and maternal aggression paradigm in rats [11]. The antiaggressive action of drugs, acting on 5-HT_2 and 5-HT_3 serotonin receptor, have been characterized by different authors as well. It has been found that the 5-HT_2 agonist, DOI, has an antiaggressive effect but only at high doses, whereas the 5-HT_2 antagonist, ritanserin, and the 5-HT_3 antagonist, ondansetron, are ineffective on isolation-induced aggression [12,16,18,21].

Serotonergic neurotransmission has profound effects on dopamine (DA)-mediated behaviours. 5-HT can modulate effects of DA in mammalian forebrain, but the interactions are complex and not fully understood. Findings of both enhanced and decreased DA release associated with increased availability of 5-HT have been reported [2–4].

Repeated treatment with low doses of an unselective DA receptor agonist, apomorphine (0.5 mg/kg, s.c., twice or 1.0 mg/kg, s.c., once daily), induces irritable aggression consisting of defensive upright postures, vocalization, and biting attacks in pairs of responsive rats. This behaviour is effectively antagonized by neuroleptics, D_2 receptor blockers, morphine, NMDA receptor antagonists, and intensified by dopaminergic agonists [1,6,15]. The apomorphine-induced aggressiveness has been proposed to be an equivalent to human pathology of aggressive or psychotic behaviour and the apomorphine-induced aggressiveness test may be classified as a ‘‘pathological’’ method of aggressive behaviour in rats [1,6].

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The development of compounds that are selective for one or more of the several 5-HT receptor subtypes may create the opportunity to determine which of the 5-HT receptor subtypes has an influence on DA-mediated behaviours.

The aim of the present study was to investigate the effects of ritanserin, ketanserin (both are 5-HT_{2A}/5-HT_{2C} receptor blockers), 5-HT_{2A}/D_2 antagonist, risperidone, and co-administration of the last compound with DOI (potent and selective 5-HT_{2A}/5-HT_{2C} receptor agonist) and haloperidol on apomorphine-induced aggressiveness in male Wistar rats.

1. Materials and methods

1.1. Ethics

The experimental protocol of the present study was approved by The Ethics Committee of the University of Tartu.

1.2. Animals

Adult male Wistar rats weighing 250–385 g were obtained from Kuopio National Animal Center, Kuopio, Finland. They were housed one per cage under standard laboratory conditions. The animal room had controlled temperature (20 ± 2°C) and light/dark cycle (lights on from 8:00 a.m. to 8:00 p.m.).

1.3. General procedure

The apomorphine-induced aggressiveness was studied as reported previously in our earlier experiments [9,18]. The standard polycarbonate, semitransparent cages were placed into the stainless steel racks. The animals were fed with a standard laboratory food used at our laboratory (Labfor R70, Lactamin, Stockholm, Sweden). For bedding, the aspen chips $4 \times 4 \times 1$ mm^3 (Tapvei, Kortteinen, Finland) were used. All animals of the present study were housed in the same room. The animals were housed singly and on the next day, the apomorphine treatment was started. After an injection, the animals were either tested for aggressiveness as described below or returned to the home cage. The same animal pairs were used throughout the study, while the animals pairs were always picked from the neighbour cages and subjected to the same drug treatment. Thus, the animals could hear and smell the opponent also during the housing, but due to the semitransparent cage walls, the visual contact was restricted. The apomorphine treatment lasted for 15 days during which the aggressiveness was scored four times (on the first, third, sixth, ninth and twelfth days); thereafter, unaggressive animals (these rats whose behaviour did not become aggressive during 15 days) were excepted from further experiment. Similarly to our previous study, the percentage of aggressive animals was about 80%. Studies of the drug effect on apomorphine-induced aggressiveness were started from 15th day on.

1.4. Measurement of aggressive behaviour

Aggressive behaviour was measured in specially designed cages with transparent plastic sidewalls ($35 \times 35 \times 55$ cm, length × width × height) and stainless steel floor, covered with aspen chips. Immediately after s.c. injection of apomorphine, the animals were put pairwise to the test cage and observed for [1] the time of the latency (the time before the first attack or the first aggressive posture) and [2] the intensity of aggressive behaviour. The animals were observed for 15 min and the rating of aggressive behaviour was scored on the 0–3 point scale (adapted from Ref. [1]):

0 — no aggressive manifestations;
1 — intermittent mild aggressive posture or attack toward other rat, no vocalizations;
2 — intermittent intensive upright aggressive posture or attack or boxing with other rat, vocalizations, but no biting or continuous fighting;
3 — continuous fighting or attempts to bite the opponent rat, loud vocalizations.

In the case of the development of the highest score of aggressive behaviour, the test was terminated to avoid injuries.

1.5. Drugs and drug administration

The following drugs were used (doses in parentheses): apomorphine hydrochloride (Reakhim, Krasnoyarsk, Russia), ketanserin tartrate, ritanserin, risperidone, haloperidol (all as pure substances), and DOI ((±)-2,5 dimethoxy-4-iodoamphetamine hydrochloride; RBI Chemicals, Natick, MA, USA). Apomorphine was dissolved in distilled water containing 0.01% L-ascorbic acid and stored as stock solution at +4°C (10.0 mg/ml). Just before the test, the apomorphine stock solution was diluted with distilled water to 1.0 mg/ml and injected s.c. in a dose of 1.0 mg/kg. Ketanserin (2.5 and 5 mg/kg), ritanserin (0.5, 1.0, 2.5, and 5.0 mg/kg), risperidone (0.05, 0.1, 0.5, and 1.0 mg/kg) and haloperidol (0.03 and 0.3 mg/kg) were suspended with a few drops of Tween-85® (polyoxyethylene-(20)-sorbitan oleate) and diluted with distilled water. Ketanserin (0.5 and 1.0 mg/kg) and DOI (0.3 and 3.0 mg/kg) were dissolved in distilled water. All drugs, except apomorphine, which was injected s.c., were injected i.p. 30 min before apomorphine treatment. In the case of co-administration of two drugs, they were injected into opposite parts of abdomen.

1.6. Statistics

For statistical analysis, the results from drug treatment experiments were subjected to one-way analysis of variance (ANOVA) and thereafter, for post-hoc data comparison,

Scheffe's test was used. Probability levels ($p < 0.05$) were always considered statistically significant. All data are expressed as means ± SEM.

2. Results

2.1. Development of apomorphine-induced aggressive behaviour

The development of aggressive behaviour was measured on the first, third, sixth, ninth, and twelfth days. The 1.0 mg/kg once daily apomorphine treatment evoked aggressive behaviour gradually, while on the 12th test day in 12 of 16 animal pairs, apomorphine-induced aggressive behaviour was observed (Fig. 1). On the first day of apomorphine treatment, none of the animals revealed to have any aggressiveness. On the third, sixth, ninth, and twelfth days, 4, 8, 15, and 24 animals, respectively, were aggressive. Whereas the remaining animals (in total, 32 animals were included into the study) did not exhibit any signs of aggressiveness. Further investigations were performed on 24 aggressive animals.

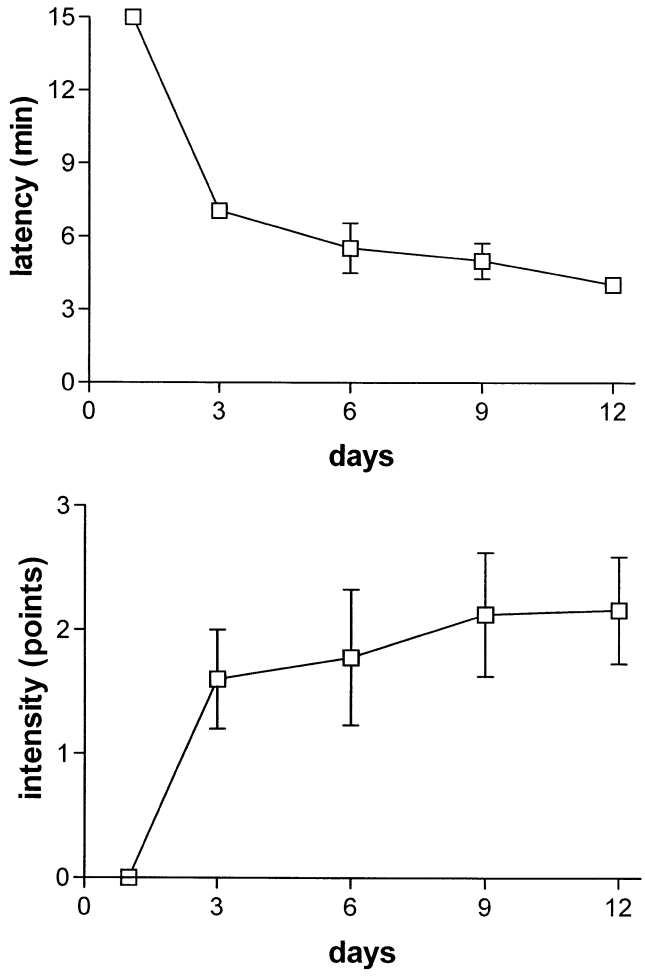

Fig. 1. Development of aggressive behaviour after repeated apomorphine treatment in male Wistar rats. Each point presents a mean (± SEM) of all animals ($N = 32$).

Table 1
The effect of ketanserin, ritanserin, and risperidone treatment on apomorphine-induced aggressive behaviour in rats

		Intensity of aggressive behaviour	Time of latency (min)
Drug (mg/kg)		APO + drug	APO + drug
Ketanserin			
Set I	0.0	2.12 ± 0.22	6.26 ± 0.87
	0.5	1.88 ± 0.12	7.00 ± 1.02
	1.0	2.00 ± 0.26	5.98 ± 0.74
Set II	0.0	2.25 ± 0.16	6.42 ± 0.88
	2.5	1.50 ± 0.32	9.46 ± 1.28
	5.0	1.75 ± 0.41	9.29 ± 1.38
Ritanserin			
Set I	0.0	1.87 ± 0.12	3.28 ± 0.42
	0.5	2.25 ± 0.16	3.10 ± 0.55
	1.0	1.62 ± 0.18	4.88 ± 0.64
Set II	0.0	1.85 ± 0.12	5.84 ± 0.29
	2.5	2.12 ± 0.12	4.48 ± 0.40
	5.0	2.37 ± 0.18	4.62 ± 0.58
Risperidone			
Set I	0.0	1.87 ± 0.12	6.17 ± 0.56
	0.05	1.62 ± 0.18	5.63 ± 0.20
	0.1	1.87 ± 0.12	6.30 ± 1.16
Set II	0.0	1.75 ± 0.16	3.67 ± 0.18
	0.5	$0.37 \pm 0.18^{\#\#\#}$	$14.27 \pm 0.72^{\#\#\#}$
	1.0	$0.25 \pm 0.16^{\#\#\#}$	$14.75 \pm 0.16^{\#\#\#}$

Means ± SEM values are presented from eight rats in each group. Significantly different values were detected by ANOVA followed by Scheffe's test.

$^{\#\#\#}$ $p < 0.001$, APO + drug vs. respective vehicle group. APO: apomorphine 1.0 mg/kg.

2.2. Effects of drugs acting at 5-HT_{2A} and D_2 receptors

Our experiments showed that ketanserin and ritanserin were without any statistically significant effect on the apomorphine-induced aggressiveness (Table 1). After acute drug treatment, the one-way ANOVA test revealed a significant drug treatment effect at the time of latency [$f(2,21) = 111.56$, $p < 0.001$] and intensity [$f(2,21) = 23.87$, $p < 0.001$] of aggressive behaviour after risperidone treatment. Subsequent Scheffe's test demonstrated a significant effect after 0.5 and 1.0 mg/kg risperidone treatment as compared with respective vehicle group. But after 0.05 and 0.1 mg/kg risperidone treatment, the one-way ANOVA test showed to have statistically no significant effect (Table 1).

In the case of concomitant treatment, ANOVA revealed a significant effect at the time of latency after risperidone 0.5 mg/kg + DOI 0.3 mg/kg, risperidone 0.5 mg/kg + DOI 3.0 mg/kg [$f(2,21) = 36.49$, $p < 0.001$], and risperidone 0.5 mg/kg + haloperidol 0.03 mg/kg, risperidone 0.5 mg/kg + haloperidol 0.3 mg/kg [$f(2,21) = 20.58$, $p < 0.001$] treatment. In the case of effect of concomitant drug treatment on the

Table 2
The effect of risperidone and DOI, risperidone, and haloperidol concomitant treatment on apomorphine-induced aggressive behaviour in rats

	Intensity of aggressive behaviour	Time of latency (min)
Drug (mg/kg)	APO + drug	APO + drug
Vehicle	1.75 ± 0.16	3.69 ± 0.39
Risperidone 0.5 + DOI 0.3	0.50 ± 0.32$^{\#\#}$	13.43 ± 1.02$^{\#\#\#}$
Risperidone 0.5 + DOI 3.0	0.37 ± 0.26$^{\#\#}$	13.24 ± 0.1.15$^{\#\#\#}$
Vehicle	1.37 ± 0.32	6.36 ± 1.90
Risperidone 0.5 + haloperidol 0.03	0.00 ± 0.00$^{\#\#\#}$	15.00 ± 0.00$^{\#\#\#}$
Risperidone 0.5 + haloperidol 0.3	0.00 ± 0.00$^{\#\#\#}$	15.00 ± 0.00$^{\#\#\#}$

Means ± SEM values are presented from eight rats in each group. Significantly different values were detected by ANOVA followed by Scheffe's test.

$^{\#\#}$ $p < 0.01$.

$^{\#\#\#}$ $p < 0.001$, APO + drug vs. respective vehicle group. APO: apomorphine 1.0 mg/kg.

intensity of aggressive behaviour, the probability levels were [$p < 0.01$, $F(2,21) = 8.54$] and [$p < 0.001$, $F(2,21) = 18.02$], respectively (Table 2).

3. Discussion

In our study, the repeated (2 weeks) administration of apomorphine gradually induced aggressive behaviour as evidenced by the step-by-step increased aggressiveness and shortened latency. First signs of aggressive behaviour were observed already on the third day of apomorphine administration. Similarly, the time of latency before the first attack shortened day-by-day, indicating the reliability of the test design. Furthermore, as found in our previous works [9,18], the ingroup deviation of the aggressiveness was quite high between the days 3 and 9. This ingroup deviation disappeared at the end of 2 weeks of apomorphine administration (as it can be seen from the data of day 12 and the following vehicle plus apomorphine groups), thereby providing additional evidence on the trustworthy of our study.

There has been considerable research in the field of identification of antiaggressive drugs over the past few years with emphasis on the discovery of better drugs, particularly those with 5-HT_2 antagonist activity [5,16,17,20].

In the present test, neither ketanserin nor ritanserin, in itself, had an antiaggressive effect in apomorphine-induced aggressiveness. Thus, as demonstrated in our experiments, none of the chosen doses (0.5, 1.0, 2.5, and 5.0 mg/kg) showed to have an antiaggressive effect. In contrast, risperidone in doses 0.5 and 1.0 mg/kg attenuated significantly the intensity of aggressive behaviour and prolonged the time before the first attack as well. Risperidone and DOI co-administration still elicited a significant decrease of aggressiveness and increased the time of latency as compared with the respective vehicle group. The expected effect of DOI in combination with risperidone would have been the antagonism at the 5-$HT_{2A/2C}$ receptors, but in the present experiment, no such effect was found. This finding indicates the involvement of dopamine D_2 antagonistic properties in the effect of risperidone. In the case of risperidone and haloperidol co-administration, already the minimal effective dose, 0.03 mg/kg, of haloperidol (whose dose was chosen on the basis of our earlier unpublished observation) potentiated the effect of risperidone and aggressive behaviour in rats was blocked completely. This additive effect of haloperidol and risperidone further confirms the idea that the D_2 receptor antagonism is the most important mechanism of action in the antiaggressive profile of risperidone. Nevertheless, since the repeated apomorphine treatment may induce disregulation of both pre- and postsynaptic DA receptors, it cannot be ruled out that the additive effect of risperidone and haloperidol might be, at least in part, associated with the nonspecific motor-suppressive properties of haloperidol.

As it has been demonstrated in our previous study, 5-$HT_{2A/2C}$ receptor agonist, DOI, had no effect on apomorphine-induced aggressiveness [18]. It has also been found that administration of ritanserin, a 5-$HT_{2A/2C}$ receptor antagonist, does not cause any significant change of DA and dihydroxyphenylacetic acid (DOPAC) outflow in the nucleus accumbens [8], neither has it any affinity for DA receptors. Furthermore, in our prior study, we found that 5-HT_{1A} agonist, buspirone, blocked the apomorphine-induced aggressiveness totally and thus, provided evidence that 5-HT_{1A} receptors may be involved in the mediation of the aggressive behaviour in rodents [9]. However, it cannot be ignored that apart from 5-HT_{1A} agonistic activity, buspirone has D_2 antagonistic property as well [5]. The antiaggressive effect of buspirone can be explained by its blocking activity on D_2 receptors. It could be supposed that risperidone blocked apomorphine-induced aggressiveness in the same manner. Risperidone and haloperidol have high affinity for dopamine D_{2A} and D_3 receptors. The last compound also has high affinity for α_2-adrenergic receptor and moderate affinity for the serotonin 5-HT_{1A} receptor, while risperidone possesses considerable affinity for the dopamine D_1 receptor [20]. Based on the affinity data, it can be proposed that risperidone and haloperidol inhibited apomorphine-induced aggressiveness by dopaminergic, but not serotonergic, mechanism.

4. Conclusion

Our experiments confirm that inhibition of the apomorphine-induced aggressive behaviour produced by drugs with DA but not with 5-HT_{2A} antagonistic activity. Moreover, it may be concluded that the serotonin 5-HT_{2A} receptor subtype does not alter the DA-mediated behaviour.

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