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Short communication

Reversal of a dizocilpine-induced disruption of prepulse inhibition of an acoustic startle response by the 5-HT_2 receptor antagonist ketanserin

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Abstract

Prepulse inhibition can be reliably disrupted by non-competitive NMDA receptor antagonists such as dizocilpine. In a recent study, we found that the potent D_2/5-HT_2 receptor antagonist, risperidone, but not the selective dopamine D_2 receptor antagonist, raclopride, could reverse this disruption. The present study was therefore designed to examine the effect of the 5-HT_2 receptor antagonist, ketanserin, against a dizocilpine-induced disruption of prepulse inhibition, as well as the behavioural stereotypy produced by this drug. Ketanserin (2 mg/kg) reversed the prepulse inhibition disruption produced by dizocilpine (0.15 mg/kg), as did the non-selective 5-HT_1/5-HT_2 receptor antagonist metergoline (1 mg/kg). Both drugs also attenuated some components of the behavioural stereotypy syndrome produced by dizocilpine (0.15 mg/kg). The present studies therefore suggest an interaction between 5-HT_2 receptors and glutamatergic systems. This may be important for the antipsychotic profile of drugs having antagonist activity at 5-HT_2 receptors.

Keywords: Prepulse inhibition, rat; 5-HT (5-hydroxytryptamine, serotonin); Glutamate; Antipsychotic; Schizophrenia

1. Introduction

Traditionally, a dysfunction of central mesolimbic dopaminergic systems has been associated with the aetiology of schizophrenia. However, more recently the contribution of other neurotransmitter systems, particularly the glutamatergic and serotonergic (5-HT, 5-hydroxytryptamine, serotonin), has been recognised. Prepulse inhibition of an acoustic startle reflex refers to the reduction in response to a startle eliciting stimulus produced by the prior presentation of a weaker intensity prepulse (Hoffman and Ison, 1980). Because prepulse inhibition is impaired in schizophrenic patients, and in rats may be disrupted by pharmacological manipulations to the dopamine mesolimbic system, this technique has become recognised as a useful means to study the aetiology of this disorder (Swerdlow et al., 1994).

Non-competitive receptor antagonists at the glutamatergic NMDA receptor, e.g. phencyclidine and dizocilpine, also disrupt prepulse inhibition (Mansbach and Geyer, 1989; Varty and Higgins, 1994). However, this disruption appears to be independent of dopamine systems, for both dopamine D_1- and D_2-like receptor antagonists are ineffective in reversing this effect (Keith et al., 1991; Hoffman et al., 1993; Wedzony et al., 1994). In a recent study (Varty and Higgins, 1995) we found that the mixed D_2/5-HT_2 receptor antagonist risperidone, produced a reliable antagonism of a dizocilpine impairment of prepulse inhibition. Thus, the purpose of the present investigation was to examine the effect of the selective 5-HT_2 receptor antagonist ketanserin, and the mixed 5-HT_1/5-HT_2 receptor antagonist metergoline, against dizocilpine-induced stereotypy (Tricklebank et al., 1989) and disruption of prepulse inhibition.

2. Materials and methods

2.1. Animals

Male Wistar rats (200–350 g) were used in all studies and allowed at least 7 days acclimatisation to the

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laboratory facility before behavioural testing. Rats were housed in groups of three, in a room controlled for constant temperature (22 ± 1°C) and humidity (50%) and allowed ad-libitum access to food and water. The lights on period was 07:00–19:00 h and all experiments were conducted between 09:00 and 17:00 h.

2.2. Prepulse inhibition experimental procedures

Prepulse inhibition studies were conducted in six identical startle chambers (SR-LAB system, San Diego Instruments, CA, USA; see Varty and Higgins, 1994, for details). The experimental session comprised of a 5 min acclimatisation period to a 70 dB background white noise (continuous throughout the session), followed by the 25 min test session. During the test session, four trial types were presented: (i) pulse alone, (ii) prepulse alone, (iii) prepulse-pulse and (iv) no stimulus. The pulse and prepulse intensities were 118 dB and 75 dB respectively, and the durations of each were 40 ms and 20 ms respectively. The interstimulus interval, the time between prepulse offset and pulse onset, was 100 ms. Each trial type was presented 20–40 s (average = 30 s) apart, in a random order, with 12 presentations of each, in addition to an initial single pulse alone trial that began the test session (data from this initial trial are not included in the analysis). For drug studies, the rats were used in no more than two startle experiments with a period of at least 5 days between each test.

2.3. Prepulse inhibition statistics

Startle data were analysed by two methods using a standard statistical package (GB-STAT, Dynamic Microsystems, MD, USA). Firstly, the mean startle amplitude data for each trial type were analysed by a three-way analysis of variance (pretreatment, treatment and trial type as factors), with trial type as the repeated measure. In the second analysis, the level of prepulse inhibition (% prepulse inhibition) was determined according to the formula (100 − [startle amplitude on prepulse-pulse trials/startle amplitude on pulse alone trials] × 100), which was then analysed by two-way analysis of variance (pretreatment and treatment as factors), following arc-sine transformation. The accepted level of significance in all cases was $P < 0.05$, and when appropriate, post-hoc comparisons between drug and vehicle groups were made using Tukey's protected t-test.

2.4. Locomotor activity and stereotypy experimental procedures

Eight Perspex activity chambers (31 × 19 × 18 cm: L × W × H; Glaxo Bioengineering, UK) were used to measure general locomotor activity. Activity was recorded by the animal interrupting infra-red beams positioned at two levels, 3 cm and 11 cm above floor level. Animals were placed into the chambers for a 30 min acclimatisation period and then following drug treatment, activity was measured over a 60 min test period, using 5 min time bins. Animal behaviour was also assessed at 10 min intervals during this test period, using the sampling method described by Fray et al. (1980). Behaviours measured were rearing, head weaving, body sway, ataxia, sniffing, grooming, forepaw treading and circling (each scored as either 1 = present, 0 = absent).

2.5. Locomotor activity and stereotypy statistics

Activity data, collected at each 5 min time bin, were analysed by three-way ANOVA, with pretreatment and treatment as between-subject factors, and time bin as the repeated measure. Additionally, total locomotor activity over the 60 min session was calculated and analysed by two-way analysis of variance (pretreatment and treatment as factors, see Table 1). Post-hoc comparisons were carried out using Tukey's protected t-test, with an accepted level of significance of $P < 0.05$. The cumulative behavioural scores for each animal during the 60 min observation period were totalled. Each behaviour was then analysed by determining the median and interquartile range for each treatment. Data were subsequently analysed by Kruskall-Wallis analysis of variance, and when appropriate, post-hoc comparisons between treatment and control groups were made using a Mann-Whitney U-test.

2.6. Drugs

The drugs used were dizocilpine maleate (Glaxo, Ware, UK), metergoline, ketanserin tartrate (both Semat, UK) and risperidone (Janssen Research Foundation, Belgium). All drugs were administered subcutaneously, except risperidone which was injected by the intraperitoneal route. Pretreatment times were: dizocilpine, 10 min; ketanserin and metergoline, 30 min; risperidone, 60 min. Doses are expressed as that of base.

3. Results

3.1. Prepulse inhibition

The dose of dizocilpine (0.15 mg/kg) used was chosen from previous studies (see Varty and Higgins, 1995), as being the minimal dose that produced a robust disruption of prepulse inhibition. In all three studies described, dizocilpine produced a highly signifi-

cant ($P < 0.01$) reduction of prepulse inhibition, which was reduced by over 80% (Fig. 1a). In two studies, dizocilpine produced a significant decrease in the amplitude of the pulse alone trial type ($P < 0.01$), whilst in the remaining study, dizocilpine produced a selective increase in the prepulse-pulse trial type ($P < 0.01$).

Risperidone (1 mg/kg, $n = 12$ per group) significantly reversed the dizocilpine-induced disruption of prepulse inhibition ($P < 0.01$, Fig. 1a(i)) indicated by significant main effects of risperidone ($F(1,44) = 8.7$, $P < 0.01$) and dizocilpine ($F(1,44) = 28.1$, $P < 0.01$) and a risperidone × dizocilpine interaction ($F(1,44) = 7.3$, $P < 0.01$). Trial type analysis revealed no main effects of either drug but a significant dizocilpine × trial type interaction ($F(3,132) = 9.1$, $P < 0.01$). This is likely due to the effect of dizocilpine to reduce the amplitude of the pulse alone trial type, an effect not reversed by risperidone pretreatment (Fig. 1b(i)).

Ketanserin (2 mg/kg, $n = 11$ per group) also reversed the dizocilpine effect ($P < 0.01$, Fig. 1a(ii)). There were significant main effects of ketanserin ($F(1,40) = 12.7$, $P < 0.01$) and dizocilpine ($F(1,40) = 35.5$, $P < 0.01$) but no interaction. Trial type analysis revealed no main effects of either drug but a significant dizocilpine × trial type interaction ($F(3,120) = 15.4$, $P < 0.01$). This reflected the decreased pulse alone am-

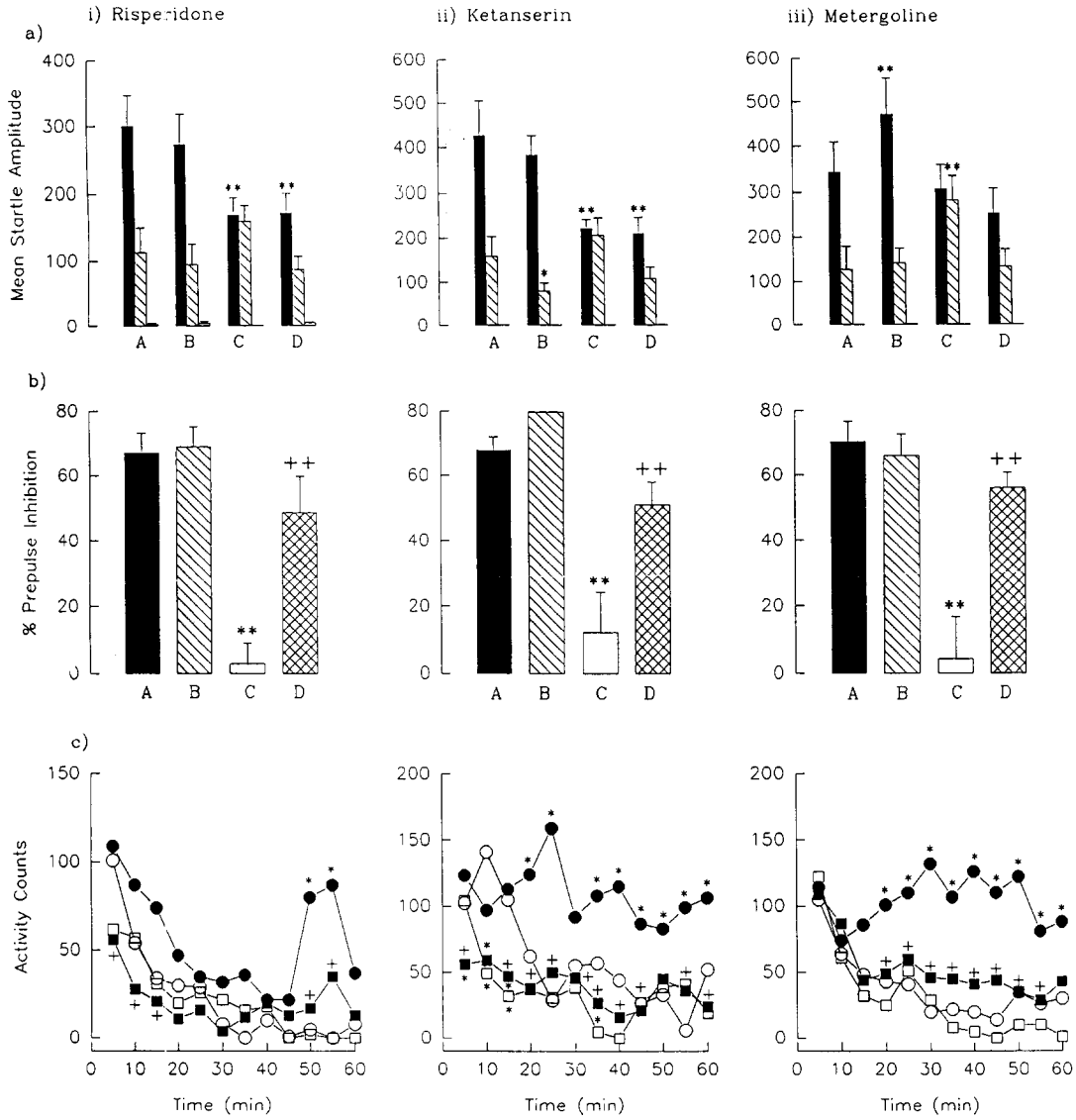

Fig. 1. Effect of (1) risperidone (1 mg/kg), (2) ketanserin (2 mg/kg) and (3) metergoline (1 mg/kg) pretreatment on the dizocilpine (0.15 mg/kg)-induced effects on (a) mean startle amplitude, (b) % prepulse inhibition and (c) locomotor activity. Graph (a): Black columns = pulse alone; hatched columns = prepulse-pulse; open columns = prepulse alone. Graph (a) and (b): A = vehicle/vehicle; B = antagonist/vehicle; C = vehicle/dizocilpine; D = antagonist/dizocilpine. Graph (c): ○ = vehicle/vehicle; □ = antagonist/vehicle; ● = vehicle/dizocilpine; ■ = antagonist/dizocilpine. * $P < 0.05$, ** $P < 0.01$ vs. vehicle/vehicle group; $^{+}$ $P < 0.05$, $^{++}$ $P < 0.01$ vs. vehicle/dizocilpine group. Values are mean ± S.E.M. Please note not all axes are to the same scale.

Table 1
Effect of risperidone (1 mg/kg), ketanserin (2 mg/kg) and metergoline (1 mg/kg) on dizocilpine (0.15 mg/kg)-induced stereotypy and hyperactivity

	Locomotor activity	Rearing		Head weaving		Body sway		Circling	
Vehicle	280 ± 62	0.5	(0–1)	0		0		0	
Risperidone	254 ± 81	0		0		0		0	
Dizocilpine	667 ± 128 [b]	1.5	(0.5–3)	4.5	(3.5–5) [b]	4	(3.5–4.5) [b]	2	(0.5–3) [b]
Risperidone + dizocilpine	244 ± 75 [d]	0	(0–0.5) [d]	1.5	(1–2.5) [b,d]	1.5	(0–2) [b,d]	1	(0–1) [a]
Vehicle	711 ± 117	1.5	(0.5–3)	0		0		0	
Ketanserin	421 ± 153	1	(0–2)	0		0		0	
Dizocilpine	1305 ± 190 [a]	3.5	(2–4)	3	(2–3) [b]	4.5	(3–5) [b]	2	(1–3) [b]
Ketanserin + dizocilpine	464 ± 122 [d]	3	(0.5–3)	0 [d]		2	(1–2.5) [b,d]	0	(0–0.5) [c]
Vehicle	468 ± 62	2	(0.5–3.5)	0		0		0	
Metergoline	354 ± 106	1	(0–1)	0		0		0	
Dizocilpine	1249 ± 382 [a]	3	(1–4.5)	2	(0.5–4) [b]	2	(1–3) [b]	2	(0.5–2) [b]
Metergoline + dizocilpine	631 ± 134	2	(0.5–2.5)	1	(0–1) [a]	1	(0–1.5) [a]	0 [c]	

All animals were behaviourally rated for 30 s at 10 min intervals for a 60 min period. Behaviour assessed as either present (score = 1) or absent (score = 0). Maximum score for a particular behaviour = 6. Locomotor activity was measured automatically and is presented as the cumulative score for the 60 min observation period. $n = 6$ rats per dose group. Data expressed as mean ± S.E.M (locomotor activity) or medians and interquartile range. [a] $P < 0.05$, [b] $P < 0.01$ vs. vehicle group; [c] $P < 0.05$, [d] $P < 0.01$ vs. dizocilpine group. Data for ataxia, forepaw treading, grooming and sniffing were not included as there was no clear effect of drug on these behaviours.

plitude following dizocilpine treatment (Fig. 1b(ii)). In a subsequent study ($n = 8$ per group), a lower dose of ketanserin (0.5 mg/kg) failed to reverse the dizocilpine disruption (prepulse inhibition data: vehicle = 59 ± 7%, ketanserin 0.5 mg/kg = 62 ± 10%, dizocilpine = 27 ± 10%, ketanserin + dizocilpine = 29 ± 10%).

Metergoline (1 mg/kg, $n = 12$ per group) reversed the dizocilpine-induced prepulse inhibition disruption ($P < 0.01$, Fig. 1a(iii)), indicated by significant main effects of metergoline ($F(1,44) = 6.5$, $P < 0.01$) and dizocilpine ($F(1,44) = 20.3$, $P < 0.01$) and a metergoline × dizocilpine interaction ($F(1,44) = 9.8$, $P < 0.01$). Trial type analysis revealed no main effects of either drug but a significant dizocilpine × trial type interaction ($F(3,132) = 5.9$, $P < 0.01$), which was due to the selective effect of this drug to increase the amplitude of the prepulse-pulse trial type (Fig. 1b(iii)).

3.2. Locomotor activity and stereotypy

In a preliminary study, dizocilpine (0.075–0.3 mg/kg, $n = 6$ per group) produced a dose-dependent increase in locomotor activity and overt behaviours, including head weaving, ataxia, body sway and circling (results not shown). Because the 0.15 mg/kg dose produced a reliable behavioural stereotypy, without ataxia, and was used in the prepulse inhibition experiments, this was selected for subsequent drug interaction studies.

Risperidone (1 mg/kg, $n = 6$ per group) significantly reversed dizocilpine-induced hyperactivity (see Table 1). Time-course analysis indicated significant main effects of risperidone ($F(1,20) = 6.2$, $P < 0.05$), dizocilpine ($F(1,20) = 4.4$, $P < 0.05$) and time ($F(11,220) = 6$, $P < 0.01$), and a significant risperidone × dizocilpine interaction ($F(1,20) = 4.9$, $P < 0.05$). Risperidone produced an initial decrease in locomotor activity compared to controls during the first 15 min of the test period, although the attenuation of the dizocilpine hyperactivity was evident throughout the 60 min test session.

Ketanserin (2 mg/kg, $n = 6$ per group) also reversed the dizocilpine-induced hyperactivity (see Table 1). Time-course analysis indicated significant main effects of ketanserin ($F(1,20) = 14$, $P < 0.01$), dizocilpine ($F(1,20) = 4.6$, $P < 0.05$) and time ($F(11,220) = 4.8$, $P < 0.01$). Thus dizocilpine tended to increase activity throughout the test session, an effect which was significantly attenuated by ketanserin pretreatment. Ketanserin alone produced a decreased activity compared to the vehicle group, although this was confined to the first 20 min of the test session.

Metergoline (1 mg/kg, $n = 6$ per group) pretreatment also reversed the dizocilpine-induced hyperactivity. Time-course analysis indicated significant main effects of dizocilpine ($F(1,20) = 6.3$, $P < 0.05$) and time ($F(11,220) = 5.5$, $P < 0.01$), but not metergoline. Dizocilpine-treated animals showed a robust hyperactivity from 20 min into the test session, an effect which was significantly attenuated by metergoline pretreatment.

Risperidone (1 mg/kg), ketanserin (2 mg/kg) and metergoline (1 mg/kg) each reduced the incidence of dizocilpine-induced head weaving and body sway, whilst ketanserin and metergoline also blocked circling behaviour (Table 1). Risperidone, ketanserin nor metergoline alone produced any overt behavioural changes (see Table 1).

4. Discussion

In this study, we have replicated the original finding described in a previous report (Varty and Higgins, 1995), that risperidone significantly reverses the effect of dizocilpine on prepulse inhibition of the acoustic startle response. This effect is most probably due to an interaction at 5-HT_2 receptors, for both the 5-HT_2 receptor antagonist ketanserin, and the 5-HT_1/5-HT_2 receptor antagonist metergoline, produced a similar effect. None of the antagonists tested had any effect on prepulse inhibition when tested alone, although metergoline produced an increase in startle responding under pulse alone trials, and ketanserin, a reduction in responding under the prepulse-pulse trial. Given this differential effect on startle, it seems unlikely that the reversal of a dizocilpine-induced prepulse inhibition disruption by each antagonist was the result of a non-specific effect on startle behaviour.

Further evidence to support an interaction between 5-HT_2 and glutamatergic systems came from the observations that risperidone, ketanserin and metergoline appeared to attenuate the locomotor hyperactivity and certain stereotyped behaviours, i.e. body sway and head weaving, which are commonly observed following dizocilpine pretreatment (Tricklebank et al., 1989). These findings are consistent with two recent studies which have reported risperidone to reverse the hyperactivity (Maurel-Remy et al., 1995) and stereotypy (Kitaichi et al., 1994) induced by phencyclidine.

Both risperidone and ketanserin have the highest affinity for the 5-HT_{2A} receptor subtype of the 5-HT_2 class (Baxter et al., 1995), making it a likely candidate for the observations described in the present report. Indeed it has recently been speculated that this subtype may be important for the antipsychotic profile of drugs having high 5-HT_2 receptor affinity (Schreiber et al., 1994; Schmidt et al., 1995). Furthermore, with relevance to the work described here, it has been shown that the ability of a range of atypical antipsychotics to reverse a phencyclidine-induced hyperactivity, correlates significantly to their affinity at the 5-HT_{2A} but not the D_2 receptor (Maurel-Remy et al., 1995). With the development of newer, more selective ligands (e.g. see Baxter et al., 1995), it should be possible to test this empirically in the prepulse inhibition model.

The present study demonstrates a novel interaction between drugs having 5-HT_2 receptor antagonist activity, with a dizocilpine-induced disruption of prepulse inhibition. The finding of a serotonin/glutamate interaction in the modulation of prepulse inhibition may have implications for the role of these neurotransmitters in the aetiology of schizophrenia. It may also indicate a mechanism by which drugs having 5-HT_2 receptor antagonist activity produce their antipsychotic effects (Schmidt et al., 1995).

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