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Pharmacology, Biochemistry and Behavior 75 (2003) 281–288
PHARMACOLOGY BIOCHEMISTRY AND BEHAVIOR
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Effect of the 5-HT$_6$ receptor antagonists Ro 04-6790 and Ro 65-7199 on latent inhibition and prepulse inhibition in the rat: comparison to clozapine

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Received 27 January 2003; received in revised form 26 March 2003; accepted 26 March 2003

Abstract

In the present study, we have investigated the effects of two selective 5-HT$_6$ receptor antagonists, Ro04-6790 and Ro65-7199, in three drug-induced models of PPI disruption and on latent inhibition (LI) utilizing a conditioned lick suppression (CLS) procedure. Clozapine was included in each experiment for comparison. Neither Ro04-6790 nor Ro65-7199 (both 30 mg/kg) affected the PPI disruption produced by PCP (1.5 mg/kg sc), apomorphine (0.1 mg/kg sc), or LSD (0.1 mg/kg sc). There was also no interaction between each drug and CS preexposure in the CLS test indicating a failure of each drug to facilitate LI. In contrast, clozapine (12 mg/kg) attenuated an apomorphine and PCP-induced PPI deficit, although the PPI disruption produced by LSD was not significantly affected. At a lower dose of 5 mg/kg, clozapine also facilitated LI. Since each of these tests bear some predictive validity for the detection of antipsychotic drugs, the present studies do not support a therapeutic potential of 5-HT$_6$ receptor antagonists in this regard.

Keywords: Latent inhibition; Prepulse inhibition; Rat; Conditioned lick suppression; Schizophrenia

1. Introduction

It is generally believed that the distinct clinical profile of the atypical antipsychotic drug class is largely attributable to high affinity antagonism of the 5-HT$_{2A}$ receptor and a more modest affinity for DA$_2$ receptors (Meltzer, 1999). Clozapine represents the prototype drug of this class, and a number of clinical studies have demonstrated its effective control of positive symptoms of psychosis, some are improvements against negative and cognitive signs, without induction of the extrapyramidal side effects so characteristic of the typical antipsychotic class (Kane et al., 1988; Meltzer, 1999). However, in addition to DA$_2$ and 5-HT$_{2A}$ receptors, clozapine has high affinity for a variety of other monoaminergic receptors, particularly serotonergic, including the 5-HT$_{1A}$, 5-HT$_{2C}$, 5-HT$_3$, 5-HT$_6$, and 5-HT$_7$ receptor subclasses (Meltzer and Nash, 1991; Roth et al., 1994).

Of these additional 5-HT receptors, the 5-HT$_6$ subclass warrants consideration as a contributory factor to the broad clinical profile of clozapine. Firstly, 5-HT$_6$ receptors are almost exclusively CNS localized, with highest density in brain areas considered important in antipsychotic drug action, i.e., nucleus accumbens, caudate putamen (Gerard et al., 1997; Roberts et al., 2002). Secondly, chronic 14-day treatment with clozapine has been reported to down-regulate hippocampal (but not striatal) 5-HT$_6$ mRNA expression in the rat (Frederick and Meador-Woodruff, 1999) suggesting a functional interaction between clozapine and the 5-HT$_6$ receptor. Thirdly, microdialysis studies report increases in frontal cortical extracellular glutamate levels following pretreatment with the selective 5-HT$_6$ antagonist SB271046 (Routledge et al., 2000; Dawson et al., 2001). This latter finding is of interest given the neurobiological evidence that schizophrenia is associated with hypofunction of the frontal lobes, particularly during cognitive challenge (Weinberger et al., 1986; Taylor, 1996; Lewis and Lieberman, 2000).

Consequently, in the present series of experiments, we have examined two selective 5-HT$_6$ receptor antagonists—Ro04-6790 (Sleight et al., 1998) and Ro65-7199 (Sleight et al., 1999; Bos et al., 2001)—in two tests widely used to detect and characterize putative antipsychotic drugs—pre-

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0091-3057/03/$ – see front matter © 2003 Elsevier Science Inc. All rights reserved.
doi:10.1016/S0091-3057(03)00082-0

pulse inhibition (PPI) and latent inhibition (LI). PPI is the normal decreased startle response to an intense acoustic stimulus (pulse) when this stimulus is immediately preceded by a weaker stimulus (prepulse) and represents an operational measure of sensorimotor gating (Graham, 1975; Braff et al., 1978; Hoffman and Ison, 1980). LI refers to the fact that prior preexposure to a nonreinforced stimulus leads to subsequent retardation of conditioning to that stimulus compared to non-preexposure in controls (Lubow, 1973, 1989). An advantage of adopting the LI paradigm in these studies is that a number of antipsychotic drugs appear to facilitate this phenomena; thus, eliminating a need to introduce a pharmacological challenge (Weiner and Feldon, 1987; Weiner et al., 1987; Feldon and Weiner, 1991; Dunn et al., 1993; for a review, see Moser et al., 2000). Because such drug-induced facilitation of PPI is not so widely described (see Depoortere et al., 1997), we tested each 5-HT_6 antagonist in three drug-induced models of PPI disruption: an LSD (likely 5-HT_{2A}r mediated; see Ouagazzal et al., 2001)-, apomorphine (likely DA_2r mediated; see Geyer et al., 2001)-, and a PCP (likely NMDAr mediated; see Mansbach and Geyer, 1989)-induced PPI disruption. We reasoned that, taken together, these approaches would maximize the chances for detecting an effect of a novel drug class, i.e., 5-HT_6 antagonist, in each paradigm.

Regarding the 5-HT_6 antagonists used in these experiments, the pharmacology and pharmacokinetic profile of Ro04-6790 in the rat has been published in some detail (see Sleight et al., 1998). Ro65-7199 has a pKi of 7.3 for 5-HT_6 receptors and in vivo at an oral dose range of 10–30 mg/kg has been reported to increase hippocampal and cortical ACh release and improve water maze learning and passive avoidance retention (Sleight et al., 1999; Bos et al., 2001). Each drug was therefore tested at doses, pretreatment times, and routes of administration shown to be effective in these reports. Clozapine was included for comparison in each study.

2. Materials and methods

The LI studies were conducted at the ETH-Zürich Laboratory of Behavioural Neurobiology, Switzerland, and the PPI experiments conducted at F. Hoffmann-La Roche, Basel, Switzerland. In each case, all experimental procedures complied with the appropriate Cantonal and Federal regulations relating to animal experimentation.

2.1. Latent inhibition

2.1.1. Subjects

The study used a total of 72 male adult Wistar rats [Zur:WIST(HanIbm); Research Unit Schwerzenbach, Switzerland] aged 10 weeks (350 g). Animals were housed individually throughout behavioral testing in Perspex Macrolon cages ($48 \times 27 \times 20$ cm^3) under reversed cycle lighting (lights on 20:00–08:00 h) in a temperature (21 ± 1 °C)- and humidity (55 ± 5%)-controlled animal facility. Food was available ad libitum in the home cages. During the experiment, water was available for only 1 hour a day in the home cage.

2.1.2. Apparatus and procedure

Rats were run in balanced squads of four. The apparatus consisted of four Coulbourn Instruments test cages (Model E10-10); each set in a ventilated sound-attenuating Coulbourn Instruments isolation cubicle (Model E10-20). A drinking bottle with a tube opening of 3-mm diameter was inserted into the chamber through a 3×4-cm hole located in the center of the right wall of the chamber, 1.5 cm above the grid floor. Licks were detected by a Coulbourn Instruments infrared optical lickometer (Model E23-01). The experiment was conducted in a dark chamber and the conditioned stimulus was a 10-s 2.9 kHz, 85dB[A]. Shock was delivered through the Coulbourn Instruments modular shock floor (Model E10-10RF) from a Coulbourn Instruments shocker (Model E13-12) and scanner (Model E13-13) set at 0.5 mA. A Coulbourn Instruments infrared activity monitor (Model E24-61) was mounted on the ceiling. It was operated in the "movement unit" mode in which a 10-ms pulse is produced each time the monitor detects a change in the animal's infrared heat pattern. This results in a series of pulses ("activity counts") at a frequency proportional to the amount of movement made by the animal. Equipment programming and data recording were controlled by a Compaq IBM-compatible personal computer (486/DX2/66).

One week prior to the beginning of the experiment, all rats were housed in single cages and a-23 h water deprivation schedule was initiated. This was followed by 5 days of training to drink in the experimental chamber. Every day for 5 days, each rat was placed into the experimental chamber and allowed to drink for 20 min. Throughout the experiment, the following data were recorded: total number of licks made by the animal and total activity counts. The LI paradigm included the following stages.

2.1.2.1. Preexposure. Each rat was placed in the experimental chamber and allowed to drink. Preexposed animals received 20 presentations of a 10-s tone with an interstimulus interval of 50 s. The parameters of the preexposure (i.e., number of preexposures) were chosen so as to allow the detection of LI enhancement. The non-preexposed (NPE) animals were confined to the chamber for an identical period of time without receiving the stimuli.

2.1.2.2. Conditioning. Each rat was placed in the experimental chamber and allowed to drink. Five light-shock pairings were given 180 s after the start of the session with an interstimulus interval of 180 s. The 1-s shock (0.5 mA) appears in the last second of the stimulus. After the fifth pairing, rats were left in the experimental chamber for an

additional 180 s. Again, as for preexposure, the parameters of conditioning were chosen so as to produce little or no LI in control animals to allow the detection of LI enhancement.

2.1.2.3. Rebaseline. Each rat was given a drinking session identical to the training sessions.

2.1.2.4. Test. Each animal was placed in the chamber and allowed to drink. The tone was presented simultaneously to all rats after 2 min for a duration of 8 min. The following data were recorded: time to complete 25 licks before stimulus onset and the time to first lick following the presentation of the stimulus.

Preexposure, conditioning, rebaseline, and test sessions were given 24 h apart.

2.1.3. Data collection and analysis

During the 5 days baseline performance, a $4 \times 2 \times 5$ ANOVA with two between-subjects factors of drug (clozapine, Ro04-6790, Ro65-7199, and vehicle) and preexposure (PE, NPE) and a within-subjects factor of 5 days was carried out on the total number of licks during the session. During the days of preexposure, conditioning, and rebaseline, the total number of licks and the total activity were analyzed by a 4×2 ANOVA with two between-subjects factors of drug (clozapine, Ro04-6790, Ro65-7199, and vehicle) and preexposure (PE, NPE) for each day separately.

For the test day, the time to complete 25 licks prior to the presentation of the stimulus was analyzed by a 4×2 ANOVA with two between-subjects factors of drug (clozapine, Ro04-6790, Ro65-7199, and vehicle) and preexposure (PE, NPE). The time to start drinking during the presentation of the conditioned stimulus (latency) was logarithmically transformed to allow a parametric statistical analysis. This logarithmic transformation of the latency was analyzed by a 4×2 ANOVA with two between-subjects factors of drug (clozapine, Ro04-6790, Ro65-7199, and vehicle) and preexposure (PE, NPE).

2.2. Prepulse inhibition

2.2.1. Subjects

Adult male (approximate body weight = 300 g) Sprague–Dawley rats were used for all PPI studies (source: RCC, Fullinsdorf, Switzerland). Following arrival in the animal facility, the animals were housed in groups of four in a light- and temperature-controlled environment (lights on: 06:00–18:00 h) with food and water available ad libitum. Animals were allowed 1 week of acclimatization before testing.

2.2.2. Apparatus and procedure

Testing was conducted in eight startle devices (SRLAB, San Diego Instruments, California, USA) each consisting of a Plexiglas cylinder (8.8-cm diameter) mounted on a Plexiglas platform in a ventilated, sound-attenuated cubicle with a high frequency loudspeaker (28 cm above the cylinder) producing all acoustic stimuli. Movements within the cylinder were detected and transduced by a piezoelectric accelerometer attached to the Plexiglas base, digitized, and stored by a computer. The session was initiated by a 5-min acclimatization period followed by 10 successive 120 dB trials (results not included in final analysis). In an initial experiment, a multiple prepulse (PP) intensity paradigm (70, 75, and 80 dB to 2, 7, and 12 dB above background, respectively) was used to investigate each 5-HT_6 antagonist alone on PPI. However, in majority of the studies, four different trial types were each presented eight times in a random manner: startle pulse alone (ST120: 120 dB, 40 ms duration); prepulse alone (75 dB, 20 ms duration); prepulse given 100 ms before onset of the startle pulse (PP75); background noise only (68 dB). Thus, the prepulse stimulus was 7 dB above background. Each trial type was administered in a randomized sequence with a mean intertrial interval (ITI) of 15 s (range 10–20 s). The mean startle amplitude (ST120 trial type) for each treatment group was determined. Percentage PPI was calculated according to the formula $\{[1\text{-}(ST120\text{-}PP75)/ST120] \times 100\}$.

Group sizes were 7–8 per dose. Prior to drug testing, all rats were exposed to a single PPI session (no pretreatment) to ensure balanced groups based on equivalent startle and PPI. In some experiments, two drug studies were conducted in the same animals. On such occasions, each experiment was separated by a 7-day interval; and in the second study, care was taken to allocate treatments based on equivalent treatment history. Data were analyzed by one- or two-way ANOVA for independent groups.

2.3. Drugs and injections

Clozapine (Novartis, Basel, Switzerland) was dissolved in 0.9% saline and 0.1 M HCl to a concentration of 5 mg/ml and then adjusted to pH 5–6 by addition of Na_2CO_3. Ro04-6790 (2,6-dimethylamino-4-sulphanilamido-pyrimidin; Hoffman-La Roche, Basel, Switzerland) was dissolved in 0.9% saline at a concentration of 30 mg/ml and Ro65-7199 (4-amino-*N*-(6-bromo-1H-indol-4-yl)-benzenesulfonamide; Hoffman-La Roche) was suspended in 0.3% Tween–saline solution at a concentration of 15 mg/ml. All drugs were given before the preexposure and the conditioning session. Clozapine was administered at a dose of 5 mg/kg ip (LI study) or 12 mg/kg (PPI studies) 30 min before test. Ro04-6790 was given at a dose of 30 mg/kg ip 15 min before and Ro65-7199 was given orally at a dose of 30 mg/kg 120 min before the respective session. Vehicle pretreated subjects received 0.9% saline as a vehicle control for the injections, given as intraperitoneal injection 30 min or 15 min before, or Tween–saline solution 120 min before as a vehicle control for the oral administration. Apomorphine (Sigma), phencyclidine, and LSD (both Hoffman-La Roche) were each dissolved in 0.9% saline and administered 30 min prior to test via the subcutaneous

route (except LSD, which had a pretreatment time of 5 min). A 0.1% ascorbic acid was added to the apomorphine vehicle.

3. Results

3.1. Latent inhibition

3.1.1. Five days baseline performance

A $4 \times 2 \times 5$ ANOVA with two between-subjects factors of drug (clozapine, Ro04-6790, Ro65-7199, and vehicle) and preexposure (PE, NPE) and a within-subjects factor of 5 days carried out on the total number of licks yielded no significant outcomes (all $P>.30$) other than that of days [$F(4,256)=14.1$, $P<.001$], which reflected the gradual increase in drinking over days (Day 1: 1480 ± 48, Day 2: 1642 ± 39, Day 3: 1643 ± 43, Day 4: 1777 ± 39, Day 5: 1780 ± 43).

3.1.2. Preexposure day: total number of licks

A 4×2 ANOVA with two between-subjects factors of drug (clozapine, Ro04-6790, Ro65-7199, and vehicle) and preexposure (PE, NPE) carried out on the total number of licks on the day of preexposure yielded only a significant main effect of drug [$F(3,64)=9.2$, $P<.001$]. This reflected the fact that the clozapine group (965 ± 163) drank significantly less than the Ro04-6790 group (2130 ± 179, $P<.001$), the Ro65-7199 group (1627 ± 179, $P<.01$), and the vehicle group (1715 ± 122, $P<.001$). The latter three groups did not differ from each other.

3.1.3. Preexposure day: total activity

A 4×2 ANOVA with two between-subjects factors of drug (clozapine, Ro04-6790, Ro65-7199, and vehicle) and preexposure (PE, NPE) carried out on the activity measure on the day of preexposure yielded only a significant main effect of drug [$F(3,64)=41.1$, $P<.001$]. This reflected the fact that the clozapine group (403 ± 67) and the Ro04-6790 group (214 ± 48) were significantly less active ($P<.001$) than the Ro65-7199 group (948 ± 128) and the vehicle group (1168 ± 44). The clozapine group did not differ from the Ro04-6790 group, and the Ro65-7199 group did not differ from the vehicle group.

3.1.4. Conditioning day: total number of licks

A 4×2 ANOVA with two between-subjects factors of drug (clozapine, Ro04-6790, Ro65-7199, and vehicle) and preexposure (PE, NPE) carried out on the total number of licks on the day of conditioning yielded only a significant main effect of drug [$F(3,64)=15.1$, $P<.001$]. This reflected the fact that the clozapine group (382 ± 79) drank significantly less than the Ro04-6790 group (1242 ± 160, $P<.001$), the Ro65-7199 group (706 ± 106, $P<.03$), and the vehicle group (936 ± 60, $P<.001$). In addition, the Ro 04-6790 group drank significantly more than the Ro65-7199 ($P<.002$) and the vehicle group ($P<.04$). The latter two groups did not differ from each other.

3.1.5. Conditioning day: total activity

A 4×2 ANOVA with two between-subjects factors of drug (clozapine, Ro04-6790, Ro65-7199, and vehicle) and preexposure (PE, NPE) carried out on activity measure on the day of conditioning yielded only a significant main effect of drug [$F(3,64)=14.8$, $P<.001$]. This reflected the fact that the clozapine group (413 ± 52) and the Ro04-6790 group (345 ± 80) were significantly less active ($P<.01$) than the Ro65-7199 group (671 ± 98) and the vehicle group (899 ± 64). The clozapine group did not differ from the Ro 04-6790 group, and the Ro65-7199 group was significantly less active ($P<.04$) than the vehicle group.

3.1.6. Rebaseline day: total number of licks

A 4×2 ANOVA with two between-subjects factors of drug (clozapine, Ro04-6790, Ro65-7199, and vehicle) and preexposure (PE, NPE) carried out on the total number of licks on the day of preexposure yielded only a significant main effect of drug [$F(3,64)=7.7$, $P<.001$]. This reflected the fact that the clozapine group (1437 ± 161) drank significantly more than the vehicle group (919 ± 72, $P<.003$). In addition, the Ro04-6790 group (1821 ± 111) drank significantly more than the Ro65-7199 (1109 ± 168, $P<.004$) and the vehicle group ($P<.001$). The latter two groups did not differ from each other.

3.1.7. Rebaseline day: total activity

A 4×2 ANOVA with two between-subjects factors of drug (clozapine, Ro04-6790, Ro65-7199, and vehicle) and

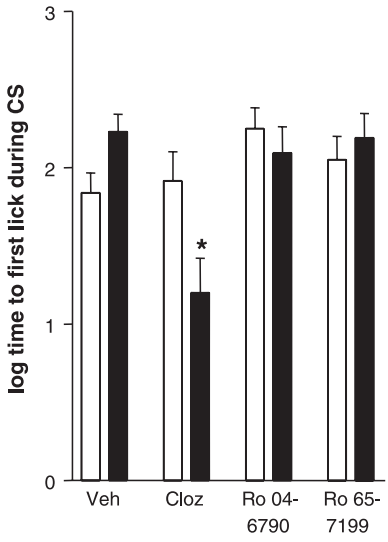

Fig. 1. Effect of clozapine (Cloz, 5 mg/kg ip), Ro 04-6790 (30 mg/kg ip), and Ro 65-7199 (30 mg/kg po) on LI using a log transformation of the latency to first lick after CS onset as a measure of conditioned suppression. □ = non-preexposed (NPE) group, ■ = preexposed group. * $P<.05$ PE versus NPE group reflecting the presence of LI in the clozapine group.

Table 1
Effect of Ro04-6790 and Ro65-7199 on baseline startle and PPI tested under varying prepulse intensities (see Materials and methods for fuller description of test protocol)

Test compound	Dose	Startle	Prepulse intensity		
			70 dB	75 dB	80 dB
Vehicle	–	494 ± 90	13 ± 7	63 ± 5	71 ± 6
Ro04-6790	3	483 ± 90	13 ± 6	57 ± 9	75 ± 5
	10	458 ± 104	15 ± 15	69 ± 6	76 ± 5
	30	304 ± 80	− 2 ± 10	55 ± 9	76 ± 4
Vehicle	–	196 ± 41	5 ± 12	56 ± 6	74 ± 5
Ro65-7199	30	164 ± 92	22 ± 8	61 ± 7	77 ± 4

preexposure (PE, NPE) carried out on the activity measure on the rebaseline day yielded only a significant main effect of preexposure [$F(1,64) = 7.8$, $P < .01$]. This reflected the fact that the preexposed groups (840 ± 61) were less active than the non-preexposed groups (1056 ± 52), probably reflecting increased levels of freezing due to enhanced contextual conditioning in the preexposed groups.

3.1.8. Test day: time to complete 25 licks prior to the presentation of the stimulus

A 4 × 2 ANOVA with two between-subjects factors of drug (clozapine, Ro04-6790, Ro65-7199, and vehicle) and preexposure (PE, NPE) carried out on the time to complete 25 licks prior to the presentation of the conditioned stimulus yielded no significant outcomes. The means and standard errors of the four groups were as follows: clozapine: 9.2 ± 1.3; Ro04-6790: 12.7 ± 3.8; Ro65-7199: 9.0 ± 2.6; vehicle: 14.3 ± 2.8.

3.1.9. Test day: time to start drinking following the presentation of the stimulus

A 4 × 2 ANOVA with two between-subjects factors of drug (clozapine, Ro04-6790, Ro65-7199, and vehicle) and

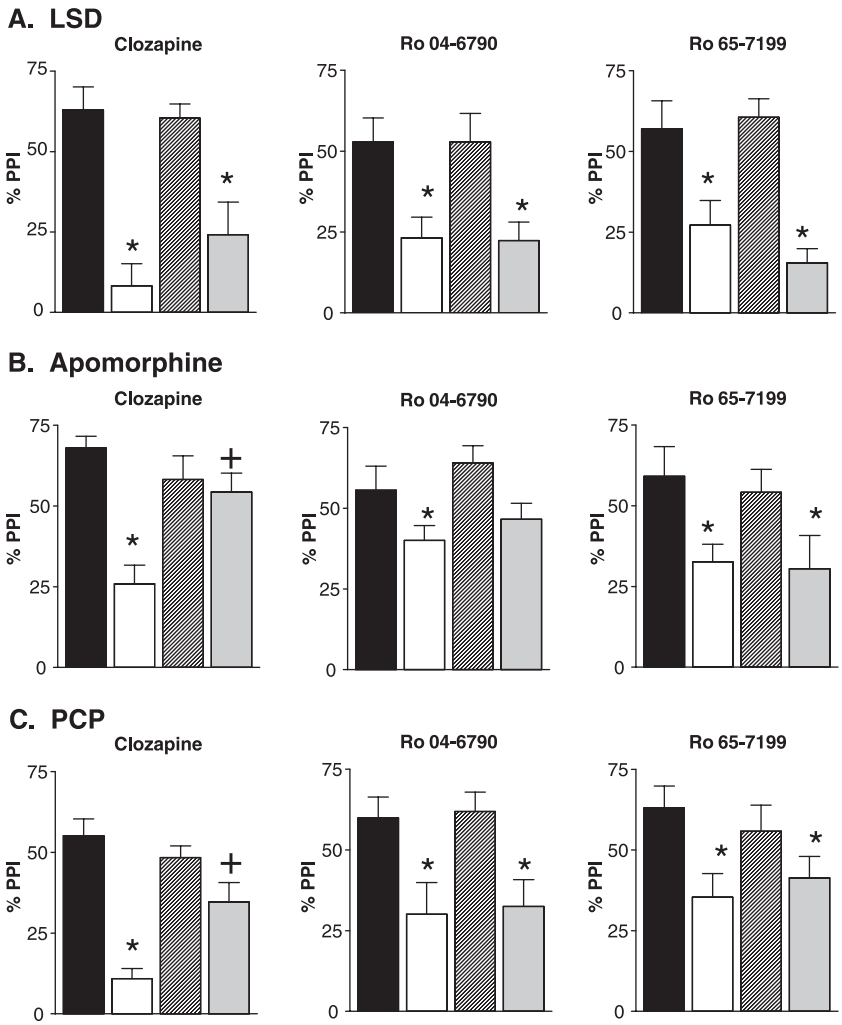

Fig. 2. Effect of clozapine (12 mg/kg ip), Ro 04-6790 (30 mg/kg ip), and Ro 65-7199 (30 mg/kg po) on PPI in experiments where PPI has been disrupted by (A) LSD (0.1 mg/kg sc), (B) apomorphine (0.1 mg/kg sc), and (C) PCP (1.5 mg/kg sc). $n = 7–8$ rats per group. $*P < .05$ versus vehicle/vehicle group, $+P < .05$ versus LSD, apomorphine, or PCP treatment alone (see Materials and methods for further detail). ■ = vehicle/vehicle, □ = agonist/vehicle, □ = antagonist/vehicle, □ = agonist/antagonist.

preexposure (PE, NPE) carried out on the logarithmic transformation of the latency to start drinking during the presentation of the conditioned stimulus yielded a significant main of drug [$F(3,64)=5.7$, $P<.002$] and a significant Drug × Preexposure interaction [$F(3,64)=4.8$, $P<.005$]. These reflected the existence of a significant LI effect, i.e., shorter latency of the preexposed as compared with the non-preexposed group only in the clozapine condition ($P<.02$). Whereas the four NPE groups did not differ from each other, the clozapine PE group exhibited a significantly shorter latency ($P<.003$) as compared with the other three PE groups, which did not differ from each other. Thus, the existence of LI only in the clozapine condition was entirely due to the PE group being less inhibited by the conditioned stimulus (Fig. 1).

3.2. Prepulse inhibition

In an initial series of experiments, Ro04-6790 (3-30 mg/kg ip) and Ro65-7199 (30 mg/kg po) were tested in a variable prepulse (70, 75, 80 dB) PPI test protocol. In each experiment, varying the prepulse markedly influenced PPI, yet in each case neither compound had any effect on baseline startle or PPI [Ro04-6790 study: startle $F(3,27)=0.9$, ns; PPI: drug $F(3,27)=0.5$, ns; Intensity × Drug $F(6,54)=0.7$, ns] [Ro65-7199 study: startle $F(1,14)=1.0$, ns; PPI: drug $F(1,14)=1.4$, ns; Intensity × Drug $F(2,28)=0.6$, ns] (see Table 1).

Subsequently, the effect of each $5HT_6$ antagonist was tested against an LSD-, apomorphine-, or PCP-induced PPI disruption. LSD (0.1 mg/kg sc) produced a significant disruption of PPI ($F>17.0$, $P<.01$) in each experiment (see Fig. 2a). Ro04-6790 and Ro65-7199 (both 30 mg/kg) each failed to affect this disruption as indicated by no main effect of test compound (Ro) or significant LSD × Ro interaction [Ro04-6790 study: $F(1,28)=0$, ns; Ro65-7199 study: $F(1,27)=1.3$, ns]. No main effects on startle were recorded in either experiment (data not shown). Although no main effect of clozapine [$F(1,26)=0.8$, ns] or Clozapine × LSD interaction [$F(1,26)=1.6$, ns] was found on PPI, there was a slight trend toward an attenuation of the LSD-induced disruption (percentage of PPI: vehicle: 63 ± 7%, LSD 8 ± 7%, clozapine + LSD 24 ± 10%).

Apomorphine (0.1 mg/kg sc) pretreatment disrupted PPI in each experiment tested ($F>7.9$, $P<.01$). Again, neither Ro04-6790 nor Ro65-7199 (both 30 mg/kg) affected this disruption (see Fig. 2b) as indicated by a lack of Apomorphine × Ro interaction [Ro04-6790 study: $F(1,26)=0.1$, ns; Ro65-7199 study: $F(1,28)=0.1$, ns]. Clozapine on the other hand reversed the apomorphine-induced deficit as shown by a significant Apomorphine × Clozapine interaction [$F(1,27)=15.3$, $P<.01$]. With the exception of a small decrease in startle following apomorphine pretreatment in the clozapine study ($P<.05$), no other effects on startle were recorded in these experiments (data not shown).

PCP (1.5 mg/kg sc) also disrupted PPI in all experiments ($F>8.2$, $P<.01$) (Fig. 2c). Ro04-6790 and Ro65-7199 (both 30 mg/kg) did not affect this disruption [PCP × Ro interaction: Ro04-6790 study: $F(1,26)=0$, ns; Ro65-7199 study: $F(1,27)=0.8$, ns]. In contrast, a significant PCP × Cloza-Clozapine interaction [$F(1,27)=11.2$, $P<.01$] confirmed that clozapine attenuated the PCP-induced deficit. No main effects on startle were recorded in these experiments, except for the Clozapine × PCP interaction being of borderline significance [$F(1,27)=4.2$, $P<.05$]. This was likely due to each drug alone producing a small nonsignificant reduction in startle amplitude, yet in combination startle was equivalent to controls (data not shown).

4. Discussion

In the present series of experiments, neither Ro04-6790 nor Ro65-7199 facilitated LI studied in a conditioned lick suppression (CLS) paradigm or reversed a PPI disruption induced by three distinct pharmacological agents—LSD, apomorphine, and PCP. By way of contrast, clozapine facilitated LI (see also Weiner et al., 1996, 1997; Trimble et al., 1998; Shadach et al., 1999, 2000) and attenuated a PPI disruption induced by apomorphine and PCP. The doses of Ro04-6790 and Ro65-7199 were selected from the literature as being behaviorally active. Thus, in the report of Sleight et al. (1998), Ro04-6790 at a dose of 30 mg/kg (intraperitoneal route) was reported to elicit a behavioral syndrome consisting of stretching and yawning and proposed to be $5\text{-}HT_6$ receptor mediated. Furthermore, cerebrospinal fluid levels attained following this dose of Ro04-6790 lead to estimates of 70% total $5\text{-}HT_6$ receptor occupancy. More recently, Routledge et al. (2000) and Woolley et al. (2001) have reported behavioral effects of this drug in a maximal electroshock seizure threshold (MEST) test and water maze test, respectively, at the 10–30 mg/kg dose level.

During the preexposure and conditioning phase of the LI experiment, while Ro65-7199 did not significantly affect either activity or licking measures relative to controls, Ro04-6790 affected each measure: activity being reduced and licking increased, i.e., the drug appeared to elicit a dipsogenic property. We have observed the rate decreasing effects of equivalent doses of Ro04-6790 in an operant VI20 schedule, yet Ro65-7199 had no such effect up to 300 mg/kg; suggesting this to be unrelated to an interaction at the $5\text{-}HT_6$ receptor (unpublished observations). Consequently, these effects may reflect an additional action of Ro04-6790.

The PPI experiments yielded similar null findings with each $5\text{-}HT_6$ antagonist. In these experiments, neither Ro04-6790 nor Ro65-7199 affected baseline startle or PPI, and there was no trend to suggest any hint of PPI reversal following disruption by apomorphine, LSD, or PCP. To our knowledge, the only other study investigating the effect of a $5\text{-}HT_6$ antagonist on PPI was reported by Pouzet et al.

(2002) using SB271046. In this study, SB271046 reversed an amphetamine but not PCP-induced disruption. Since an amphetamine-induced PPI disruption is blocked by DA_2 antagonists (e.g., Geyer et al., 2001), this result may seem inconsistent with the present null findings of Ro04-6790 and Ro65-7199 in the apomorphine PPI model. However, there are various differences between the pharmacology of an amphetamine and apomorphine PPI challenge (for a recent review, see Geyer et al., 2001), and further studies are necessary to resolve these differences. The ineffectiveness of Ro04-6790 against an LSD-induced disruption was previously reported (Ouagazzal et al., 2001) and has now been extended to a second 5-HT_6 antagonist.

In each experimental set, we included clozapine as a comparator compound. Clozapine facilitated LI and attenuated an apomorphine and PCP-induced PPI deficit. With respect to PPI interaction studies, rat strain seems to be an important factor in determining result outcome. Thus, a number of groups have reported clozapine reversing an apomorphine PPI deficit in the Sprague–Dawley but not necessarily the Wistar strain where doses above 5 mg/kg become disruptive (Varty and Higgins, 1995; for a review, Geyer et al., 2001). The reversal of a PCP-induced PPI deficit is also consistent with other groups using the Sprague–Dawley strain (Bakshi et al., 1994). Perhaps the most surprising result from these experiments was the failure of clozapine to reverse an LSD PPI deficit, although there was a slight trend toward reversal. Since we have previously found the selective 5-HT_{2A} receptor antagonist M100,907 to fully reverse this effect of LSD (Ouagazzal et al., 2001), the high affinity shared by clozapine for this receptor might have been expected to produce a similar effect. A higher dose of clozapine may be necessary to achieve appropriate pharmacological blockade, or alternatively an additional aspect of its pharmacology may confound this interaction. In this respect, one plausible locus may be the 5-HT_{2C} receptor, which under certain circumstances may functionally oppose effects mediated through the 5-HT_{2A} site (e.g., Fletcher et al., 2002).

In summary, through the use of Ro04-6790 and Ro65-7199 as tool compounds to investigate the 5-HT_6 receptor, we can find no evidence that antagonism at this site influences behavior measured in an LI paradigm and a range of PPI tests. These conclusions are of course dependent on the value of each drug as a pharmacological tool at this receptor, and higher affinity 5-HT_6 antagonists with improved bioavailability are now becoming available, e.g., SB357134 (Stean et al., 2002). Nonetheless, each drug was tested at behaviorally active doses and the behaviors produced have been described as 5-HT_6 mediated (Sleight et al., 1998, 1999; Routledge et al., 2000; Woolley et al., 2001; Bos et al., 2001). Although the present data may not support an involvement of 5-HT_6 receptors in the antipsychotic profile of clozapine or suggest that 5-HT_6 antagonism alone may result in a clinically effective antipsychotic, alternative studies may have been more fruitful. The findings of Dawson et al. (2001) suggest that 5-HT_6 antagonism may increase extracellular glutamate levels in the frontal cortex. Hypofunction of the frontal cortex is associated with poor executive function characterized by impaired performance in tasks of set shifting across distinct stimulus dimensions (Robbins, 2000). In schizophrenic patients, this may be manifested as a perseverative tendency in a Wisconsin card sort test (Weinberger et al., 1986; Weinberger, 1988). It is therefore intriguing to note the recent preliminary studies of Hatcher et al. (2002) showing SB271046 improving ED shifting in a rat attentional set shifting task (Birrel and Brown, 2000). If confirmed, particularly in a higher species, this might suggest that 5-HT_6 receptor antagonism contributes to the cognitive improvement reported in schizophrenic patients following clozapine treatment (Meltzer, 1999).

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