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The effects of the dopamine D2 agonist sumanirole on prepulse inhibition in rats

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Abstract

Dopamine agonists reduce prepulse inhibition (PPI) of startle in rats. While it is used to predict antipsychotic efficacy, the specific receptor subtypes mediating this effect of dopamine agonists remains unclear. We characterized the effects of sumanirole, a highly selective D2 agonist, on PPI in rats. Sumanirole decreased PPI at 60-120 ms prepulse intervals, and increased PPI at 10-20 ms intervals. PPI deficits were antagonized by low doses of the preferential D2 antagonist L741626, supporting a D2 mechanism of action. Sumanirole is a valuable tool for parsing the role of dopamine receptor subtypes in the regulation of PPI.

Keywords

Dopamine; D2 receptor; prepulse inhibition; schizophrenia; sumanirole

Introduction

The ability of compounds to prevent the disruption of prepulse inhibition (PPI) by non-selective dopamine (DA) agonists like apomorphine (APO) in rats is widely used to identify compounds with antipsychotic properties (c.f. Swerdlow et al., 2008). In the absence of both receptor-specific knock-out rats and subtype-selective DA receptor compounds, however, relatively little is known about the role of DA receptor subtypes in regulating these PPI deficits in this species. For example, it is difficult to distinguish DA D2- vs. D3-receptor linked effects in these models. *In vivo* screens have utilized the ability to oppose PPI deficits induced by pramipexole (PRA) or PD128907 as a basis for identifying D3-preferential receptor antagonists for clinical applications (Zhang et al., 2007; Weber et al., 2009). In these studies, greater sensitivity to oppose PPI deficits induced by D3 agonists than by non-specific DA agonists is used to suggest D3-preferential receptor blockade. The sensitivity of this *in vivo* assay to

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Contributors: The study was designed by MW, WLC, and NRS. Experiments were carried out by MW, WLC, MB, and AY. MW and NRS analyzed and interpreted the data. MJM provided L741626 and critical discussion. MW wrote the first version of the manuscript, WLC, MJM, and NRS revised the manuscript. All authors have contributed to and approved the final manuscript.

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distinguish antagonists with primary D3 vs. D2-linked mechanisms would be enhanced by highly D2-preferential agonists that reliably disrupt PPI. Here, we assessed the PPI-disruptive effects of sumanirole (SUM), a novel D2-selective agonist (Heier et al., 1997).

Based on *in vitro* receptor binding, the D2 affinity of SUM exceeds that of other DA receptor subtypes by over 200-fold (c.f. de Paulis, 2003; Wuts et al., 2002; McCall et al., 2005); accordingly, studies have used SUM to parse D2 vs. D3-receptor effects in assays of hypothermia and drug discrimination (Collins et al., 2007; Koffarnus et al., 2008; Achat-Mendes et al., 2009). To our knowledge, no study has evaluated SUM in animal models for schizophrenia.

Experimental procedures

Animals

Adult male Sprague Dawley rats (n = 49; 225-250 g; Harlan, Livermore, CA) were handled 1d after arrival, housed in groups of 2-3, and maintained on a reversed light/dark schedule with water and food *ad libitum*. Testing occurred during the dark phase. Experiments were conducted in accordance with the NIH Guide for the Care and Use of Laboratory Animals and approved by the UCSD Animal Subjects Committee (protocol #S01221).

Drugs

Ascorbic acid and APO hydrochloride hemihydrate (Sigma; St. Louis, MO), PRA hydrochloride (TRC; North York, Canada), and L741626 (Tocris; Ellisville, MO) were used. Sumanirole maleate was supplied by the NIMH Chemical Synthesis and Drug Supply Program. SUM and PRA were dissolved in saline, APO in 0.01 % ascorbate/saline, and L741626 in 0.03% lactic acid/water (w/v; pH ≥5 with NaOH). Doses (mg/kg salt) were administered subcutaneously in 1 ml/kg. L741626 was administered 30 min prior to SUM. SUM was administered immediately before the “time course” experiment, and 15 min prior to testing in all subsequent experiments. Based on previous studies (Weber et al., 2008, 2009), PRA and APO were administered 15 and 0 min before testing, respectively.

Apparatus

Startle chambers (SD Instruments, San Diego, CA) (Weber et al., 2008, 2009) recorded 100 1-ms readings beginning at stimulus onset. Startle magnitude was the average of these 100 readings.

Testing

All studies used a continuous 70 dB(A) background noise (all sound calibrated on the “A” scale); after 5 min, trials were presented in pseudorandom order. Approximately 7 days after arrival, rats completed a brief startle test; based on the results, rats were assigned to drug dose groups with matched baseline %PPI (Weber et al., 2008, 2009). Inter-test intervals were 3 - 7 days. Variable inter-trial intervals averaged 15s. Interspersed between active trials were trials in which no stimulus was presented, but cage displacement was measured (NOSTIM trials).

Protocol 1 (65 min) established the time course of SUM effects on PPI, using a within-subject balanced dose order design. After the acclimation period, rats were exposed to six 10 min blocks consisting of 5 min of startle trials followed by 5 min without trials. Blocks began with a P120 stimulus (a 40 ms - 120 dB noise burst; not included in the calculation of PPI), followed by a mixture of 6 P120, 6 PP12+P120 (P120 preceded 100 ms (onset-to-onset) by a 20 ms noise burst of 12 dB above background), and 4 PP12 stimuli (PP12 pulses *not* followed by P120).

Protocol 2 (18.25 min) tested the dose-response properties of SUM using a between-subject design, and the effects of SUM vs. L741626 using a mixed-model, balanced dose-order design with L741626 as the between- and SUM as the within-factor. Twenty-three rats were first tested in the dose-response experiment, then redistributed to dose groups according to baseline PPI and SUM drug group. After a "washout" period of 5 d, these rats were tested in the SUM × L741626 experiment. The session began with 4 and ended with 3 consecutive P120 trials; between these trials were 2 blocks, each consisting of 8 P120 trials, 5 PP5+P120 trials, 5 PP10 +P120 trials, and 5 PP15dB+P120 trials, i.e. trials in which the P120 was preceded 100 ms (onset to onset) by a prepulse of 20 ms duration and an intensity of either 5, 10, or 15 dB above background, respectively.

Protocol 3 (15.5 min) evaluated the effects of SUM, APO and PRA at 10 - 120 ms prepulse intervals. Twenty rats first used for the SUM experiment (above) were redistributed into balanced dose groups; after a "washout" period of 16 d, these rats were used in the APO/PRA experiment. The session began and ended with 3 consecutive P120 trials. Between these trials were 6 P120, and 6 PP10ms+ P120, 6 PP20ms+ P120, 6 PP30ms+P120, 6 PP60ms+ P120, or 6 PP120ms+ P120 trials, i.e. trials in which the P120 was preceded 10, 20, 30, 60, or 120 ms by a 5 ms prepulse that was 15 dB above background.

Data analysis

PPI was defined as 100-[(startle magnitude on prepulse trials / startle magnitude on P120 trials) × 100], and was analyzed by ANOVAs. Post-hoc comparisons used ANOVAs or Fisher's PLSD. Data were collapsed across prepulse intensities and blocks (protocol 2). Alpha was 0.05.

Results

1. Time course study

This study was conducted to identify adequate pretreatment times for SUM (0 vs. 3.0 mg/kg). ANOVA of %PPI revealed a main effect of SUM dose ($F = 7.1$; df 1,5, $p<0.05$), but no significant effects of time ($F = 1.6$; df 1,5; n.s.), or time × dose interaction ($F < 1$). Based on inspection of the data (Fig. 1A), subsequent studies utilized a SUM pretreatment interval of 15 min.

2. Dose-response study

The effects of SUM (0, 0.3, 1.0, 3.0 mg/kg) were tested next. ANOVA of %PPI revealed a main effect of SUM dose ($F=3.1$; df 3,19; $p=0.05$). Post-hoc tests revealed that each active dose of SUM significantly reduced PPI relative to the vehicle condition, ($p<0.05$ for 0.3 and 1.0 mg/kg; $p<0.005$ for 3.0 mg/kg of SUM) (Fig. 1B).

3. L741626 study

The effects of SUM (0, 3.0 mg/kg) on PPI were tested after pretreatment with the D2-preferential antagonist L741626 (0, 0.3, 0.6 mg/kg). ANOVA of %PPI revealed a significant effects of SUM ($F=16.4$; df 1,20; $p<0.001$) and a SUM × L741626 interaction ($F=9.5$; df 2,20; $p<0.005$). No other effects were significant. Post-hoc analyses revealed that SUM decreased %PPI in rats pretreated with 0 mg/kg of L741626 ($p<0.005$), and this effect was opposed in animals pretreated with 0.3 ($p<0.05$) or 0.6 ($p<0.0005$) mg/kg of L741626 (Fig. 1C).

4. Interval study

The effects of SUM (0, 3.0 mg/kg) were tested at varying prepulse intervals. ANOVA of % PPI revealed significant effects of prepulse interval ($F=18.6$; df 4,72; $p<0.0001$), and a significant interaction of SUM dose × prepulse interval ($F=7.5$; df 4,72; $p<0.0001$). Post-hoc

analyses revealed significant PPI-*increasing* effects of SUM at short prepulse intervals (10 - 20 ms; $p<0.05$) and significant PPI-*decreasing* effects of SUM at long prepulse intervals (60 ms and 120 ms; $p<0.0001$) (Fig. 1D).

We next compared these effects of SUM to those of APO and PRA, using doses that disrupt long interval PPI by magnitudes comparable to that produced by 3 mg/kg of SUM. ANOVA of % PPI revealed significant effects of prepulse interval ($F=24.5$; df 4,68; $p<0.0001$) and interval × drug interaction ($F=2.1$; df 4,68; $p<0.05$). Post-hoc analyses revealed no PPI-enhancing effects of PRA or APO at short (10 - 20 ms) prepulse intervals, but PPI-reducing effects of both PRA ($p<0.001$) and APO ($p<0.0005$) at long (60 - 120 ms) prepulse intervals (Fig. 1E).

In all protocols, SUM effects on startle magnitude were not statistically significant; when nonsignificant trends towards a SUM effect on startle magnitude were observed, simple regression analyses revealed that these trends could not account for SUM effects on PPI. All main or interaction effects of SUM, APO or PRA on NOSTIM or prepulse only activity (protocol 1) were not statistically significant. Startle magnitude for APO and PRA followed previously published patterns (Table 1; Weber et al., 2008,2009).

Discussion

In this study, SUM disrupted PPI under testing conditions widely used in published studies that assessed antipsychotic potency (c.f. Swerdlow et al., 2008) in a dose range linked to D2 receptor activation in rats (Collins et al., 2007; Koffarnus et al., 2008). While having marked preferences for D2 receptors over all other types of DA receptor subtypes (>200 fold) – SUM has only a moderate (~8 fold) binding preference for the D2 vs. 5-HT_{1A} receptors (c.f. de Paulis 2003; McCall et al., 2005). Hence, it was critical to test SUM against the preferential D2 antagonist L741626, a compound with ~10 fold binding preference for D2 receptors relative to D3 receptors, and 80-200 fold preferences relative to all other DA receptors, and non-DA receptors, including the 5-HT_{1a} receptor (Cussac et al., 2000; Millan et al., 2000, 2004). The fact that *very low* doses of L741626 favoring D2 receptor blockade fully antagonized SUM-induced PPI deficits strongly supports a D2 (rather than a D3 and/or 5HT1a) receptor linked mechanism of action of SUM in measures of PPI.

SUM both *decreased* PPI at long prepulse intervals and *increased* PPI at short prepulse intervals; at doses that generated a comparable degree of long-interval PPI-reduction, the non-selective DA agonist APO, and the D3 preferential agonist PRA did not increase short-interval PPI. Importantly, studies testing higher doses of APO (0.5 mg/kg), or using PRA in more sensitive within-subjects designs both have detected increased short-interval PPI (Swerdlow et al. 2004; Swerdlow et al. 2009), suggesting that the differences vs. SUM detected here are ones of degree, i.e. compared to PRA and APO, SUM has a more potent effect on the substrate responsible for the increased short interval PPI.

The present findings extend previous studies with non-selective DA agonists like APO and quinpirole (c.f. Swerdlow et al., 2008; Millan et al., 2002; Zhang et al., 2007; Weber et al., 2008, 2009). While these studies showed that co-activation of D2-receptors with either D1, and/or D3 receptors potently disrupts PPI in rats, the present findings demonstrate that D2 receptor activation is sufficient to disrupt PPI deficits in rats, even at SUM doses that would be predicted to have *no appreciable co-activation of either D1 and/or D3 receptors*. Such a selective D2-receptor linked mechanism of action of SUM on PPI will be valuable in parsing the neurobiological basis of antipsychotic-like effects in rodent PPI models. In particular, SUM may be valuable in interpreting findings in *in vivo* assays that use PPI to detect D3-preferential antagonists (Zhang et al., 2007; Weber et al., 2009) or other novel antipsychotics (e.g.

Wadenberg et al. 2000), and in clarifying the PPI-regulatory role of intracellular signaling pathways linked to D2 vs. D3 receptor activation (Chang et a. 2009; Saint Marie et al. 2007).

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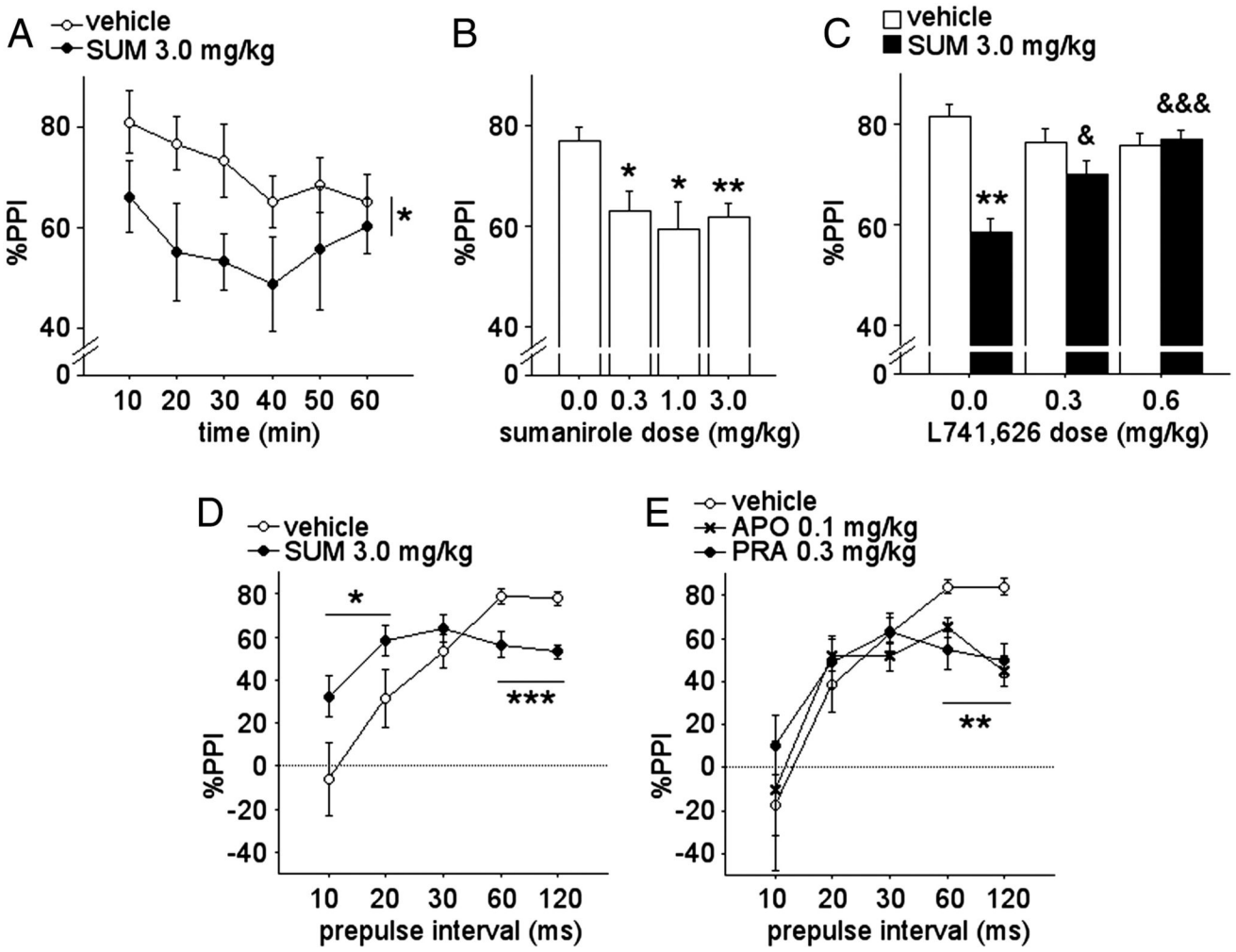

Fig. 1. The effects of SUM (A-D), and APO or PRA (E) on PPI

(A) Time course of SUM effects. **(B)** Dose-response effects of SUM. **(C)** L741626 antagonized SUM-induced PPI deficits. **(D)** SUM *decreased* PPI at long prepulse intervals, but *increased* PPI at short prepulse intervals. **(E)** APO and PRA decreased PPI at long prepulse intervals, but PPI at short prepulse intervals was unaffected. * denotes significant differences for treatment with SUM, APO, or PRA vs. vehicle, & denotes significant antagonism of the SUM-induced PPI deficit by L741626; *,& $p<0.05$; ** $p<0.005$; ***,&&& $p<0.0005$.

Table 1
Effects of SUM, APO, or PRA on startle magnitude

SUM effects on startle magnitude were independent from SUM effects on PPI (see results).

EXPERIMENT	Drug(s)	Startle Magnitude Mean (SEM)
1. Time course	Vehicle:	400 (79)
	SUM (3 mg/kg):	237 (49)
2. SUM Dose-response	Vehicle:	187 (6)
	SUM (0.3 mg/kg):	213 (54)
	SUM (1.0 mg/kg):	270 (65)
	SUM (3.0 mg/kg):	242 (31)
3. L741626 × SUM	Vehicle/Vehicle:	182 (60)
	Vehicle/L741626 (0.3 mg/kg):	206 (43)
	Vehicle/L741626 (0.6 mg/kg):	267 (34)
	SUM/Vehicle:	174 (34)
	SUM/L741626 (0.3 mg/kg):	226 (35)
	SUM/L741626 (0.6 mg/kg):	290 (32)
4. Prepulse Intervals	Vehicle:	259 (45)
	SUM (3.0 mg/kg):	243 (33)
	Vehicle:	246 (32)
	APO (0.1 mg/kg):	161 (33)
	PRA (0.3 mg/kg):	140 (26)