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Effects of D_3/D_2 Dopamine Receptor Agonists and Antagonists on Prepulse Inhibition of Acoustic Startle in the Rat

S. Barak Caine, Ph.D., Mark A. Geyer, Ph.D., and Neal R. Swerdlow, M.D., Ph.D.

Prepulse inhibition (PPI) is the normal reduction in a startle response that occurs when a weak stimulus ("prepulse") precedes the startling stimulus by 30 to 500 msec. Schizophrenic patients are deficient in this operational measure of sensorimotor gating; therefore, animal models of deficient PPI may provide information useful in the understanding and treatment of schizophrenia. Prepulse inhibition is disrupted in rats by systemic administration of direct dopamine agonists having affinity for the D_2 subtype family (D_2, D_3, and D_4) of dopamine receptors. This study tested the hypothesis that dopamine agonists and antagonists with different affinities for D_3 and D_2 receptors differ in their relative potencies to modulate PPI. The dopamine agonists quinpirole, 7-hydroxy-N,-N-di-n-propyl-2-aminotetralin (7-OH-DPAT) and apomorphine were approximately equipotent in decreasing PPI. Pretreatment with haloperidol (13 to 130 nmol/kg sc), but not equimolar doses of UH 232, prevented the disruption of PPI produced by the highest dose (0.6 μmol/kg sc) of each agonist. Given the 100-fold higher affinity of haloperidol relative to UH 232 for D_2 receptors, and equal relative affinities of these antagonists for D_3 receptors, these data are consistent with previous studies suggesting that dopamine agonists may modulate PPI in the rat through the D_2 subtype of dopamine receptors.
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KEY WORDS: *Prepulse inhibition; Sensorimotor gating; Startle; Schizophrenia; Dopamine receptor; Antipsychotic*

The startle reflex to a startling stimulus is inhibited when a weak lead stimulus ("prepulse") precedes the startling stimulus by 30 to 500 msec (Hoffman and Searle 1968; Graham 1975). This "prepulse inhibition" (PPI) of the startle reflex can be used as an operational measure of sensorimotor gating in both animals and humans (Swerdlow et al. 1986; Braff and Geyer 1990; Geyer et al. 1990; Braff et al. 1992). Schizophrenic patients exhibit deficient sensorimotor gating as measured by PPI (Braff et al. 1978, 1992), an observation consistent with the hypothesis that deficient sensory gating contributes to the cognitive fragmentation and related symptomatology associated with schizophrenia (McGhie and Chapman 1961). Studies of PPI in rats provide a valuable model for testing hypotheses about the neural substrates of deficient sensory gating in schizophrenic patients (Swerdlow et al. 1986, 1991a, 1992), as well as providing a preclinical screen for novel pharmacotherapeutic approaches for schizophrenia (Geyer et al. 1990; Rigdon and Viik 1991; Swerdlow et al. 1991b, 1994a,b; Swerdlow and Geyer 1993).

Prepulse inhibition is disrupted in rats by treatment with direct dopamine agonists having high affinities for D_2 family receptors and not by D_1 dopamine agonists (Peng et al. 1990). The disruption of PPI produced by dopamine agonists can be prevented by pretreatment with "typical" neuroleptic agents such as spiperone (Swerdlow et al. 1991b) and haloperidol (Swerdlow and

From the Departments of Neuroscience (SBK, MAG, NRS) and Psychiatry (NRS, MAG), University of California, San Diego, La Jolla, California.

Address correspondence to: Neal Swerdlow, M.D., Ph.D., Department of Psychiatry, 0804, University of California, San Diego, La Jolla, CA, 92093.

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Geyer 1993) and by pretreatment with "atypical" antipsychotics such as clozapine (Swerdlow and Geyer 1993), risperidone (Rigdon and Viik 1991), and seroquel (Swerdlow et al. 1994b). The D_1 antagonist SCH23390 does not restore PPI in rats treated with apomorphine (Swerdlow et al. 1991b), although conflicting data was recently reported (Hoffman and Donovan 1994). Such studies suggest that activation of the D_2 family (D_2, D_3, and D_4) of dopamine receptor subtypes but not the D_1 family (D_1, D_5) is responsible for the disruption of PPI produced by dopamine agonists. It has been demonstrated recently that three D_2 family dopamine agonists—apomorphine, quinpirole, and 7-OH-DPAT—have affinities for the D_3 receptor that correlate with their relative potencies to elicit behavioral effects (Caine and Koob 1993). The present study explored the relative contributions of specific D_2 family receptors to the neural modulation of PPI, using dopamine agonists and antagonists with different affinities for D_3 and D_2 receptors.

METHOD

Subjects

Eighty male Sprague-Dawley rats (225 to 250 g; Harlan, Indianapolis, IN) were housed in pairs and maintained on a reversed 12 hour: 12 hour light/dark schedule (lights off at 0700 hours) with food and water provided ad libitum. Testing occurred during the dark phase, between 0900 and 1500 hours. Animals were handled within three days of arrival, and daily thereafter.

Chemicals

Apomorphine hydrochloride and ascorbate were obtained from Sigma Chemical Co., St. Louis, MO. Quinpirole hydrochloride and 7-OH-DPAT hydrobromide were obtained from Research Biochemicals International, Natick, MA. Haloperidol was obtained from Solopak Laboratories, Franklin Park, IL. UH 232 was generously provided by George F. Koob. All chemicals were dissolved in physiological saline except for apomorphine, which was dissolved in saline with 0.1% ascorbate, and haloperidol, which was obtained in aqueous solution containing lactic acid.

Drug Treatments

Dopamine agonists and antagonists were administered subcutaneously 5 and 10 minutes prior to testing, respectively.

Apparatus

Four startle chambers (SR-LAB, San Diego Instruments, San Diego, CA) were used, each consisting of a Plexiglas cylinder 8.2 cm in diameter, resting on a Plexiglas frame within a ventilated enclosure. Acoustic noise bursts were presented via a loudspeaker mounted 24 cm above the animal. A piezoelectric accelerometer (assembled using a Blatek Audio Transducer Model 6030, Blatek Inc.) mounted below the Plexiglas frame detected and transduced motion within the cylinder. Stabilimeter readings were rectified and recorded by a microcomputer and interface assembly (San Diego Instruments), with 100 1-ms readings collected beginning at the stimulus onset. Startle amplitude was defined as the average of the 100 readings.

Test Session

The test session in all experiments consisted of five consecutive blocks of nine test trials each (45 trials total) with an average of 15 seconds separating each trial. A 65 dB background noise was constant throughout the entire test session. After a 5-minute acclimation period in the test chamber, three different trial types were delivered in pseudorandom order: startle stimulus alone (a 118 dB [A] 40 ms broad band burst); no stimulation; or startle stimulus preceded 100 ms earlier by a prepulse (a 70 dB [A] 20 ms broad band burst).

Experiment 1: Agonist Treatments

One week after arrival, the animals were tested in the startle session 5 minutes after subcutaneous injection of a dopamine agonist or vehicle. Every animal received a single dose (between-subject design, $n = 5$/dose), and each animal was used for tests with only one of the agonists.

Experiment 2: Antagonist Pretreatments Prior to Agonist Treatments

Five days after Experiment 1, the animals were split into two pretreatment groups and retested after pretreatment with either haloperidol or UH 232 (0 to 130 nmol/kg sc), and treatment (10 minutes after the pretreatment) with 0.6 μmol/kg of the agonist they had received in Experiment 1. Five days later, the animals were tested a third time in an identical fashion except that the pretreatment groups were reversed, so that every animal received each antagonist pretreatment once. An additional group of animals was tested with apomorphine as in Experiment 1, and then tested with UH 232, but not haloperidol, during the second startle test, and with UH 232 alone (no apomorphine) during the third startle test.

Data Analysis

Startle amplitude was analyzed using analysis of variance with repeated measures on block. Level of sig-

nificance was $p < 0.05$. A significant main effect of dose was followed by individual comparisons of each dose with the vehicle treatment (Experiments 1 and 3) or vehicle pretreatment (Experiment 2) using Neuman-Keul's a posteriori tests. The amount of PPI is expressed as the percentage decrease in the startle response caused by presentation of the prepulse, and was calculated using the following equation: ([startle amplitude caused by pulse alone − startle amplitude caused by pulse preceded by prepulse] / startle amplitude caused by pulse alone) × 100. Using this description of PPI, a high degree of sensorimotor gating is reflected in a high "% PPI" value, whereas less or no gating results in a small or negative "% PPI" value. Relative potencies were calculated and analyzed using a "Manual of Pharmacologic Calculations with Computer Programs" (Tallarida and Murray 1987).

RESULTS

Experiment 1: The Dopamine Agonists 7-OH-DPAT, Quinpirole, and Apomorphine Disrupted PPI with Similar Relative Potencies

Although none of the dopamine agonists significantly altered the amplitude of the startle reflex to an acoustic startling stimulus (Figure 1A; 7-OH-DPAT, [F(3,17) = 0.99]; quinpirole, [F(3,17) = 2.43]; apomorphine, [F(3,36) = 0.81]), each of the agonists dose-dependently decreased PPI of startle (Figure 1B; 7-OH-DPAT, [F(3,17) = 11.6, $p < .001$]; quinpirole, [F(3,17) = 15.9, $p < .0001$]; apomorphine, [F(3,36) = 16.7, $p < .0001$]).

The relative potencies of the three agonists to decrease PPI (i.e., the ratio of the amounts of each drug needed to decrease PPI) were calculated using a parallel line assay of the regression lines for the dose (μmol/kg) versus effect function (the correlation coefficient of the regression line for each potency estimate was $r \geq 0.7$). With the most potent drug, quinpirole, being assigned unit potency, the relative potencies of apomorphine and 7-OH-DPAT were 1.11 and 1.26, respectively. The relative potency values for the three agonists were not significantly different from each other (upper and lower confidence limits, 2.1 and 0.8, respectively).

Experiment 2: Haloperidol, but Not UH 232, Prevented the Disruption of PPI by Each of the Three Agonists

Pretreatment with haloperidol (13 to 130 nmol/kg sc) dose-dependently reduced the disruption of PPI produced by treatment with 0.6 μmol/kg sc of each of the agonists (Figure 2A; haloperidol/7-OH-DPAT, [F(3,17) = 3.94, $p < .05$]; haloperidol/quinpirole, [F(3,17) = 3.2, $p < .05$]; haloperidol/apomorphine, [F(3,17) = 14.8, $p < .0001$]. In contrast, pretreatment with UH 232 (13 to 130 nmol/kg sc) did not alter PPI after treatment with 0.6 μmol/kg sc of 7-OH-DPAT or quinpirole (Figure 2B; UH 232/7-OH-DPAT, [F(3,17) = 0.21]; UH 232/quinpirole, [F(3,17) = 1.48]. An intermediate dose of UH 232 (26 nmol/kg sc) increased PPI after treatment with 0.6 μmol/kg sc apomorphine in a group of 10 animals [F(3,17) = 3.56, $p < .05$]. However, this effect was not replicated in another group of ten animals [F(3,17) = 0.143], and was not reliable across the 20 animals tested (Figure 2B; [F(3,36) = 1.53]). Treatment with haloperidol alone under these conditions does not significantly alter PPI (Swerdlow and Geyer 1993). UH 232 alone (0 to 130 nmol/kg sc) had no effect on PPI [F(3,17) = 1.20] (data not shown).

DISCUSSION

This study examined the relative potencies to modulate PPI of startle among D_2 family dopamine receptor agonists and antagonists having different affinities for D_3 and D_2 receptors. Although quinpirole and apomorphine have affinities for the D_3 receptor that are approximately sevenfold and 26-fold weaker, respectively, than that of 7-OH-DPAT (K_i for D_3 receptor: 7-OH-DPAT < quinpirole < apomorphine; Sokoloff et al. 1990; Levesque et al. 1992), the three agonists dose-dependently disrupt PPI with equivalent relative potencies. This finding suggests that D_3 receptors may not be responsible for the disruption of PPI produced by dopamine agonists. However, the three agonists also differ in their affinities for the D_2 receptor, 7-OH-DPAT and quinpirole being approximately threefold and 24-fold weaker than apomorphine (K_i for D_2 receptor: apomorphine < 7-OH-DPAT < quinpirole). Thus no significant correlation could be found between the affinities of the three agonists for either the D_3 or the D_2 receptor and their relative potencies to disrupt PPI.

Importantly however, the potent D_2 dopamine receptor antagonist haloperidol, but not the preferential D_3 antagonist UH 232, prevented the disruption of PPI produced by the dopamine agonists. Neither antagonist alone altered PPI under these conditions (Swerdlow and Geyer 1993; this study), suggesting that the effects of haloperidol observed here represent a specific blockade of the PPI disruption produced by the agonists. The 100-fold higher affinity of haloperidol relative to UH 232 for D_2 receptors despite the equivalent affinities of these antagonists for D_3 receptors (Sokoloff et al. 1990; Levesque et al. 1992) supports the hypothesis that dopaminergic agents modulate PPI through blockade of D_2 receptors. This hypothesis is consistent with the observation that PPI is disrupted by dopamine infusion into the anteromedial striatum (Swerdlow et al. 1992), where D_2 but not D_3 receptors are localized (Levesque et al. 1992).

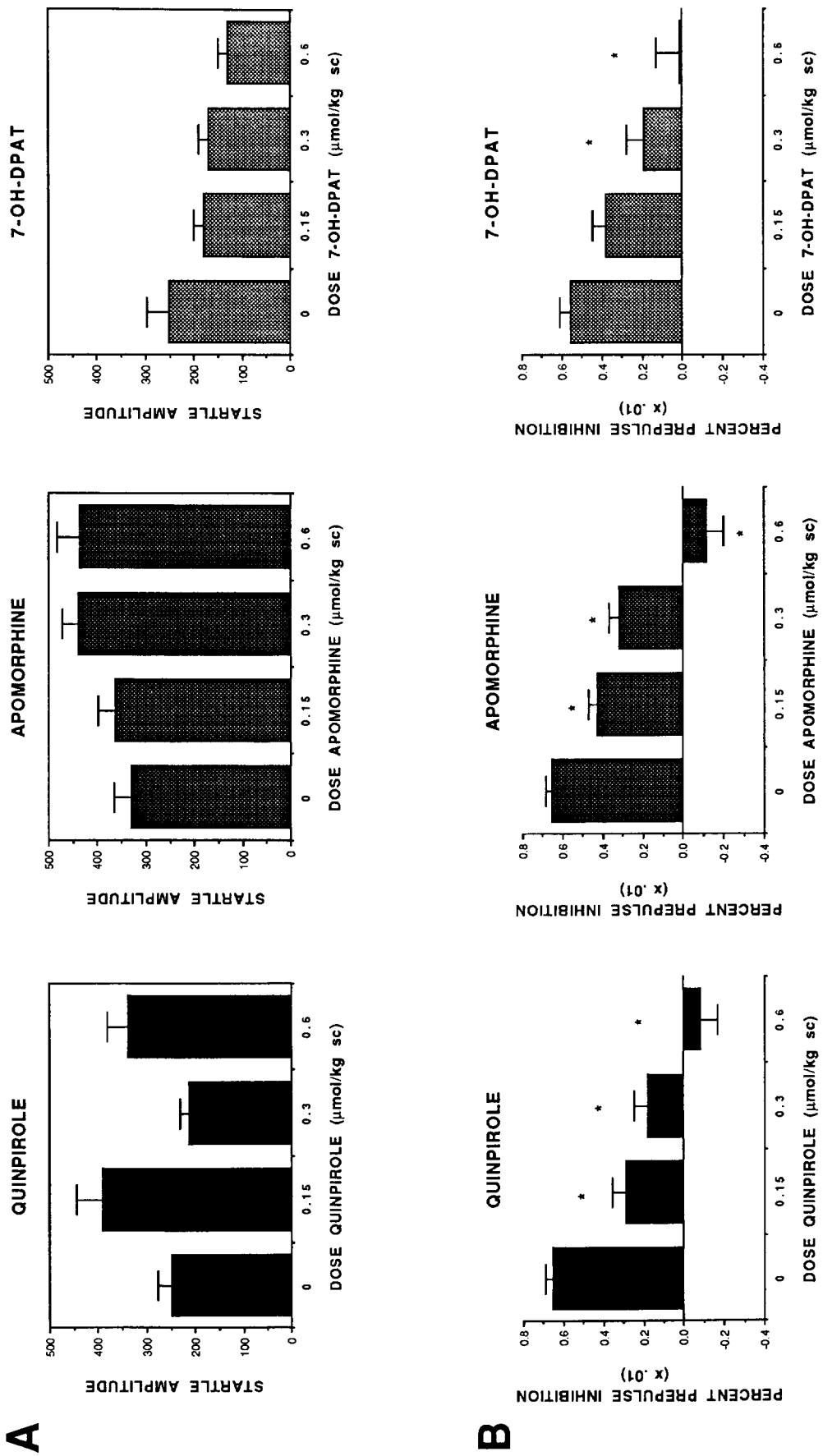

Figure 1. Effects of D_2/D_3 dopamine receptor agonists on the acoustic startle reflex (**A**) and prepulse inhibition of startle (**B**). Note that prepulse inhibition is calculated such that large positive values indicate a high degree of sensorimotor gating (i.e., the prepulse greatly inhibits the response to the startling stimulus). Asterisks indicate significantly ($p < .05$) different from vehicle control following significant main effect of dose (ANOVA).

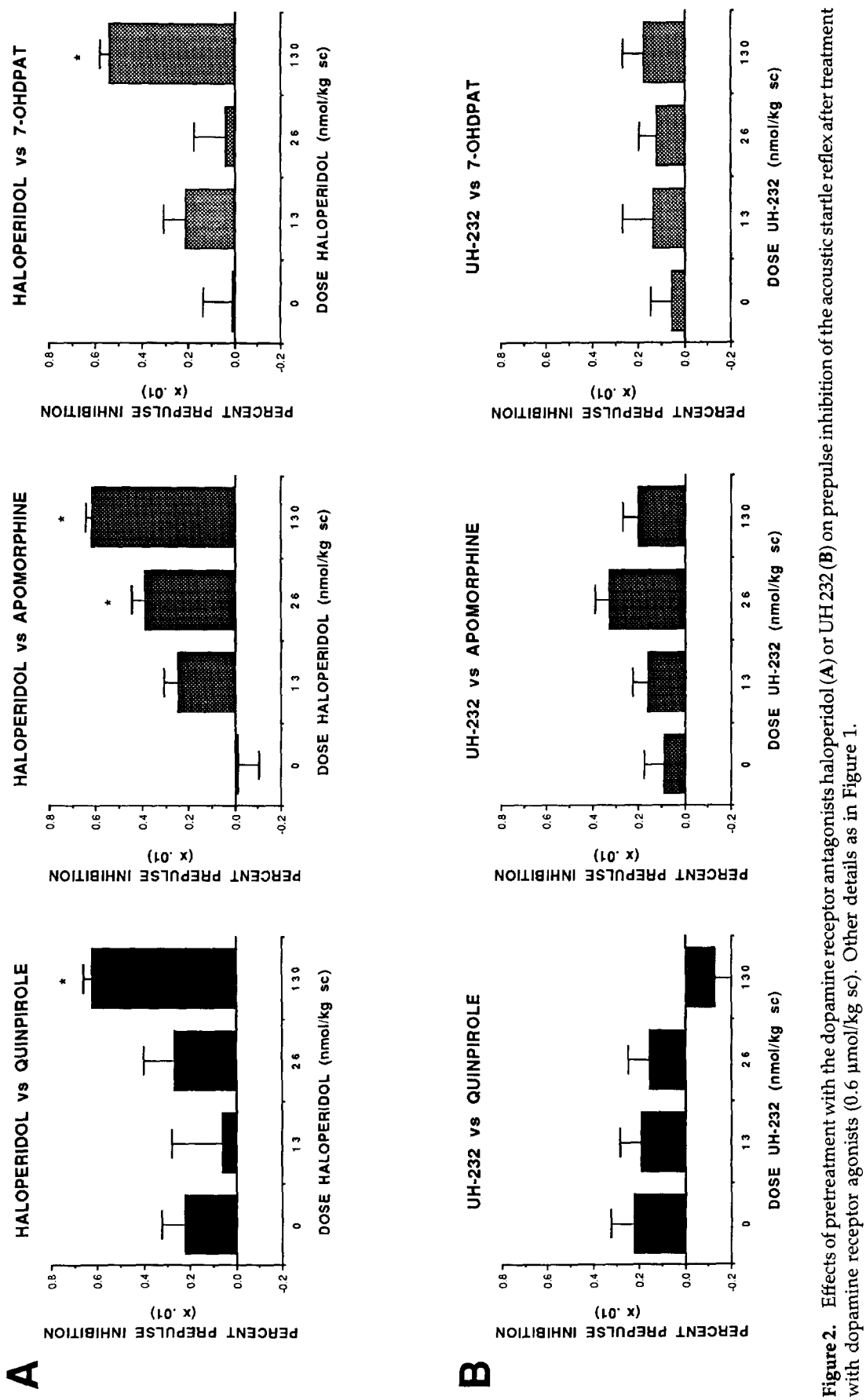

Figure 2. Effects of pretreatment with the dopamine receptor antagonists haloperidol (**A**) or UH 232 (**B**) on prepulse inhibition of the acoustic startle reflex after treatment with dopamine receptor agonists (0.6 µmol/kg sc). Other details as in Figure 1.

Several issues warrant consideration regarding such an interpretation of the present data. First, the affinities of agonists for D_3 versus D_2 receptors are modulated by guanine nucleotides, EDTA, magnesium, and sodium concentrations (Sokoloff et al. 1990; Levesque et al. 1992; Castro and Strange 1993; Boundy et al. 1993; Burris et al. 1994; Chio et al. 1994; Freedman et al. 1994; Tang et al. 1994); therefore, extrapolations from specific in vitro conditions to activity in vivo require caution. Two issues of particular importance are whether the three agonists tested here differ from each other in their affinities for D_3 and D_2 receptors, and whether any of the dopamine agonists (e.g., 7-OH-DPAT, quinpirole) are at all "selective" for D_3 versus D_2 receptors (Sokoloff et al. 1990; Levesque et al. 1992; Boundy et al. 1993; Burris et al. 1994; Chio et al. 1994; Freedman et al. 1994; Tang et al. 1994). Second, given the preliminary nature of information regarding the coupling of D_3 receptors to known dopaminergic effector mechanisms, the functional significance of the D_3 receptor in general remains unknown (Boundy et al. 1993; Freedman et al. 1994; Levesque et al. 1993; Chio et al. 1994). Finally, although the binding profiles of the antagonists haloperidol and UH 232 are not subject to many of the factors that modulate agonist binding, these agents may differ from each other in pharmacokinetic factors. Alternatively, UH 232 has been reported to possess stimulant-like properties, perhaps as a result of its preferential action at dopaminergic autoreceptors (Svennson et al. 1986; Piercey et al. 1992). These properties may account for the differences observed here between UH 232 and the classical neuroleptic haloperidol. Indeed, others have reported UH 232 to be inactive in behavioral tests that are sensitive to classical neuroleptics (Callahan et al. 1992).

In summary, comparisons of the dopamine receptor subtype affinities of three dopamine agonists with their relative potencies to disrupt PPI failed to conclusively implicate either the D_3 or the D_2 receptor in this effect. In contrast, of two antagonists with equal affinities for the D_3 receptor, only the antagonist with 100-fold higher affinity for the D_2 receptor prevented the disruption of PPI by the dopamine agonists, supporting the hypothesis that dopaminergic agents modulate PPI through D_2 receptors. Nevertheless, given the paucity of information regarding D_3 receptor function and the lack of selective ligands to adequately discriminate between D_3 and D_2 receptors in vivo, conclusions drawn from these data need to be considered cautiously. The development of ligands with greater selectivity for specific dopamine receptor subtypes under a wide range of conditions or perhaps the use of molecular or genetic approaches (Gold et al. 1994; Wahlestedt 1994) in combination with behavioral and neurochemical techniques, may help confirm the relative contribution of specific dopamine receptor subtypes to the modulation of PPI. These issues may be important for determining the neural bases of deficient sensorimotor gating in schizophrenic patients, and may facilitate the development of novel pharmacotherapies for the treatment of schizophrenia.

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