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ORIGINAL INVESTIGATION

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8-OH-DPAT disruption of prepulse inhibition in rats: reversal with (+)WAY 100,135 and localization of site of action

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Abstract Recent studies have implicated central serotonergic systems in the modulation of prepulse inhibition (PPI), an operational measure of sensorimotor gating, which has been used to identify gating deficits in psychiatric disorders, such as schizophrenia, Huntington's disease, and obsessive compulsive disorder. Both serotonin (5-HT) releasers and agonists at 5-HT_{1A}, 5-HT_{1B}, and 5-HT_2 receptors reduce PPI in the rat. The present experiments demonstrate that the disruption of PPI in rats induced by the systemic administration of the 5-HT_{1A} agonist, 8-OH-DPAT (8-hydroxy-2(di-*n*-propylamino)tetralin; 0.2 mg/kg), can be attenuated by the novel, selective 5-HT_{1A} antagonist (+)WAY 100,135, (20.0 mg/kg), *N*-tert-butyl -3-(4-(2-methoxyphenyl)-piperazin-1-yl)-2-phenyl-propanamide. Further experiments addressing the central site of action of 8-OH-DPAT revealed that the microinjection of 8-OH-DPAT (5.0 µg/0.5 µl) into either the median raphe nucleus (MR) or dorsal raphe nucleus (DR) disrupts PPI. The reduction in PPI produced by intra-raphe microinjections of 8-OH-DPAT was prevented by a systemic injection of (+)WAY 100,135. These results support the hypothesis that somatodendritic 5-HT_{1A} autoreceptors within the midbrain raphe subserve the PPI-disruptive effects of systemically administered 8-OH-DPAT. The decrement in PPI after intra-raphe infusions of a high dose of 8-OH-DPAT, however, was substantially less than the decrement in PPI after systemic administration of the drug. Hence, sites in addition to the somatodendritic autoreceptors may also play an important role in 8-OH-DPAT-induced disruption of PPI. Together with previous reports that 5-HT releasers and other 5-HT agonists also disrupt PPI, the results support the hypothesis that the serotonergic system modulates PPI through multiple receptor and anatomical systems.

Key words 8-OH-DPAT 8(-hydroxy-2(di-*n*-propylamino)tetralin · (+)WAY 100,135 · 5-HT_{1A} · Median raphe Dorsal raphe · Prepulse inhibition · Startle · Serotonin

Introduction

Prepulse inhibition (PPI) of the startle reflex refers to the reduction of the startle response due to the prior presentation of a stimulus that is below startle threshold. This effect is presumed to reflect sensorimotor gating mechanisms (Braff and Geyer 1990). PPI is of particular interest to clinical researchers because it is deficient in schizophrenia (Braff et al. 1992; Grillon et al. 1992). In addition, evidence suggests that PPI is deficient in Huntington's disease (Swerdlow et al. 1992a) and obsessive compulsive disorder (Swerdlow et al. 1993a). Theoretically, a disturbance in such gating mechanisms may permit the intrusion of unwanted sensory information, behaviors, or thoughts into ongoing behavioral patterns and may manifest itself as clinical syndromes (Swerdlow et al. 1993b).

Studies of PPI in animals have revealed some of the neural substrates underlying sensorimotor gating deficits. This work has demonstrated that multiple anatomical and neurotransmitter systems modulate PPI in the rat. Briefly, a deficit in PPI can be produced in rats by altering dopaminergic activity within the nucleus accumbens and anteromedial striatum (Swerdlow et al. 1992b), cholinergic activity within the hippocampus (HPC) (Caine et al. 1991), or GABAergic activity in the ventral pallidum (Swerdlow et al. 1990). Furthermore, as lesions of the pedunculopontine tegmental nucleus reduce PPI (Swerdlow and Geyer

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1993), this nucleus may be a final common output pathway by which these forebrain structures affect the primary startle circuit (Davis 1984).

Recently, Rigdon (Rigdon and Weatherspoon 1992) and others (Kucharik et al. 1991; Sipes and Geyer 1994) identified a serotonergic contribution to PPI by demonstrating that systemic administrations of 5-HT_{1A} receptor agonists, such as 8-OH-DPAT, ipsapirone and buspirone, disrupt PPI in the rat. The effects of the 5-HT_{1A} agonists could be attenuated with the mixed β-adrenergic/5-HT_1 antagonist propranolol. With the recent introduction of a selective 5-HT_{1A} antagonist, (+)WAY 100,135 (Cliffe et al. 1993), however, it is now possible to determine the pharmacological specificity of the 8-OH-DPAT-induced disruption of PPI. The experiments undertaken here were designed to characterize further the pharmacological specificity and the neural substrates underlying the 8-OH-DPAT-induced disruption of PPI.

One aim of these studies was to identify a possible central site of action for the PPI-disruptive effects of 8-OH-DPAT. Presumably, 8-OH-DPAT acts on the 5-HT_{1A} receptors in the forebrain, which have been found in such structures as the median raphe (MR) and dorsal raphe (DR), HPC, septal nuclei, amygdala, and the cortex (Palacios et al. 1991). The 5-HT_{1A} receptors located in the MR and DR function as somatodendritic inhibitory autoreceptors (Verge et al. 1985), whereas those receptors localized in other structures are postsynaptic. Given the effectiveness of low doses of 8-OH-DPAT to disrupt PPI (Rigdon and Weatherspoon 1992), it was hypothesized that 8-OH-DPAT acts preferentially upon the inhibitory 5-HT_{1A} autoreceptors located in the MR or DR to disrupt PPI.

The cell bodies of the ascending serotonin neurons lie in the midbrain and form two distinct cell groups, MR and DR, which have differential ascending projection patterns (Azmitia and Segal 1978). Some researchers have argued that the different projection systems of the MR and DR may be related to different behavioral functions (Geyer et al. 1976b; Fletcher 1993). For example, tactile startling stimuli have been found selectively to activate the MR but not the DR (Geyer et al. 1982). Anatomically, the MR innervates limbic structures such as the HPC and septal nuclei, forming what has been called the mesolimbic serotonergic pathway (Geyer et al. 1976a). The DR has been shown to innervate the basal ganglia such as the dorsal and ventral striatum (Azmitia and Segal 1978) and nucleus accumbens, forming the mesostriatal serotonergic pathway (Geyer et al. 1976a). While these two systems demonstrate distinct anatomical projection patterns, there exists some overlap in their innervation targets, as both nuclei project to cortical structures and both send projections to the HPC (Azmitia and Segal 1978). Given that the MR is selectively activated by startling stimuli and that components of the limbic system have been implicated in the modulation of PPI (Swerdlow et al.1993b), it was hypothesized that the mesolimbic projection from the MR may be the site which is responsible for the serotonergic involvement in the modulation of PPI. Some of the experiments presented here have appeared in abstract form (Sipes and Geyer 1993).

Materials and methods

Animals

One hundred and twenty male Sprague-Dawley rats (225–250 g, Harlan, Ind.) were used in these studies. Animals were housed in pairs and maintained on a reversed 12 h/12 h light/dark cycle (lights off 0700–1900 hours) with food and water provided ad libitum. Testing occurred during the dark phase, between 0900 and 1500 hours. Animals were handled within 3 days after arrival, and daily thereafter. All animal testing was carried out in accordance with NIH regulations of animal care, as described in "Principles of laboratory animal care" (NIH publication 85–23).

Drugs

8-OH-DPAT (8-hydroxy-2(di-*n*-propylamino)tetralin) was obtained from Research Biochemicals, Natick, Mass. 8-OH-DPAT was dissolved in 0.9% saline and made fresh each test day. (+)WAY 100,135 (*N*-tert-butyl-3-(4-(2-methoxyphenyl)-piperazin-1-yl)-2-phenylpro panamide) was a gift from Wyeth-Ayerst Research, (Princeton, N.J.) and was dissolved in distilled water.

Surgery

Animals were anesthetized with 2.0% halothane in air and placed in a Kopf stereotaxic device with toothbar set level with the interaural line. For injections, stainless steel 23 gauge cannulae were positioned at a 20 degree angle (to the vertical) 3.0 mm above the target structure. Coordinates used for DR were (from interaural point; Paxinos and Watson 1986): AP + 2.0 mm; DV +4.0 mm; and ML 0.0 mm. For MR, the coordinates were: AP + 1.5 mm; DV +2.0 mm; and ML 0.0 mm. Cannulae were held in place with light-cured filled resin (Sun Schein, Henry Schein, Port Washington, N. Y.), anchored with three skull screws, and filled with a removeable stylet wire. Animals were allowed 1 week recovery from surgery before behavioral testing.

Infusions

Intracerebral injections were made by replacing the stylet with a 30 gauge needle fashioned to extend 3.0 mm beyond the tip of the cannulae. A 0.5-μl volume of drug or vehicle was delivered through the injector from a Hamilton syringe via polyethylene tubing at a rate of 1.0 μl/90 s. Injectors were left in place for 1 min after injection to allow for diffusion of drug; after the stylets were replaced, animals were tested within 10 min of injection. For experiment 3, each animal was tested on four occasions with 1 week between injections. For experiment 4, each animal was tested twice. Pilot studies indicated that up to four repeated exposures to the injectors has minimal impact on drug-induced behavioral changes.

Histology

All animals that received intracerebral injections were killed by IP injection of pentobarbital sodium (120 mg/kg). To estimate the extent of drug diffusion, 0.5 µl Evans blue dye was injected into the MR or DR prior to intracardiac infusion of 50 ml 10% formalin. Brains were removed and cannula placements were verified histologically. Animals were removed from the analyses if the extent of dye diffusion was > 2.0 mm from the nucleus of interest, i.e., if the dye diffused to the other nucleus.

Apparatus

Four startle chambers (SR LAB San Diego Instruments, San Diego, Calif.) were used. Each chamber consists of a Plexiglas cylinder 8.2 cm in diameter, resting on a Plexiglas frame in a sound-attenuated, ventilated enclosure. Acoustic noise bursts were presented via a loudspeaker mounted 24 cm above the animal. A piezoelectric accelerometer (Blatek Audio Transducer Model 6030, Blatek) mounted below the frame detects and transduces the motion in the cylinder. Stabilimeter readings were rectified and recorded by microcomputer and interface assembly (San Diego Instruments), with 100 1-ms readings collected beginning at stimulus onset. Startle amplitude was defined as the average of the 100 readings. Cross-modal PPI was measured using acoustic prepulses and a tactile startling stimulus consisting of an air-puff (20 psi, 30 ms duration) directed 2.0 cm above the animal's back.

Test sessions

The test session for experiments 1 and 2 consisted of five blocks with a total of 91 trials. The session consisted of 11 trial types which were presented in pseudorandom order against a continuous background noise of 70 dB. The trial types were as follows: no stimulus; 75 dB prepulse, 85 dB prepulse, 120 dB alone (P 120–1 and P 120–2 for blocks 1 and 2); 105 dB stimulus alone (P 105); 75 dB prepulse followed by 120 dB pulse (pp75p120); 75 dB prepulse followed by 105 dB (pp75p105); 85 dB prepulse followed by 120 dB (pp85p120); puff alone, 75 dB prepulse followed by puff, and 85 dB prepulse followed by puff. Individual trials were separated by a variable intertrial interval averaging 15 s. The interstimulus interval for prepulse + pulse/puff trials was 100 ms. The test session for experiments 3 and 4 comprised three blocks with a total of 45 trials. The first and third blocks consisted of four high pulse intensity trials (p120 dB) and were used to monitor changes in startle reactivity across the test session. The middle block consisted of 37 trials and was designed to assess PPI. Six trial types were presented in pseudorandom order: no stimulus; 105 dB stimulus alone; 120 dB alone; pp75p120; pp75p105; pp85p120.

Data analyses

Behavioral data from experiments 1 were analyzed with a single factor (dose of 8-OH-DPAT) ANOVA. Data from experiment 2 were analyzed by a two-factor ANOVA with dose of (+)WAY 100,135 and dose of 8-OH-DPAT as factors. Data from experiment 3 were analyzed using a mixed ANOVA with site of injection (MR or DR) as between subjects factor and with repeated measures on dose of 8-OH-DPAT. Data from experiment 4 were analyzed with a mixed ANOVA with systemic pretreatment as between subjects factor and with repeated measures on dose of intra-raphe injection. When significant main effects or interactions were revealed in the ANOVA, post hoc comparisons were done with either the Tukey Studentized Range Method (experiments 1 and 2) or Dunnett's test (experiments 3 and 4). The criterion for significance was set at $P < 0.05$. Data used in these analyses were the percent prepulse inhibition (%PPI) score, which was calculated as the difference between pulse alone and prepulse + pulse trials, divided by the pulse alone, multiplied by 100. For example, in experiments 1 and 2, the %PPI measures were calculated as follows: 75–120 = (P120–1 -pp75p120)/P120–1*100; 75–105 = (P105 -pp75p105)/P 105*100; 85–120 = (P 120–2 -pp85p120)/P 120–2*100. Percent scores are typically used in order to minimize the effect of individual variation of startle amplitude on PPI (Mansbach et al. 1988). A large value of the %PPI measure indicates that the prepulse inhibited the response to the startling stimulus, whereas a low value indicates less PPI. Three measures of PPI were employed in these studies: 75–120; 85–120; and 75–105. These measures differ with respect to the intensity of the prepulse (5 or 15 dB above background) and pulse stimuli (105 or 120 dB). Multiple measures using different prepulse and pulse intensities permit assessment of abnormal PPI even in the presence of drug-induced floor and/or ceiling effects on startle reactivity or PPI.

Experiment 1: dose-response of 8-OH-DPAT

Forty naive animals were separated into four groups and given a single subcutaneous (SC) injection of one of four doses of 8-OH-DPAT: 0.0, 0.02, 0.06 or 0.2 mg/kg. Ten minutes after the injection, animals were placed in the startle chamber and allowed to acclimate for 5 min. Testing began immediately after the acclimation period. This procedure was identical for all subsequent experiments.

Experiment 2: (+)WAY 100,135 antagonism of 8-OH-DPAT

Thirty-two naive animals were separated into four groups, with eight animals per group. The dose of 8-OH-DPAT chosen for this experiment (0.2 mg/kg) was an effective dose determined from experiment 1. Animals were pretreated with either saline (n = 16) or 20 mg/kg (+)WAY 100,135 (SC n = 16) 30 min prior to treatment with 0.2 mg/kg 8-OH-DPAT or saline. Animals were placed in the startle chambers 10 min after the treatment injection.

Experiment 3: intra-median and intra-dorsal raphe infusions

To localize the central site of action of the effects of systemically administered 8-OH-DPAT, 32 experimentally naive animals were fitted with an MR (n = 16) or a DR (n = 16) cannula and tested for PPI of the startle response during four, weekly test sessions. Immediately prior to a session, animals received either saline vehicle, 0.5 µg, 1.0 µg or 5.0 µg 8-OH-DPAT in 0.5 µl volume. Every animal received each dose once with order of dose counterbalanced across sessions. Testing of the DR group was identical to MR infusions except that 4 days prior to initial testing, animals were anesthetized and an infusion needle was inserted into the DR to reduce the behavioral reactions to the injection on test day (T. Sipes, unpublished observations).

Experiment 4: (+)WAY 100,135 (systemic) versus intra-median raphe 8-OH-DPAT

To determine if the activation of 5-HT_{1A} autoreceptors was directly responsible for the effects found in experiment 3, systemic administration of (+)WAY 100,135 was employed to antagonize the PPI-disruptive effects of DPAT infused into the MR. Sixteen experimentally naive animals received MR cannulae as in

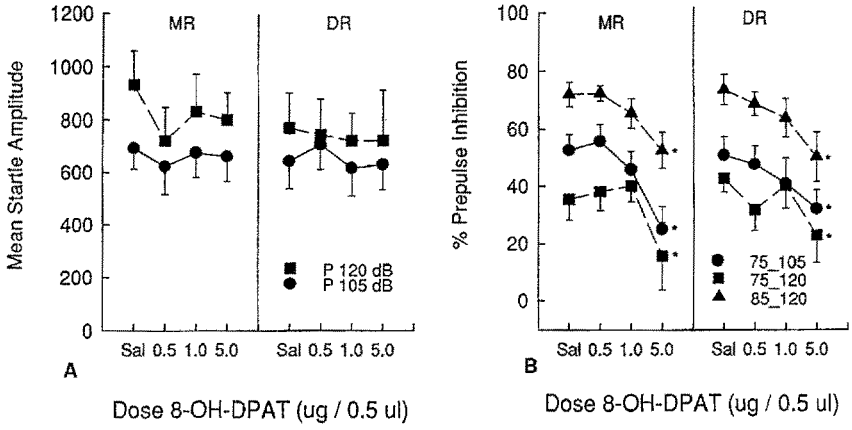


Fig. 1 Effects of systemic administration of 8-OH-DPAT (0.02–0.2 mg/kg) and saline on startle reactivity (**a**) and PPI (**b**). Three measures of startle reactivity are: low intensity (P 105 dB); high intensity block 1; and high intensity block 2 (P 120–1 dB, P 120–2 dB). Three measures of %PPI are: low (75–105: 75 db prepulse followed by 105 dB pulse); high block 1 (75–120: 75 dB prepulse followed by 120 dB pulse); and high block 2 (85–120: 85 dB prepulse followed by 120 dB pulse). Significant differences from saline controls represented by $^*P < 0.05$

experiment 3 and were tested during two weekly sessions. Thirty minutes prior to intra-raphe injection of either saline or 5.0 µg/0.5 µl 8-OH-DPAT, animals were given either 20 mg/kg (+)WAY 100,135 (n = 8) or saline (n = 8). Immediately after the intra-raphe injection, animals were placed in the startle chambers and the test session began. The following week, animals received the identical pretreatment but reversed treatment.

Results

The experimental design included measures of tactile startle and cross-modal PPI. Because no significant drug effects were found on these measures at the doses and parameters used, these data are not described.

Experiment 1: 8-OH-DPAT dose dependently disrupts PPI

The effects of 8-OH-DPAT on PPI and startle reactivity are presented in Fig. 1. Treatment with the 5-HT_{1A} agonist 8-OH-DPAT (0.0–0.2 mg/kg) failed to affect startle reactivity to high intensity pulse in block 1 [P 120–1: $F(3,36) = 1.58$]. Nevertheless, animals treated with 8-OH-DPAT demonstrated an increase in startle reactivity to the high intensity pulse in block 2 [P 120–2: $F(3,36) = 3.01$, $P < 0.05$] and to the low intensity pulse in block 3 (P 105: $F(3,36) = 4.30$, $P < 0.01$). Animals treated with 8-OH-DPAT demonstrated a significant disruption of PPI, as seen in Fig. 1b, for all three measures of %PPI: 75–105: $F(3,36) = 11.02$, $P < 0.001$; 75–120: $F(3,36) = 10.36$, $P < 0.001$; and 85–120: $F(3,36) = 6.41$, $P < 0.01$.

Experiment 2: (+)WAY 100,135 antagonizes the effect of 8-OH-DPAT on PPI

The results of (+)WAY 100,135 versus 8-OH-DPAT on startle reactivity and %PPI are shown in Fig. 2. In contrast to the dose-response study, animals treated with 8-OH-DPAT (0.2 mg/kg) failed to show an alteration in startle reactivity to the high intensity pulse in block 1 or block 2 [P 120–1: $F(1,28) = 0.04$; P 120–2: $F(1,28) = 0.21$] or to the low intensity pulse [P 105: $F(1,28) = 0.59$]. Similarly, the 5-HT_{1A} antagonist (+)WAY100,135 (20.0 mg/kg) had no effect on responses to the pulse-alone stimuli: P 120–1: $F(1,28) = 0.50$; P 120–2: $F(1,28) = 0.32$; P 105: $F(1,28) = 1.39$. Animals treated with 8-OH-DPAT demonstrated a significant disruption on all measures of %PPI: 75–105: $F(1,28) = 8.49$, $P < 0.01$; 75–120: $F(1,28) = 9.18$, $P < 0.01$; and 85–120: $F(1,28) = 9.33$, $P < 0.01$. Pretreatment with (+)WAY 100,135 successfully attenuated the disruptive effects of 8-OH-DPAT on %PPI as indicated by significant interactions: 75–105: $F(1,28) = 20.02$, $P < 0.001$; 75–120: $F(1,28) = 8.22$, $P < 0.01$; and 85–120: $F(1,28) = 14.44$, $P < 0.001$.

Experiment 3: 8-OH-DPAT infusion into either MR or DR disrupts PPI

Data from five animals in the DR group and three animals in the MR group were removed prior to analyses because of irregular dye diffusion and cannulae placement. The location of the cannula in the remaining animals can be seen in Fig. 3a. The effects of 8-OH-DPAT infusion into both nuclei on PPI and startle reactivity are summarized in Fig. 4. The infusion of 8-OH-DPAT (0.0–5.0 µg/0.5 µl) into MR or DR had no effect on startle reactivity: P 120: $F(3,66) = 0.48$; P 105: $F(3,66) = 0.05$. Infusion of 8-OH-DPAT into MR or DR disrupted all measures of %PPI: 75–105: $F(3,66) = 6.79$, $P < 0.001$; 75–120: $F(3,66) = 3.59$, $P < 0.01$; and 85–120: $F(3,66) = 7.72$, $P < 0.001$. There were neither significant differences between site of injection (MR versus DR) nor

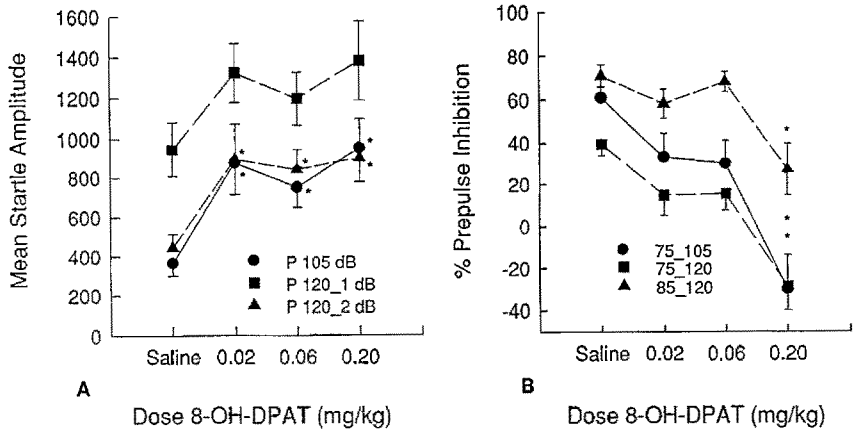


Fig. 2 Effects of systemic administration of 8-OH-DPAT (0.20 mg/kg) and saline versus (+)WAY 100,135 (20.0 mg/kg, SC) on startle reactivity (**a**) and PPI (**b**). Three measures of startle reactivity are: low intensity (P 105 dB); high intensity block 1; and high intensity block 2 (P 120–1 dB, P 120–2 dB). Three measures of %PPI are: low (75–105: 75 dB prepulse followed by 105 dB pulse); high block 1 (75–120: 75 dB prepulse followed by 120 dB pulse); and high block 2 (85–120: 85 dB prepulse followed by 120 dB pulse). Significant differences from corresponding treatment control represented by * and significant differences from corresponding pretreatment control represented by #. Level of significance is set at $P < 0.05$

interactions with dose of 8-OH-DPAT on startle reactivity or PPI.

Experiment 4: systemic (+)WAY 100,135 prevents intra-raphe 8-OH-DPAT disruption of PPI

The location of the cannula in these animals can be seen in Fig. 3b. The results of (+)WAY 100,135 versus 8-OH-DPAT on startle reactivity and %PPI are shown in Fig. 5. Again, animals treated with 8-OH-DPAT (5.0 µg/0.5 µl) failed to show an alteration in startle reactivity to either the high [P 120: $F(1,12) = 0.00$] or the low intensity pulse [P 105: $F(1,12) = 0.12$]. As expected, the 5-HT_{1A} antagonist (+)WAY 100,135 (20.0 mg/kg) failed to affect startle reactivity to either stimulus: P 120: $F(1,12) = 0.03$; P 105: $F(1,12) = 0.11$. Animals treated with 8-OH-DPAT demonstrated a significant disruption on all measures of %PPI: 75–105: $F(1,12) = 5.52$, $P < 0.05$; 75–120: $F(1,12) = 8.39$, $P < 0.05$; and 85–120: $F(1,12) = 6.66$, $P < 0.05$. As expected from experiment 2, (+)WAY 100,135 pretreatment successfully attenuated the disruptive effects of intra-raphe 8-OH-DPAT on all three measures of %PPI, as confirmed by post hoc comparisons (Fig. 5b).

Discussion

The experiments presented in this paper were designed to further characterize the ability of the 5-HT_{1A} agonist, 8-OH-DPAT, to disrupt PPI of the acoustic startle response. Specifically, these studies found that: (1) the novel 5-HT_{1A} antagonist (+)WAY 100,135 attenuated the PPI-disruptive effects of 8-OH-DPAT; and (2) the activation by 8-OH-DPAT of the 5-HT_{1A} autoreceptors localized within the MR and DR disrupts PPI. These findings confirm and extend the previous findings by Rigdon and Weatherspoon (1992) that 5-HT_{1A} agonists disrupt PPI in rats. The findings also corroborate the report of Kucharik et al. (1991), indicating that the selective 5-HT_{1A} antagonist (+)WAY 100,135 prevents the disruption of PPI

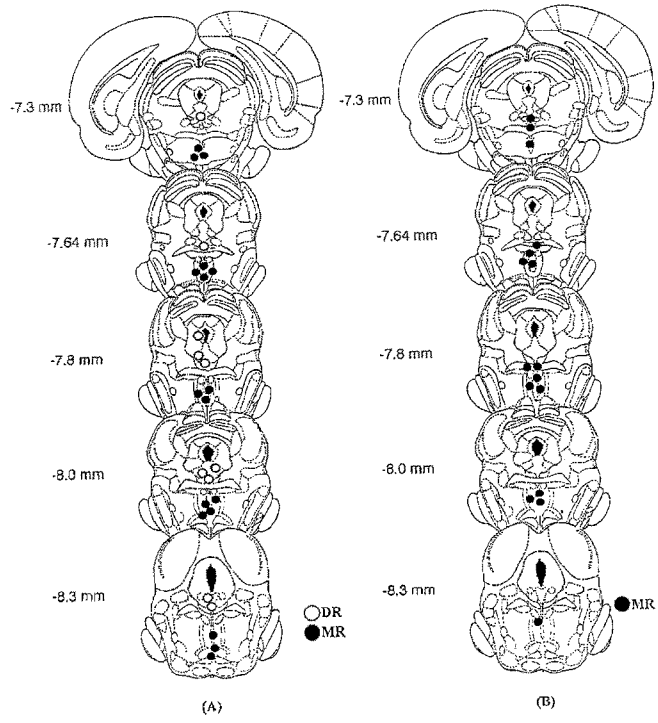


Fig. 3 Location of the cannula tips for infusion of 8-OH-DPAT into the DR and MR for experiment 3 (**a**). Location of the cannula tips for infusion of 8-OH-DPAT into the MR for experiment 4 (**b**). Brain plates obtained and modified from Paxinos and Watson (1986)

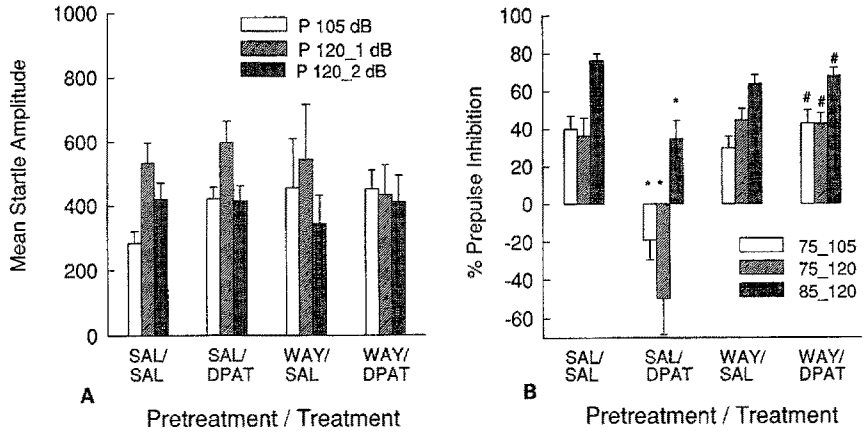


Fig. 4 Effects of 8-OH-DPAT (0.5–5.0 μg/0.5 μl) infused into the DR or MR on startle reactivity (**a**) and PPI (**b**). Two measures of startle reactivity are: low intensity (P 105 dB); and high intensity (P 120 dB). Three measures of %PPI are: low (75–105: 75 dB prepulse followed by 105 dB pulse); high block 1 (75–120: 75 dB prepulse followed by 120 dB pulse); and high block 2 (85–120: 85 dB prepulse followed by 120 dB pulse). Significant main effect represented by $^*P < 0.05$

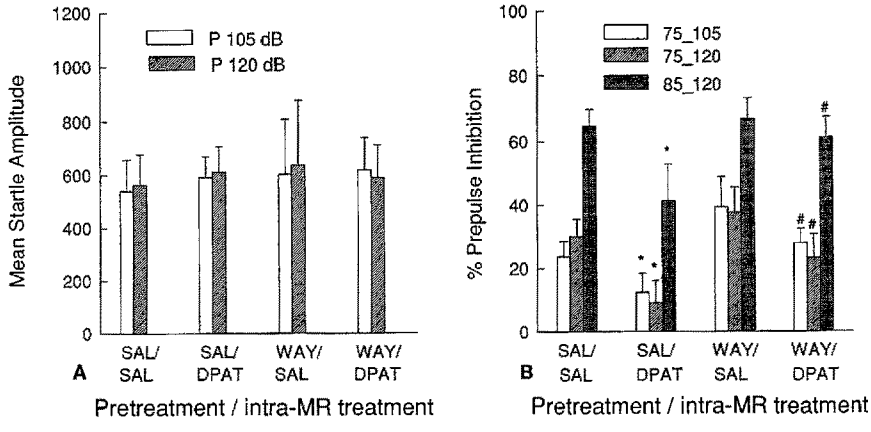
induced by 8-OH-DPAT, and extend this observation to another strain of rats. In combination with findings indicating that serotonin releasers (Mansbach et al. 1989) or the activation of 5-HT_{1B} and 5-HT_2 serotonin receptor subtypes similarly reduce the amount of PPI (Sipes and Geyer 1994), these results support the hypothesis that central serotonergic systems play an important role in the modulation of sensorimotor gating in the rat.

In concert with the reports of others (Kucharik et al. 1991; Rigdon and Weatherspoon 1992), the present experiments found that low doses of 8-OH-DPAT appear to be specific to the inhibition of startle by prepulse stimuli as opposed to a general effect on startle reactivity. Experiments 2–4 demonstrate the consistent PPI-disruptive effects of 8-OH-DPAT, but fail to show consistent drug-induced alterations in startle reactivity to low or high intensity startling stimuli. Similarly, results of the first experiment demonstrate the potent PPI-disruptive effects of 8-OH-DPAT in all blocks of trials despite an increase in the startle response only late in the session, during blocks 2 and 3.

The identification of the serotonin receptor subtypes that are responsible for the behavioral effects of serotonergic agonists such as 8-OH-DPAT has been hampered by the lack of a suitably selective 5-HT_{1A} antagonist. The present experiments found that the selective 5-HT_{1A} antagonist, (+)WAY 100,135 prevented the disruption in PPI by 8-OH-DPAT, without producing effects on startle or PPI by itself. Because (+)WAY 100,135 displays greater selectivity for the 5-HT_{1A} receptor than other serotonergic, adrenergic or dopaminergic receptors (Fletcher 1991), these results provide the strongest evidence to date that the PPI-disruptive effects of 8-OH-DPAT are attributable to its actions as a 5-HT_{1A} agonist.

A central aim of these studies was to identify a component of the serotonergic system responsible for the PPI-disruptive effects of 8-OH-DPAT. It was hypothesized that the effectiveness of low doses of systemically administered 8-OH-DPAT to reduce PPI was due to the activation of 5-HT_{1A} inhibitory autoreceptors localized within the MR or the DR. More specifically,

Fig. 5 Effects of 8-OH-DPAT (5.0 μg/0.5 μl) infused into the MR versus systemic (+)WAY 100,135 (20.0 mg/kg) on startle reactivity (**a**) and PPI (**b**). Two measures of startle reactivity are: low intensity (P 105 dB); and high intensity (P 120 dB). Three measures of %PPI are: low (75–105: 75 dB prepulse followed by 105 dB pulse); high block 1 (75–120: 75 dB prepulse followed by 120 dB pulse); and high block 2 (85–120: 85 dB prepulse followed by 120 dB pulse). Significant differences from corresponding treatment control represented by * and significant differences from corresponding pretreatment control represented by #. Level of significance is set at $P < 0.05$



it was hypothesized that activation of 5-HT_{1A} receptors within the MR, which forms a mesolimbic serotonergic pathway, but not 5-HT_{1A} receptors within the DR, which forms the mesostriatal serotonergic pathway (Geyer et al. 1976a), was responsible for the 8-OH-DPAT-induced PPI deficit. It was found, however, that infusion of 8-OH-DPAT into *either* the MR or DR disrupted PPI. While this result does not support the specific hypothesis that activation of the 5-HT_{1A} receptors within the mesolimbic serotonergic system is solely responsible for the 8-OH-DPAT-induced PPI deficit, it does support the general conclusion that activation of 5-HT_{1A} autoreceptors is responsible for the disruption of PPI by intra-raphe injections of 8-OH-DPAT.

Because the PPI-disruptive effects of 8-OH-DPAT injected into either the MR or DR were found at only the highest dose tested (5.0 μg/0.5 μl), the possibility was considered that the PPI deficit may have been due to a non-specific effect of 8-OH-DPAT. Systemic administration of (+)WAY 100,135, however, prevented the PPI-disruptive effects of 8-OH-DPAT infused into the MR. This result indicates that it is the activation of the inhibitory 5-HT_{1A} autoreceptors within the MR, and not a non-specific effect, which is responsible for the 8-OH-DPAT-induced PPI deficit.

The finding that infusion of 8-OH-DPAT into either the MR or the DR disrupts PPI fails to support the hypothesis that there exists a functional (e.g., behavioral) distinction between these nuclei. Although great care was taken during these studies to exclude animals based on the diffusion of dye away from the target structure, it is possible that diffusion of 8-OH-DPAT away from the DR may have affected the MR, thus leading to disruption of PPI. This reasoning, however, must also be applied to the MR infusion of 8-OH-DPAT, to support a DR site of action. Another possibility is that infusion into either nucleus permits the diffusion of 8-OH-DPAT to some other, unknown structure in the vicinity of the MR and DR, to disrupt PPI. This latter possibility is currently being examined. Another hypothesis is that 8-OH-DPAT -induced alteration of the neuronal activity of the MR and DR leads to a disruption in PPI because both the MR and DR project to a common output structure, which is responsible for the serotonergic modulation of PPI.

Because infusion of 8-OH-DPAT into either the MR or DR disrupts PPI, the results suggest a possible overlap between these two nuclei in their influences on sensorimotor gating. Anatomically, such a hypothesis may be supported by the combined innervation of the HPC by the MR, which projects to the dorsal HPC, and the DR, which projects to the ventral HPC (Azmitia and Segal 1978). Given that intra-raphe infusions of 8-OH-DPAT disrupt PPI, the hypothesis may be entertained that the disruption of PPI is due to a decrease in serotonergic tone in the HPC. Raphe infusions of 8-OH-DPAT have been shown to decrease electrophysiological activity of serotonergic neurons (Sprouse and Aghajanian 1987) and decrease serotonin release in terminal areas such as the HPC (Sharp et al. 1989; Bonvento et al.1992). Thus, it is conceivable that 8-OH-DPAT disrupts PPI via a decrease in serotonergic activity in the HPC. Further explorations of the neural substrates responsible for the 8-OH-DPAT disruption of PPI will be needed to examine these additional hypotheses.

Interest in the possible role for serotonin dysfunction in the pathophysiology of schizophrenia has increased in the last few years. Serotonergic mechanisms have been hypothesized to underlie the efficacy of atypical antipsychotics such as clozapine, which has greater binding affinity for serotonin receptors than for dopamine receptors (Meltzer and Nash 1991). Of particular interest to the present studies are indications that an increase in the number of serotonin uptake sites and 5-HT_{1A} and 5-HT_{2A} receptors have been found in the nucleus accumbens, striatum and hippocampi of schizophrenic patients (Joyce et al. 1993).

In summary, the ascending serotonergic projections arising from the MR and DR modulate sensorimotor gating as evidenced by the disruption of PPI by the local infusion of the 5-HT_{1A} receptor agonist, 8-OH-DPAT. The novel compound, (+)WAY 100,135, demonstrates its 5-HT_{1A} antagonist ability by attenuating the PPI-disruptive effects of 8-OH-DPAT. Finally, it appears that multiple serotonergic pathways and serotonergic receptor subtypes are involved in the modulation of PPI. Further study of the serotonergic involvement in PPI should help to clarify the role of this neurotransmitter in the pathophysiology of schizophrenia and related psychiatric disorders.

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