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Effects of scopolamine in comparison with apomorphine and phencyclidine on prepulse inhibition in rats

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Abstract

The potential involvement of the muscarinic cholinergic system in the underlying mechanisms of prepulse inhibition of the acoustic startle reflex was evaluated in male Sprague–Dawley rats under conditions of varying dose, prepulse intensity, and interstimulus interval. The effects of scopolamine on prepulse inhibition were also directly compared with the effects observed using apomorphine and phencyclidine under the same test parameters. Scopolamine (0.03–1.0 mg/kg) produced a significant dose-dependent decrease in prepulse inhibition, but had no effect on startle amplitude over the dose range tested. Apomorphine (0.03–1.0 mg/kg) and phencyclidine (0.1–5.6 mg/kg) produced significant dose-dependent decreases in prepulse inhibition and changes in startle amplitude. The scopolamine-induced decrease in prepulse inhibition varied with prepulse intensity in that the changes produced by scopolamine became smaller in magnitude as the prepulse intensity was increased from 9 to 30 dB above background. On the other hand, apomorphine and phencyclidine decreased prepulse inhibition to approximately the same magnitude across all prepulse intensities tested. The observed decreases in prepulse inhibition produced by scopolamine, apomorphine, and phencyclidine were also dependent on interstimulus interval duration. Scopolamine produced marked decreases in prepulse inhibition at the 100- and 300-ms interstimulus interval durations, but had little or no effect on prepulse inhibition at the 30- and 1000-ms interstimulus interval durations. In contrast, apomorphine decreased prepulse inhibition across all interstimulus interval durations while phencyclidine decreased prepulse inhibition across the 30- to 300-ms interstimulus interval durations. The present findings support the hypothesis that the muscarinic cholinergic system, like the dopaminergic and glutamatergic systems, is directly involved in the mechanisms of prepulse inhibition. However, these three neurotransmitter systems appear to modulate different aspects of prepulse inhibition.

Keywords: Scopolamine; Muscarinic acetylcholine receptor; Prepulse inhibition; Startle; Apomorphine; Phencyclidine; Dopamine receptor; Glutamate receptor; (Rat)

1. Introduction

Prepulse inhibition of the acoustic startle reflex is an inhibitory mechanism for the gating or filtering of sensory acoustic stimuli. Prepulse inhibition is functionally defined as the reduction in startle response produced by a low intensity stimulus presented before a high intensity, startle-inducing stimulus (Ison and Hammond, 1971; Graham, 1975; Hoffman and Ison, 1980). Failure of prepulse inhibition is speculated to account, at least in part, for the sensory information overload and subsequent fragmentation of normal cognitive functions observed in a number of psychiatric conditions including schizophrenia and schizotypal personality disorder (Braff et al., 1978, 1992; Cadenhead et al., 1993).

While the exact neuronal substrates mediating prepulse inhibition remain unclear, several neurotransmitter systems have been linked to the underlying mechanisms of this sensory information gating process. For example, systemic administration of dopamine receptor agonists such as apomorphine and quinpirole produce dose-related decreases in prepulse inhibition which can be reversed by dopamine

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receptor antagonists, demonstrating that the dopaminergic system is directly involved in mediating prepulse inhibition (Mansbach et al., 1988; Peng et al., 1990; Swerdlow and Geyer, 1993a). Noncompetitive NMDA receptor antagonists, including phencyclidine and dizocilpine, also disrupt prepulse inhibition (Mansbach and Geyer, 1989), indicating that the glutamatergic system is also directly involved in the mechanisms of prepulse inhibition.

In addition to the dopaminergic and glutamatergic systems, limited evidence suggests that the muscarinic cholinergic system may also be involved in the mechanisms of prepulse inhibition of the acoustic startle reflex. Wu et al. (1993) found that the nonselective muscarinic receptor antagonist scopolamine, at a single dose of 1.0 mg/kg, produced a significant decrease in prepulse inhibition. Further, chronic treatment with the cholinergic false precursor *N*-aminodeanol and a choline-free diet produced significant reductions in prepulse inhibition as well as corresponding decreases in brain acetylcholine levels of greater than 60%, and this decrease in prepulse inhibition was partially reversed by the muscarinic receptor agonist arecoline (Wu et al., 1993). In addition, lesions of the pedunculopontine tegmental nucleus, a primarily cholinergic brainstem nucleus expressing numerous muscarinic receptor subtypes, also attenuate prepulse inhibition (Koch et al., 1993; Swerdlow and Geyer, 1993b). Taken together, these data suggest that the muscarinic cholinergic system may have a role in mediating prepulse inhibition.

In order to further assess the involvement of the muscarinic cholinergic system in mediating prepulse inhibition, one purpose of the present study was to systematically evaluate the effects of the muscarinic receptor antagonist scopolamine on prepulse inhibition of the acoustic startle reflex. To this end, a complete dose–response curve was determined for the effects of scopolamine on prepulse inhibition and startle amplitude. In addition, dose–response curves were determined for the effects of scopolamine on prepulse inhibition under conditions of varying prepulse intensity (9, 20, 30 dB [A] above background) and interstimulus interval (interstimulus interval durations; 30, 100, 300, 1000 ms). Another purpose of the present study was to compare the effects of scopolamine on prepulse inhibition with the effects observed using apomorphine and phencyclidine, two drugs which have been well-documented to affect prepulse inhibition (e.g., Mansbach and Geyer, 1989; Mansbach et al., 1988). Thus, we also determined dose–response curves for the effects of apomorphine and phencyclidine on both prepulse inhibition and startle amplitude, as well as dose–response curves for these two drugs on prepulse inhibition under conditions of varying prepulse intensity and interstimulus interval duration. Data from the present direct, side-by-side comparisons of scopolamine with apomorphine and phencyclidine indicate that the muscarinic cholinergic system modulates prepulse inhibition, but in a manner different from either the dopaminergic or glutamatergic systems.

2. Materials and methods

2.1. Subjects

Adult male Sprague–Dawley rats (Harlan Sprague–Dawley, Indianapolis, IN) weighing 325–350 g, were housed in pairs in a large colony room under a 12-h light/dark cycle (lights on at 0600 h). Each rat was maintained on 15 g of food per day with water ad libitum. Test sessions were performed between 0800 and 1800 h. Each rat was used in three to five experiments with at least a 1-week interval between test sessions. The repeated use of each rat was balanced according to their previous drug and testing history. All experiments were conducted in accordance with the NIH regulations of animal care covered in ''Principles of Laboratory Animal Care'', NIH publication 85-23 and were approved by the Institutional Animal Care and Use Committee.

2.2. Apparatus

All test sessions were performed in a Coulbourn acoustic startle apparatus (Coulbourn Instruments, Allentown, PA) consisting of two ventilated, sound attenuated chambers with four force transducer platforms per chamber. Data were recorded on-line utilizing a Compac Deskpro 386 computer (Compac Computer) and Lablinc interface modules (Coulbourn Instruments), with 200, 1-ms readings collected beginning at trial onset.

2.3. Procedure

All rats were adapted to the startle chambers for 30 min on each of two consecutive days. On the third day, in order to pre-expose each rat to the acoustic stimuli before the first drug test session, rats were placed in the startle chambers and, after a 5-min acclimation period, presented with a test session consisting of eight counterbalanced presentations of the following four trial types (total of 32 trials/session): no stimulus, startle pulse alone (106 dB [A] 20 ms broad band burst), prepulse tone alone (77 dB [A] 20 ms, 10 kHz), and prepulse + startle pulse. The intertrial interval was varied pseudorandomly between 15 and 45 s. The interstimulus interval duration was 120 ms. An ambient background noise of 50 dB [A] was present throughout the test session. Identical test sessions were used for the dose–response studies.

In the prepulse intensity studies, after a 5-min acclimation period, rats were exposed to six counterbalanced presentations of the following six trial types (total of 36 trials/session): no stimulus, startle pulse alone (106 dB [A] 20 ms broad band burst), prepulse tone alone (59 dB [A], 10 kHz, 20 ms), and three prepulse (59, 70, or 80 dB [A] 20 ms, 10 kHz) + startle pulse combinations. The intertrial interval was varied between 15 and 45 s, the interstimulus interval duration was 120 ms, and the ambi-

ent background noise was 50 dB [A]. In a separate experiment, the effect of prepulse tones presented alone on startle amplitude was evaluated. Rats were exposed to five counterbalanced presentations of the following four trial types (total of 20 trials/session): no stimulus or a prepulse tone alone (59, 70, or 80 dB [A] 20 ms, 10 kHz). The intertrial interval was varied between 15 and 45 s and the ambient background noise was 50 dB [A].

For the interstimulus interval studies, after a 5-min acclimation period, rats were exposed to seven counterbalanced presentations of each of the following seven trial types (total of 49 trials/session): no stimulus, startle pulse alone (106 dB [A] 20 ms broad band burst), prepulse tone alone (77 dB [A], 10 kHz), and prepulse + startle pulse trials with the following four interstimulus interval durations (30, 100, 300, 1000 ms). The intertrial interval was varied between 15 and 45 s and the ambient background noise was 50 dB [A]. Interstimulus interval duration was defined as the interval from the prepulse tone offset to the startle pulse onset.

2.4. Drugs

R(−)-Apomorphine hydrochloride (Research Biochemicals International, Natick, MA) was administered s.c. 5 min before the start of a test session. (−)-Scopolamine hydrobromide (Sigma, St. Louis, MO) and phencyclidine hydrochloride (Lilly Research Laboratories, Indianapolis, IN) were injected s.c. 30 min prior to testing. All doses refer to the salt and were injected in a 1.0 ml/kg volume s.c. Scopolamine and phencyclidine were dissolved in double deionized water; apomorphine, freshly prepared within 30 min of administration, was dissolved in a minimal amount of lactic acid and diluted to the appropriate concentration with deionized water.

2.5. Data analysis

Startle amplitude was defined as the peak of the 200 1-ms readings. For the dose–response studies, data were analyzed by a between-groups analysis of variance (ANOVA) with comparison to the vehicle control group using a Dunnett's test and/or a Student's *t*-test. A repeated measures two-way ANOVA with prepulse intensity as a within-subjects factor was performed on data collected in the prepulse intensity studies. For the interstimulus interval studies, data were analyzed by a repeated measures two-way ANOVA with interstimulus interval as a within-subject factor. Prepulse inhibition was calculated using the following equation: $100 \times [(\text{mean startle amplitude in startle pulse trials} - \text{mean startle amplitude in prepulse + pulse trials})/(\text{mean startle amplitude in startle pulse trials})]$. Calculations were performed using JMP v 3.2 (SAS Institute, Cary, NC) statistical software.

3. Results

3.1. Dose–response studies

Scopolamine produced a dose-dependent decrease in prepulse inhibition with doses of 0.3 and 1.0 mg/kg producing a significant disruption of prepulse inhibition (Fig. 1, upper left panel). However, scopolamine had no effect on startle amplitude over the dose range tested (Fig. 1, lower left panel). Apomorphine also produced a dose-dependent decrease in prepulse inhibition with a significant disruption of prepulse inhibition at doses of 0.3 and 1.0 mg/kg (Fig. 1, upper middle panel). Startle amplitude was modestly, but not significantly, increased by the lowest dose of apomorphine tested (0.03 mg/kg); at higher doses, apomorphine produced a decrease in startle amplitude which was non-significant by Dunnett's test but was significant at the 0.3 mg/kg dose by a Student's *t*-test compared to the vehicle control group (Fig. 1, lower middle panel). Phencyclidine also produced a dose-dependent decrease in prepulse inhibition, with doses of 3.0 and 5.6 mg/kg producing a significant disruption of prepulse inhibition (Fig. 1, upper right panel). Startle amplitude was first increased and then decreased with increasing doses of phencyclidine (Fig. 1, lower right panel): startle amplitude was significantly increased at 0.3 and 1.0 mg/kg by Dunnett's test; a dose of 5.6 mg/kg of phencyclidine non-significantly decreased amplitude by Dunnett's test, but significantly decreased amplitude by a Student's *t*-test compared with the vehicle control group.

3.2. Prepulse intensity studies

In rats administered vehicle alone, there was an increase in the magnitude of prepulse inhibition as prepulse inten-

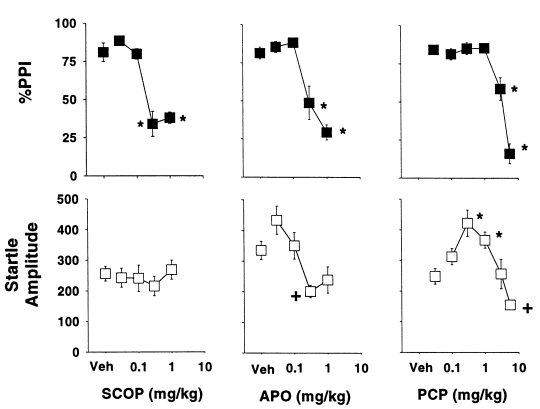

Fig. 1. Dose–response curves for scopolamine, apomorphine, and phencyclidine on prepulse inhibition (upper panels) and startle amplitude (lower panels). Each point represents the mean of eight rats. The vertical lines represent ±S.E.M. and are absent when less than the size of the point. (Abscissa) Dose of drug in milligrams/kilogram. (Ordinates) Upper panels, percentage of prepulse inhibition; lower panels, startle amplitude in arbitrary units. $^{*}P < 0.05$ vs. vehicle, Dunnett's test. $^{+}P < 0.05$ vs. vehicle, Student's *t*-test.

sity was increased from 9 to 30 dB relative to the 50 dB [A] ambient background noise. For example, in the vehicle-treated groups for the scopolamine dose–response curve, there was an increase in the magnitude of prepulse inhibition from approximately 60% to 85% to 90% at 9, 20, and 30 dB above background, respectively (Fig. 2, left panel, points above vehicle). As shown in Table 1, the prepulse tones of 9, 20, and 30 dB above background used in the present studies did not produce a significant startle response when presented alone.

Scopolamine produced a dose-dependent decrease in prepulse inhibition at each of the three prepulse intensities tested, which was significant after 0.3 and 1.0 mg/kg (Fig. 2, left panel). The magnitude of the scopolamine-induced decrease in prepulse inhibition varied as a function of the magnitude of the prepulse intensity, as confirmed by a significant dose × prepulse intensity interaction [$F(8,70) = 3.6$, $P = 0.0015$]. For example, the difference between the magnitude of prepulse inhibition after the 0.3 mg/kg dose and the magnitude of prepulse inhibition after vehicle was approximately 50%, 45%, and 30% at 9, 20, and 30 dB above background. Higher doses of scopolamine were not tested due to increased motor behaviors, which were incompatible with the accurate measurement of prepulse inhibition. Scopolamine had no significant effect on startle amplitude over the dose range tested in the prepulse intensity studies (Table 2).

Apomorphine also produced a dose-dependent decrease in prepulse inhibition at each of the three prepulse intensities tested, which was significant after doses of 0.1 mg/kg (9 dB) or 0.3 mg/kg and higher (all three prepulse intensities) (Fig. 2, middle panel). The magnitude of the apomorphine-induced disruption of prepulse inhibition was not dependent on prepulse intensity in that, at the highest doses tested, prepulse inhibition was reduced to less than 25% irrespective of prepulse intensity (Fig. 2, middle panel). However, there was a significant dose × prepulse intensity interaction [$F(10,76) = 3.8$, $P = 0.0004$]. This interaction was most likely due to a floor effect in the disruption of prepulse inhibition observed over the 0.3 to 2.0 mg/kg dose range of apomorphine at the prepulse intensity of 9 dB above background. Apomorphine significantly decreased startle amplitude after doses of 0.03 to 1.0 mg/kg in the present experiment (Table 2).

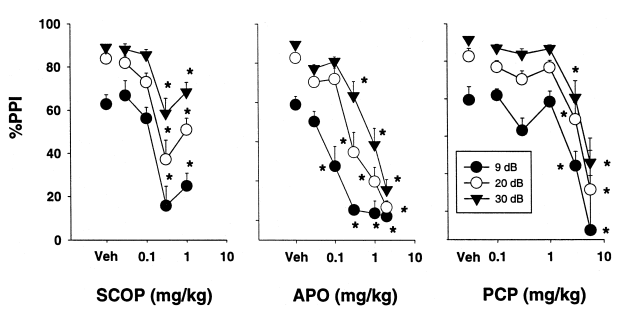

Fig. 2. The effects of varying the intensity of the prepulse stimulus on the dose-related effects of scopolamine-, apomorphine-, and phencyclidine-produced decreases in prepulse inhibition. Each point represents the mean of eight rats. The vertical lines represent ±S.E.M. and are absent when less than the size of the point. (Abscissa) Dose of drug in milligrams/kilogram. (Ordinate) Percentage of prepulse inhibition. $^{*}P < 0.05$ vs. respective control group (Dunnett's).

Table 1
The lack of effects of prepulse tones presented alone on startle amplitude in rats. Each value represents the mean ±S.E.M. of eight rats. Ambient background noise was 50 dB [A]

Prepulse tone intensity	Startle amplitude (Arbitrary units)
No stimulus	5.6 ± 1.7
9 dB above background	4.1 ± 1.0
20 dB above background	3.7 ± 1.9
30 dB above background	6.9 ± 2.3

Phencyclidine produced a dose-related decrease in prepulse inhibition, which was significant after doses of 3.0 and 5.6 mg/kg across all prepulse intensities (Fig. 2, right panel). The magnitude of the phencyclidine-induced disruption of prepulse inhibition did not vary as a function of prepulse intensity as the dose × prepulse intensity interaction term was not significant. Phencyclidine produced a significant decrease in startle amplitude after the 5.6 mg/kg dose (Table 2).

3.3. Interstimulus interval studies

In rats administered vehicle alone, the magnitude of prepulse inhibition decreased as interstimulus interval duration was increased from 30 to 1000 ms (Fig. 3, points above vehicle in all three panels). For example, in the vehicle-treated groups for the scopolamine dose–response curve (Fig. 3, left panel), prepulse inhibition decreased from greater than 90% at the 30-ms interstimulus interval duration to less than 45% at the 1000-ms interstimulus interval duration. In each of the experiments described below, the main effect for interstimulus interval duration was highly statistically significant with all F-ratios having P values < 0.001.

Scopolamine produced a dose-dependent as well as an interstimulus interval-dependent decrease in prepulse inhibition (Fig. 3, left panel) as indicated by a significant main effect for dose [$F(4,96) = 12.67$, $P < 0.0001$) and a significant dose × interstimulus interval interaction [$F(12,96) = 3.47$, $P = 0.0003$]. The significant interaction between the dose of scopolamine and the interstimulus interval duration was due to the disruption produced by scopolamine at the middle interstimulus interval durations (100 and 300 ms) as compared with the lack of effect at the shortest (30 ms) and longest (1000 ms) interstimulus interval durations (Fig. 3, left panel). Scopolamine had no significant effect on startle amplitude over the dose range tested (Table 2).

Table 2
The effects of scopolamine, apomorphine and phencyclidine on startle amplitude in the experiments where prepulse intensity or interstimulus interval were varied. Each startle amplitude value represents the mean of eight rats ± S.E.M.

Drug	Dose (mg/kg)	Startle amplitude (prepulse intensity experiments, arbitrary units)	Startle amplitude (interstimulus interval experiments, arbitrary units)
Scopolamine	Vehicle	294.0 ± 22.4	315.6 ± 22.7
	0.03	323.1 ± 39.4	
	0.1	289.3 ± 40.5	302.7 ± 35.0
	0.3	301.3 ± 18.3	255.3 ± 28.6
	1.0	267.6 ± 21.6	264.1 ± 29.3
Apomorphine	Vehicle	302.6 ± 20.3	393.7 ± 31.2
	0.03	181.8 ± 21.1[a]	
	0.1	159.4 ± 19.8[a]	
	0.3	200.0 ± 42.7[a]	274.1 ± 44.7
	1.0	239.5 ± 38.7[a]	320.6 ± 20.5
	2.0	296.7 ± 46.8	195.2 ± 38.8[a]
Phencyclidine	Vehicle	286.0 ± 28.6	326.7 ± 22.0
	0.1	294.4 ± 33.3	
	0.3	385.8 ± 53.0	
	1.0	341.7 ± 65.7	392.7 ± 26.5
	3.0	263.4 ± 32.6	261.3 ± 32.1
	5.6	123.1 ± 31.2[a]	185.9 ± 22.6[a]

[a] $P < 0.05$ vs. respective control group (Dunnett's).

Apomorphine also produced a dose-dependent as well as interstimulus interval-dependent decrease in prepulse inhibition (Fig. 3, middle panel), as indicated by a significant main effect for dose [$F(4,105) = 12.7$, $P < 0.0001$] and a significant dose × interstimulus interval interaction [$F(12,105) = 3.4$, $P = 0.003$]. In contrast with scopolamine, the dose-dependent decrease in prepulse inhibition produced by apomorphine occurred across all interstimulus interval durations, including the shortest and longest interstimulus interval durations. The significant dose × interstimulus interval interaction was primarily due to a decrease in prepulse inhibition to the same value (~ 15%) produced by the two highest doses of apomorphine, which resulted most likely from a floor effect (Fig. 3, middle panel). In this experiment, apomorphine produced a significant decrease in startle amplitude at the 2.0 mg/kg dose (Table 2).

In addition, phencyclidine produced a dose-dependent as well as interstimulus interval-dependent decrease in prepulse inhibition (Fig. 3, right panel), as indicated by a significant main effect for dose [$F(4,105) = 15.0$, $P < 0.0001$] and a significant dose × interstimulus interval interaction [$F(12,105) = 5.8$, $P = 0.0001$]. The significant dose × interstimulus interval interaction was primarily due to a lack of effect on prepulse inhibition by any dose of phencyclidine at the 1000-ms interstimulus interval duration (Fig. 3, right panel). Phencyclidine again produced a significant decrease in startle amplitude after 5.6 mg/kg (Table 2).

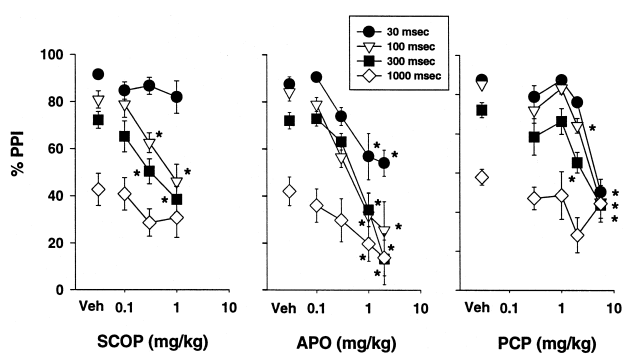

Fig. 3. The effects of varying the duration of the interstimulus interval on the dose-related effects of scopolamine-, apomorphine-, and phencyclidine-produced decreases in prepulse inhibition. Each point represents the mean of eight rats. The vertical lines represent ± S.E.M. and are absent when less than the size of the point. (Abscissa) Dose of drug in milligrams/kilogram. (Ordinate) Percentage of prepulse inhibition. * $P < 0.05$ vs. respective control group (Dunnett's).

4. Discussion

In the present study, scopolamine, a nonselective muscarinic receptor antagonist, produced a significant dose-dependent decrease in prepulse inhibition. The scopolamine-induced disruption of prepulse inhibition was similar in magnitude to that produced by both the dopamine receptor agonist apomorphine and the noncompetitive NMDA receptor antagonist phencyclidine. However, there were several differences in the effects of these three drugs. First, scopolamine had no effect on startle amplitude over the dose range tested, while both apomorphine and phencyclidine significantly altered startle amplitude. Second, as

prepulse intensity was increased relative to background, the maximum magnitude of the scopolamine-induced disruption of prepulse inhibition decreased. In contrast, the maximum magnitude of the disruption of prepulse inhibition produced by apomorphine and phencyclidine achieved comparable values across all prepulse intensities. And finally, while the effects of scopolamine, apomorphine, and phencyclidine on prepulse inhibition were dependent on the duration of the interstimulus interval, the nature of the interaction between interstimulus interval duration and dose was qualitatively different for each drug. Scopolamine only disrupted prepulse inhibition at moderate, but not extreme, prepulse inhibition durations. On the other hand, apomorphine disrupted prepulse inhibition across all prepulse inhibition durations while phencyclidine disrupted prepulse inhibition across the 30–300-ms prepulse inhibition durations, but not the 1000-ms prepulse inhibition duration. Taken together, our data demonstrate that the muscarinic cholinergic system, like the dopaminergic and glutamatergic systems, is involved in the mechanisms of prepulse inhibition of the acoustic startle reflex. However, the role of the muscarinic cholinergic system in modulating prepulse inhibition appears to be different from that of either the dopaminergic or glutamatergic systems.

The present findings represent the first report of a dose-dependent decrease in prepulse inhibition produced by scopolamine, extending the report by Wu et al. (1993) that a single dose of scopolamine (1.0 mg/kg) produced a significant disruption of prepulse inhibition. In the present studies, scopolamine was approximately equipotent to apomorphine and phencyclidine. Further, all three drugs were approximately equiefficacious in that they produced comparable reductions in prepulse inhibition at the highest doses tested, particularly when the prepulse intensity was 9 dB above background. The dose-dependent decreases in prepulse inhibition produced by apomorphine and phencycylidine observed in our study have been previously well-documented (e.g., Mansbach and Geyer, 1989; Mansbach et al., 1988), and have led several investigators to propose that the dopaminergic and glutamatergic systems play a critical role in the normal mechanisms of prepulse inhibition. Furthermore, investigators have speculated that alterations in the dopaminergic and glutamatergic systems may contribute to the gating deficits observed in patients with schizophrenia (e.g., Braff et al., 1978) and other central nervous system disorders (Grillon et al., 1996; Pouretemad et al., 1998). Our data indicate that the muscarinic cholinergic system also contributes to the normal mechanisms of prepulse inhibition and suggest the possibility that deficits in the muscarinic cholinergic system could contribute to gating deficits observed in individuals with schizophrenia or other central nervous system disorders.

The dose–response curves obtained for apomorphine and phencyclidine in the present studies, which were collected by testing multiple rats simultaneously within the same enclosure, are very similar to dose–response curves obtained previously using rats tested in individual enclosures (e.g., Mansbach and Geyer, 1989; Mansbach et al., 1988). Previous investigators have found that rats may emit ultrasonic vocalizations following exposure to startle stimuli (e.g., Kaltwasser, 1990). One may speculate that ultrasonic vocalizations might alter the responses of rats when tested as a group. However, the high degree of similarity of our data to previous studies indicates that testing multiple animals within the same startle enclosure does not appear to substantially alter the effects of drugs on startle amplitude or prepulse inhibition.

Scopolamine disrupted prepulse inhibition while having no effect on startle amplitude over a broad range of doses. Our findings are consistent with a previous report by Williams et al. (1974) that scopolamine, over the dose range of 0.5 to 2.0 mg/kg, produced no significant effects on startle amplitude. On the other hand, Davis (1980) demonstrated that scopolamine decreased startle amplitude when the ambient background level was 70 dB but had no effect on amplitude when the ambient background level was less than 70 dB. Thus, ambient background level is an important determinant of the magnitude of the effects of scopolamine on startle amplitude. Our data are consistent with the findings of Davis (1980) in that the ambient background noise in the present study was less than 70 dB (i.e., 50 dB [A]) and scopolamine had no effect on startle amplitude.

Unlike scopolamine, apomorphine and phencyclidine produced variable, and often statistically significant, alterations in startle amplitude, as has been seen in previous studies (e.g., Mansbach and Geyer, 1989; Young et al., 1991). Alterations in startle amplitude can potentially complicate the interpretation of the effects of drugs on prepulse inhibition, although many investigators have noted a lack of correlation between the magnitude of prepulse inhibition and startle amplitude (e.g., Mansbach and Geyer, 1989; Mansbach et al., 1988). However, the changes in startle amplitude produced by apomorphine and phencyclidine indicate that these two drugs affect not only the sensory information processing events involved in prepulse inhibition, but also the motor output in response to a startling stimulus. In contrast, scopolamine did not alter startle amplitude, and therefore the disruption of prepulse inhibition by scopolamine is most likely due only to the disruption of the sensory information processing events in prepulse inhibition. Together with previous reports, the present evidence suggests that the dopaminergic and glutamatergic systems modulate both the sensory information processing and the motor output components of prepulse inhibition, whereas the muscarinic cholinergic system primarily modulates only the sensory information processing events underlying prepulse inhibition.

One way of modulating prepulse inhibition is to modulate signal-to-noise ratio and thereby the detection of the prepulse stimulus. Signal-to-noise ratio can be modulated by increasing prepulse intensity relative to background. If

a drug decreases prepulse inhibition primarily by decreasing signal-to-noise ratio, then it would be expected that increasing prepulse intensity relative to background would surmount, or reverse, the drug-induced disruption of prepulse inhibition. In the present studies, the disruptive effects of scopolamine on prepulse inhibition were largely surmounted by increasing the intensity of the prepulse stimulus, consistent with the interpretation that scopolamine affected prepulse inhibition by lowering the signal-to-noise ratio for the detection of the prepulse stimulus. Our interpretation is consistent with a number of previous studies showing that the cholinergic system can modulate the detection of a target stimulus by modulating the signal-to-noise ratio (e.g., Drachman and Sahakian, 1979; Robbins, 1997).

In contrast to scopolamine, the disruption of prepulse inhibition by apomorphine and phencyclidine in the present studies was not surmounted by increasing the intensity of the prepulse stimulus, suggesting that apomorphine and phencyclidine did not disrupt prepulse inhibition by altering signal-to-noise ratio. Our findings with apomorphine, in particular, are consistent with previous studies in which apomorphine disrupted prepulse inhibition when prepulse intensities were as high as 15 dB above background (e.g., Swerdlow and Geyer 1993a; Swerdlow et al., 1991). In contrast, Davis et al. (1990) reported that the disruptive effects of apomorphine were surmounted by increasing prepulse intensity relative to background and concluded that apomorphine does not disrupt prepulse inhibition when the prepulse intensity is greater than 10 dB above background. The reasons for the apparent discrepancies between Davis et al. (1990) and other reports, including the present findings, are not entirely clear, but seem unlikely to be related to procedural differences such as differences in the magnitude of the startle stimulus intensity (105 dB in the present study and 118 dB by Swerdlow et al., 1991 vs. 115 or 120 dB by Davis et al., 1990), and/or background noise levels (50 dB in the present study and 65 dB by Swerdlow et al., 1991 vs. 65 dB by Davis et al., 1990). Further experiments are needed to clarify apparent differences between the present, as well as other studies, with those of Davis et al. (1990). Nevertheless, the majority of the evidence suggests that the muscarinic cholinergic system is primarily involved in modulating the signal-to-noise ratio for the detection of the prepulse stimulus. On the other hand, the dopaminergic and glutamatergic systems appear to modulate prepulse inhibition by affecting the detection of the prepulse stimulus through mechanisms other than alteration of signal-to-noise ratio. Such mechanisms cannot be determined based on the present data, but may involve a more direct modulation of the neuronal circuits necessary for the prepulse stimulus to impact or gate the startle stimulus.

The magnitude of prepulse inhibition is dependent not only on the intensity of the prepulse stimulus relative to background, but also on the interval between the prepulse and startle stimuli. Thus, another way to modulate prepulse inhibition is to alter the interstimulus interval duration. In general, prepulse inhibition is largest in magnitude at interstimulus interval durations less than or equal to approximately 100 ms and decays with increasing interstimulus interval duration (e.g., Ison and Hammond, 1971; Swerdlow et al., 1991). In the present studies, the disruption of prepulse inhibition by scopolamine, apomorphine, and phencyclidine varied dependent upon interstimulus interval duration, as evidenced by a significant dose × interstimulus interval interaction term for each drug. However, the nature of the interaction was qualitatively different for each drug. Scopolamine did not affect prepulse inhibition at the 30- or 1000-ms interstimulus interval durations, but produced dose-dependent decreases in prepulse inhibition at both the 100- and 300-ms interstimulus interval durations. On the other hand, apomorphine produced dose-dependent decreases in prepulse inhibition at all of the interstimulus interval durations, while phencyclidine produced dose-dependent decreases in prepulse inhibition across the 30–300-ms interstimulus interval durations. The lack of effects of scopolamine at the 30-ms interstimulus interval duration indicates that under the present experimental conditions (i.e., prepulse stimulus 27 dB above background), scopolamine did not disrupt the detection of the prepulse stimulus. Rather, it appears that scopolamine influenced the rate of decay of the gating of the startle stimulus by the prepulse stimulus. However, the significant effects of apomorphine and phencycylidine at the 30-ms interstimulus interval duration provide further support for the idea that these two drugs interfered with the detection of the prepulse stimulus, albeit through a mechanism other than alteration of the signal-to-noise ratio. Moreover, the significant interstimulus interval-dependent effects of apomorphine and phencyclidine on prepulse inhibition suggest that both drugs may have also increased the rate of decay of the gating of the startle stimulus by the prepulse stimulus. In fact, the present studies cannot rule out the possibility that apomorphine and phencyclidine produced such a rapid decay that the prepulse stimulus no longer gated the startle stimulus at the 30-ms interstimulus interval duration; however, such a possibility seems unlikely. If, as suggested above, apomorphine and phencyclidine affect primarily the neuronal circuits necessary for the prepulse stimulus to gate the startle stimulus, then the results of the present interstimulus interval duration experiments might be expected. Further experiments are needed to better understand the relative effects of scopolamine, apomorphine, and phencyclidine on the processes involved in the detection of the prepulse stimulus as well as the rate of decay of the gating of the startle stimulus by the prepulse stimulus.

In the present study, scopolamine, apomorphine, and phencyclidine were demonstrated to produce different profiles of effects on prepulse inhibition, suggesting that the muscarinic cholinergic, dopaminergic, and glutamatergic

systems may mediate different components of the sensory information processing events involved in prepulse inhibition. Taken together, the present data indicate that the muscarinic cholinergic system appears to be primarily involved in enhancing the impact of salient sensory information (i.e., prepulse stimuli) through an increase in the signal-to-noise ratio. On the other hand, our data suggest that the dopaminergic and glutamatergic systems primarily modulate the temporal coupling and/or rate of decay of the impact, or gating, of the prepulse stimulus on the startle stimulus in the production of prepulse inhibition. In conclusion, the present findings support the hypothesis that the muscarinic cholinergic as well as the dopaminergic and glutamatergic systems are involved in the mechanisms of prepulse inhibition of the acoustic startle reflex. Additional studies are needed to further clarify the respective roles of these three neurotransmitter systems in the mechanisms of prepulse inhibition of the acoustic startle reflex.

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References

Braff, D.L., Grillion, C., Geyer, M.A., 1992. Gating and habituation of the startle reflex in schizophrenic patients. Arch. Gen. Psychiatry 49, 206–215.

Braff, D., Stone, C., Callaway, E., Geyer, M., Glick, I., Bali, L., 1978. Prestimulus effects of human startle reflex in normals and schizophrenics. Psychophysiology 15, 339–343.

Cadenhead, K.S., Geyer, M.A., Braff, D.L., 1993. Impaired startle prepulse inhibition and habituation in patients with schizotypal personality disorder. Am. J. Psychiatry 150, 1862–1867.

Davis, M., 1980. Neurochemical modulation of sensory–motor reactivity: acoustic and tactile startle reflexes. Neurosci. Biobehav. Rev. 4, 241–263.

Davis, M., Mansbach, R.S., Swerdlow, N.R., Campeau, S., Braff, D.L., 1990. Apomorphine disrupts the inhibition of acoustic startle induced by weak prepulses in rats. Psychopharmacology 102, 1–4.

Drachman, D.R., Sahakian, B.J., 1979. The effects of cholinergic agents on human learning and memory. In: Growdon, T.H., Wurtman, R.J. (Eds.), Nutrition in the Brain. Raven Press, New York, p. 351.

Graham, F.K., 1975. The more or less startling effects of weak prestimulation. Psychophysiology 12, 238–248.

Grillon, C., Morgan, C.A., Southwick, S.M., Davis, M., Charney, D.S., 1996. Baseline startle amplitude and prepulse inhibition in Vietnam veterans with posttraumatic stress disorder. Psychiatry Res. 64, 169–178.

Hoffman, H.S., Ison, J.R., 1980. Reflex modification in the domain of startle: I. Some empirical findings and their implications for how the nervous system process sensory input. Psychol. Rev. 87, 175–189.

Ison, J.R., Hammond, G.R., 1971. Modification of the startle reflex in the rat by changes in the auditory and visual environments. J. Comp. Physiol. Psychol. 75, 435–452.

Kaltwasser, M.T., 1990. Startle-inducing acoustic stimuli evoke ultrasonic vocalization in the rat. Physiol. Behav. 48, 13–17.

Koch, M., Kungel, M., Herbert, H., 1993. Cholinergic neurons in the pedunculopontine tegmental nucleus are involved in the mediation of prepulse inhibition of the acoustic startle response in the rat. Exp. Brain Res. 97, 71–82.

Mansbach, R.S., Geyer, M.A., 1989. Effect of phencyclidine and phencyclidine biologs on sensorimotor gating in the rat. Neuropsychopharmacology 2, 299–308.

Mansbach, R.S., Geyer, M.A., Braff, D.L., 1988. Dopaminergic stimulation disrupts sensorimotor gating in the rat. Psychopharmacology 94, 507–514.

Peng, P.Y., Mansbach, R.S., Braff, D.L., Geyer, M.A., 1990. A D2 Dopamine receptor agonist disrupts sensorimotor gating in rats: implications for dopaminergic abnormalities in schizophrenia. Neuropsychopharmacology 3, 211–218.

Pouretemad, H.R., Thompson, P.J., Fenwick, P.B., 1998. Impaired sensorimotor gating in patients with non-epileptic seizures. Epilepsy Res. 31, 1–12.

Robbins, T.W., 1997. Arousal systems and attentional processes. Biol. Psychol. 45, 57–71.

Swerdlow, N., Geyer, M.A., 1993a. Clozapine and haloperidol in an animal model of sensorimotor gating deficits in schizophrenia. Pharmacol. Biochem. Behav. 44, 741–744.

Swerdlow, N., Geyer, M.A., 1993b. Prepulse inhibition of acoustic startle in rats after lesions of the pedunculopontine tegmental nucleus. Behav. Neurosci. 107, 104–117.

Swerdlow, N., Keith, V.A., Braff, D.L., Geyer, M.A., 1991. Effects of spiperone, raclopride, SCH 23390 and clozapine on apomorphine inhibition of sensorimotor gating of the startle response in the rat. J. Pharmacol. Exp. Ther. 256, 530–536.

Williams, J.M., Hamilton, L.W., Carlton, P.L., 1974. Pharmacological and anatomical dissociation of two types of habituation. J. Comp. Physiol. Psychol. 87, 724–732.

Wu, M., Jenden, D.J., Fairchild, M.D., Siegel, J.M., 1993. Cholinergic mechanisms in startle and prepulse inhibition: effects of the false cholinergic precursor *N*-aminodeanol. Behav. Neurosci. 107, 306–316.

Young, K.A., Randall, P.K., Wilcox, R.E., 1991. Dose and time response analysis of apomorphine's effect on prepulse inhibition of acoustic startle. Behav. Brain Res. 42, 43–48.