Muscarinic Cholinergic Modulation of Prepulse Inhibition of the Acoustic Startle Reflex

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ABSTRACT

The purpose of the present study was to determine the effects of muscarinic cholinergic receptor antagonists and agonists on prepulse inhibition (PPI) of the acoustic startle reflex in rats. The muscarinic receptor antagonist scopolamine (0.03–1.0 mg/kg) produced a significant dose-dependent decrease in PPI without affecting startle amplitude. In contrast, N-methyl scopolamine, the quaternary analog of scopolamine, had no effect on PPI, indicating that scopolamine disrupted PPI through a central cholinergic mechanism. Two other muscarinic receptor antagonists, trihexyphenidyl (0.3–10 mg/kg) and benztropine (0.03–10 mg/kg), produced significant decreases in PPI similar to scopolamine. On the other hand, the muscarinic receptor antagonists dicyclomine (0.03–10 mg/kg) and biperiden (0.03–10 mg/kg) had no effect on PPI but significantly decreased startle amplitude. Mecamylamine (0.1–10 mg/kg), a nicotinic receptor antagonist, also had no effect on PPI. Administered alone, the muscarinic receptor agonists pilocarpine (0.03–10 mg/kg), oxotremorine (0.01–0.3 mg/kg), RS-86 (0.1–3.0 mg/kg), and arecoline (0.3–10 mg/kg), as well as the cholinesterase inhibitors physostigmine (0.01–0.3 mg/kg) and tacrine (0.03–10 mg/kg), had no effect on PPI, but each produced significant decreases in startle amplitude at the highest doses tested. In addition, the disruption of PPI by scopolamine was reversed in a dose-dependent manner by the muscarinic receptor agonist oxotremorine. The present findings demonstrate that the muscarinic cholinergic system plays an important role in the normal mechanisms of PPI.

Multiple lines of evidence indicate that the muscarinic cholinergic system constitutes part of the neuronal circuitry important for normal cognition. For example, muscarinic receptor antagonists, such as scopolamine, are well known to produce or exacerbate impairments in attention, learning, and memory in rats (Beatty et al., 1986; Shannon et al., 1990a,b), primates (Bartus et al., 1976; Aigner et al., 1993; Callahan et al., 1993), and humans (Petersen et al., 1977; Rusted and Warburton, 1988). In addition, muscarinic receptor agonists and cholinesterase inhibitors have been shown to enhance normal cognition and/or reverse deficits in cognitive functions produced by muscarinic receptor antagonists in both animals (Aigner and Mishkin, 1986; Bartus et al., 1988; Rupniak et al., 1989) and humans (Drachman et al., 1977; Mohs et al., 1985; Bodick et al., 1997). Taken together, these studies have lead researchers to speculate that a loss of, or deficits in, the muscarinic cholinergic system may account, at least in part, for the cognitive impairments observed in individuals with schizophrenia as a deficit in sensorimotor gating as assessed by prepulse inhibition (PPI) of the acoustic startle reflex (Braff et al. 1992). There is limited evidence suggesting the possible involvement of the muscarinic cholinergic system in the mechanisms of PPI of the acoustic startle reflex. Wu et al. (1993) reported that PPI was significantly decreased in rats treated chronically with N-aminodeanol, a cholinergic false precursor, and a choline-free diet and that the muscarinic receptor agonist arecoline partially reversed the observed deficits in PPI. Lesions of primarily cholinergic brainstem nuclei expressing numerous muscarinic receptor subtypes, including the pedunculopontine tegmental nucleus and laterodorsal tegmental nucleus, have also been reported to attenuate PPI (Koch et al., 1993; Swerdlow et al., 1993; Jones and Shannon, 1998). We have previously demonstrated in rats that the muscarinic receptor antagonist scopolamine produced a significant dose-dependent decrease in PPI (Jones and Shannon, 2000). At present, it is unknown whether the disruption of PPI by scopolamine is unique or is produced by other muscarinic receptor antagonists. Moreover, the effects of muscarinic receptor agonists on PPI have not yet been evaluated.

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ABBREVIATIONS: PPI, prepulse inhibition; ITI, intertrial interval; ISI, interstimulus interval.

One of the cognitive impairments observed in individuals with schizophrenia is a deficit in sensorimotor gating as assessed by prepulse inhibition (PPI) of the acoustic startle reflex (Braff et al. 1992). There is limited evidence suggesting the possible involvement of the muscarinic cholinergic system in the mechanisms of PPI of the acoustic startle reflex. Wu et al. (1993) reported that PPI was significantly decreased in rats treated chronically with N-aminodeanol, a cholinergic false precursor, and a choline-free diet and that the muscarinic receptor agonist arecoline partially reversed the observed deficits in PPI. Lesions of primarily cholinergic brainstem nuclei expressing numerous muscarinic receptor subtypes, including the pedunculopontine tegmental nucleus and laterodorsal tegmental nucleus, have also been reported to attenuate PPI (Koch et al., 1993; Swerdlow et al., 1993; Jones and Shannon, 1998). We have previously demonstrated in rats that the muscarinic receptor antagonist scopolamine produced a significant dose-dependent decrease in PPI (Jones and Shannon, 2000). At present, it is unknown whether the disruption of PPI by scopolamine is unique or is produced by other muscarinic receptor antagonists. Moreover, the effects of muscarinic receptor agonists on PPI have not yet been evaluated.

The purpose of the present study was to investigate the role
of the muscarinic cholinergic system in PPI of the acoustic startle reflex by determining the effects of the systemic administration of muscarinic receptor antagonists and agonists, from a variety of chemical classes, on both PPI and the amplitude of the startle reflex. Accordingly, dose-response curves were determined for the muscarinic receptor antagonists scopolamine and N-methyl scopolamine, the quaternary analog of scopolamine that does not readily cross the blood-brain barrier, on PPI and startle reflex amplitude. Dose-response curves were also determined for the muscarinic receptor antagonists trihexyphenidyl, benzatropine, dicyclomine, and biperiden, which are frequently used clinically. For purposes of comparison, mecamylamine, a nicotinic receptor antagonist, was also evaluated. In addition, dose-response curves were determined for the effects of the muscarinic receptor antagonists pilocarpine, oxotremorine, RS-86 ([2-ethyl-8-methyl-2,8-diazaspiro(4.5) decane-1,3-dione]hydrochloride), and arecoline, as well as the cholinesterase inhibitors physostigmine and tacrine, on both PPI and startle reflex amplitude. Finally, to demonstrate that the effects of scopolamine on PPI were mediated through muscarinic cholinergic receptors, the effects of scopolamine (1.0 mg/kg) were determined alone and in the presence of varying doses of oxotremorine.

Materials and Methods

Subjects. Adult male Sprague-Dawley rats (Harlan Sprague-Dawley, Indianapolis, IN), weighing 325 to 350 g, were housed in pairs in a large colony room under a 12-h light/dark cycle (lights on at 6:00 AM). Each rat was maintained on 15 g of food per day with water available ad libitum. Test sessions were performed between 8:00 AM and 6:00 PM. Each rat was used in two or three experiments with at least a 1-week interval between test sessions. All experiments were conducted in accordance with the National Institutes of Health 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.

Apparatus. All test sessions were performed in a Coulbourn Instruments (Allentown, PA) acoustic startle apparatus consisting of two ventilated, sound-attenuated chambers with four force transducer platforms per chamber. Each chamber was a testing holder that measured 16.5 × 8.5 × 7.6 cm with a top made of aluminum rods of 0.5 cm in diameter spaced 1.25 cm center-to-center, which allowed full exposure to acoustic stimuli. Each holder was positioned on an individual force transducer platform. The background decibel level in each chamber was determined to be 50 dBA using a Radio Shack Digital Sound Level Meter (catalog no. 33-2055). Sound levels were calibrated in each chamber using a five-point calibration curve. There was no significant difference between the two chambers in sound delivery or response amplitude. Data were recorded on-line using a Compaq Deskpro 386 computer (Compaq Computer Corp.) and Labline interface modules (Coulbourn Instruments), with 200 1-ms readings collected beginning at trial onset. In a separate experiment, core body temperature was recorded rectally (model BAT 8; Bailey Instruments, Brooks, NJ).

Procedure. All rats were adapted to the startle chambers for 30 min on each of 2 consecutive days. On the 3rd day, to preexpose 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 broadband burst), prepulse tone alone (77 dB [A] 20 ms, 10 kHz), and prepulse plus startle pulse. The intertrial interval (ITI) was varied pseudorandomly between 15 and 45 s. The interstimulus interval (ISI) was 120 ms. An ambient background noise of 50 dB[A] was present throughout the test session.

Core body temperature (°C) was measured before and 30 min after the administration of vehicle, scopolamine alone (1.0 mg/kg), oxotremorine alone (0.03–1.0 mg/kg), or scopolamine (1.0 mg/kg) plus varying doses of oxotremorine (0.1–10 mg/kg).

Drugs. Arecoline hydrobromide (Sigma Chemical Co., St. Louis, MO) was administered s.c. 5 min before the start of a test session. Pilocarpine hydrochloride, (−)scopolamine hydrobromide, mecamylamine hydrochloride, dicyclomine hydrobromide, benzatropine methanesulfonate, oxotremorine sesquifumarate, RS-86 [2-ethyl-8-methyl-2,8-diazaspiro(4.5) decane-1,3-dione]hydrochloride, (−)-scopolamine methyl bromide, physostigmine hemisulfate, tacrine hydrochloride (Sigma Chemical Co.), biperiden hydrochloride (Knoll AG, Ludwigshaffen, Germany), and trihexyphenidyl hydrochloride (Lederle Lab, American Cyanamid Company, Pearl River, NY) were injected s.c. 30 min before testing. All doses refer to the salt and were injected in a 1.0 ml/kg volume. Each compound was dissolved in double deionized water.

Data Analysis. Startle amplitude was defined as the peak of the 200 readings of 1 ms. Percentage PPI was calculated using the equation: 100 × [mean startle amplitude in prepulse trial − mean startle amplitude in prepulse + pulse trial]/(mean startle amplitude in startle pulse alone trial)]. Percentage PPI and startle amplitude data were analyzed by a one-way ANOVA with comparison of the vehicle control group using Dunnett’s test. In the body temperature study, data were expressed as the difference in body temperature before and after the administration of vehicle, scopolamine alone, oxotremorine alone, or scopolamine in the presence of varying doses of oxotremorine. Body temperature data were analyzed by a one-way ANOVA with comparison of dose groups with the vehicle-treated group using Dunnett’s test. Calculations were performed using JMP v 3.2 (SAS Institute Inc., Cary, NC) statistical software.

Results

Muscarinic Cholinergic Antagonists. The muscarinic receptor antagonist scopolamine produced a dose-dependent decrease in PPI that was significant after doses of 0.3 and 1.0 mg/kg (Fig. 1, top left). Scopolamine had no effect on startle amplitude over the dose range tested (Fig. 1, bottom left). In contrast, N-methyl scopolamine, the quaternary analog of scopolamine that crosses the blood-brain barrier poorly, had no effect on PPI over the dose range of 0.03 to 1.0 mg/kg (Fig. 1, top right). N-Methyl scopolamine also had no effect on startle amplitude over the dose range tested (Fig. 1, bottom right).

The muscarinic receptor antagonists trihexyphenidyl and benzatropine produced decreases in PPI that were significant for both compounds after doses of 10 mg/kg (Fig. 2, top). Trihexyphenidyl and benzatropine also produced significant decreases in startle amplitude after doses of 10 and 3 mg/kg, respectively (Fig. 2, bottom). On the other hand, the muscarinic cholinergic receptor antagonists dicyclomine and biperiden had no effect on PPI over the dose ranges of 0.3 to 10 mg/kg (Fig. 3, top left and right). Dicyclomine and biperiden produced dose-related decreases in startle amplitude that were significant after 10 mg/kg dicyclomine and after 1.0, 3.0, and 10 mg/kg biperiden (Fig. 3, bottom left and right).

Mecamylamine. Mecamylamine, a nicotinic cholinergic receptor antagonist, had no effect on PPI over the dose range tested (Fig. 4, top). Mecamylamine also had no significant effect on startle amplitude over the dose range tested (Fig. 4, bottom). Higher doses of mecamylamine were not tested be-
cause they produced marked behavioral sedation, which precluded testing.

**Muscarinic Cholinergic Agonists.** The muscarinic cholinergic receptor agonists pilocarpine, oxotremorine, RS-86, and arecoline had no effect on PPI over the dose ranges tested (Fig. 5, top). However, oxotremorine, RS-86, and arecoline produced dose-dependent decreases in startle amplitude, which were significant after doses of 0.1 and 0.3 mg/kg oxotremorine, 3.0 mg/kg RS-86, and 10.0 mg/kg arecoline (Fig. 5, bottom). Higher doses of all four muscarinic agonists produced substantial motor side effects, which prevented testing.

**Cholinesterase Inhibitors.** Physostigmine and tacrine had no significant effect on PPI over the dose ranges of 0.01 to 0.1 and 0.3 to 10 mg/kg, respectively (Fig. 6, top). Physostigmine had no effect on startle amplitude over the dose range tested (Fig. 6, bottom left), whereas tacrine produced a dose-dependent decrease in startle amplitude that was significant after the 10 mg/kg dose (Fig. 6, bottom right). Higher doses of both drugs produced lethality in pilot studies and were not tested here.

**Scopolamine-Oxotremorine Interactions.** To determine doses of oxotremorine that might be expected to reverse the scopolamine-induced disruption of PPI, we initially evaluated the interaction between oxotremorine and scopolamine on body temperature changes produced by oxotremorine. Accordingly, dose-response curves were determined for oxotremorine alone and in the presence of scopolamine (1.0 mg/kg; a dose that produced maximal disruption of PPI) on body temperature. Oxotremorine administered alone produced dose-dependent decreases in body temperature that were significant after doses of 0.1, 0.3, and 1.0 mg/kg (Fig. 7).

Scopolamine (1.0 mg/kg) administered alone had no effect on body temperature (Fig. 7, point above V/S). In the presence of scopolamine (1.0 mg/kg), the oxotremorine dose-response curve for changes in body temperature was shifted to the right by approximately 10-fold (Fig. 7). Thus, doses of 3.0 to 10 mg/kg oxotremorine were required to surmount the antagonism of the oxotremorine-induced hypothermia by 1.0 mg/kg scopolamine.

To determine whether oxotremorine reversed the effects of scopolamine on PPI, scopolamine was administered alone and in the presence of varying doses of oxotremorine. Scopolamine (1.0 mg/kg) in the presence of vehicle produced a significant decrease in PPI but had no effect on startle amplitude (Fig. 8, points above V/S). Oxotremorine produced a dose-dependent reversal of the disruption of PPI by scopolamine (1.0 mg/kg) with significant reversals at doses of 3.0 and 5.6 mg/kg oxotremorine (Fig. 8). Scopolamine in the presence of increasing doses of oxotremorine had no effect on startle amplitude (Fig. 8).

**Discussion**

The present findings confirm and extend our previous observations that the muscarinic cholinergic system is involved in mediating PPI of the acoustic startle reflex (Jones and Shannon, 2000). As in our previous study, the muscarinic receptor antagonist scopolamine produced a significant dose-dependent decrease in PPI without affecting startle amplitude. Moreover, N-methyl scopolamine, the quaternary analog of scopolamine that does not readily cross the blood-brain barrier, had no effect on PPI when tested at approximately equimolar doses to scopolamine. The disruption of PPI by
scopolamine was also reversed in a dose-related manner by the muscarinic agonist oxotremorine. Although the doses of oxotremorine required to reverse scopolamine were relatively high (3.0 to 5.6 mg/kg), these doses of oxotremorine were pharmacologically relevant because identical doses were required to surmount the antagonism by scopolamine (1.0 mg/kg) of body temperature in the present study. Taken together, our findings indicate that the disruption of PPI by scopolamine was mediated by the antagonism of central muscarinic cholinergic receptors.

To determine whether the disruption of PPI was unique to scopolamine or also produced by other muscarinic receptor antagonists, we evaluated the effects on PPI of four additional muscarinic receptor antagonists from a variety of chemical classes. Trihexyphenidyl and benztropine were approximately equiefficacious to scopolamine in that all three drugs produced substantial reductions in the magnitude of PPI from approximately 80% to as low as 25%. Unlike scopolamine, however, trihexyphenidyl and benztropine significantly decreased PPI at doses that also significantly decreased startle amplitude. In contrast, the muscarinic receptor antagonists dicyclomine and biperiden had no effect on PPI at doses that significantly decreased startle amplitude. The doses of dicyclomine and biperiden used in the present study were apparently sufficient to achieve substantial brain levels because both drugs decreased startle amplitude and higher doses of each drug produced substantial motor behaviors that precluded testing. Thus, the muscarinic receptor antagonists scopolamine, trihexyphenidyl, and benztropine disrupted PPI; however, only scopolamine decreased PPI without affecting startle amplitude.

One possible explanation for the differences observed among the five muscarinic receptor antagonists in affect on PPI might be differences in muscarinic receptor subtype selectivity. Five muscarinic receptor subtypes, belonging to a superfamily of G protein-coupled receptors, have been identified by molecular cloning techniques and are referred to as the M1, M2, M3, M4, and M5 receptor subtypes (Buckley et al., 1989). If only one or a few of the muscarinic receptor subtypes are involved in modulating PPI, then it might be expected that the muscarinic antagonists with the greatest selectivity for those receptor subtypes involved would produce the largest effects on PPI. In particular, because the M1 muscarinic receptor subtype has been previously postulated to be important in cognition (e.g., Mash et al., 1985; Bymaster et al., 1993), it might be expected that M1-preferring antagonists would be most effective in disrupting PPI. In reviewing the in vitro binding profiles of the five muscarinic receptor antagonists tested, scopolamine is relatively nonselective; however, scopolamine has been shown to have a slightly higher affinity for the M3 receptor subtype with a 2- to 3-fold lower affinity for the other muscarinic receptor subtypes (Bolden et al., 1992). Trihexyphenidyl, benztropine, and biperiden have the highest affinity, based on $K_i$ values, for the M1 receptor subtype with a range of approximately 2- to 13-fold lower affinities for the other muscarinic receptor subtypes (Bolden et al., 1992). On the other hand, dicyclomine has the highest affinity for both the M1 and M5 receptor subtypes, based on IC$_{50}$ values, with an approximately 2- to 8-fold lower affinity for the other muscarinic receptor subtypes (Buckley et al., 1989). Thus, based on in vitro binding data, scopolamine, trihexyphenidyl, and benztropine do
not share a preferential selectivity for one or more of the five muscarinic receptor subtypes relative to dicyclomine and biperiden, indicating that in vitro muscarinic receptor subtype selectivity cannot readily explain the differences in effects on PPI we observed among the five muscarinic antagonists.

There are, however, other important considerations with regard to interpreting the present findings relative to muscarinic receptor subtype selectivity. First, the degree of separation in muscarinic receptor selectivity is greater in native tissue receptor populations for a number of muscarinic receptor antagonists (Waelbroeck et al., 1990) than has been reported using receptors expressed in nonneuronal cell lines (Buckley et al., 1989; Bolden et al., 1992). Although the muscarinic antagonists tested in the present study were not investigated by Waelbroeck et al. (1990), it is possible that the antagonists in the present studies could have greater selectivity.

Fig. 5. Dose-dependent effects of pilocarpine, oxotremorine, RS-86, and arecoline on PPI (top) and startle amplitude (bottom). Each point represents the mean of eight rats. The vertical lines represent ±S.E.M. values and are absent when less than the size of the point. Abscissa, dose of drug in milligrams per kilogram. Ordinates: top, percentage PPI; bottom, startle amplitude in arbitrary units. *P < .05 versus vehicle.

Fig. 6. Dose-dependent effects of physostigmine and tacrine on PPI (top left and right) and startle amplitude (bottom left and right). Each point represents the mean value for eight rats. The vertical lines represent ±S.E.M. values and are absent when less than the size of the point. Abscissa, dose of drug in milligrams per kilogram. Ordinates: top, percentage PPI; bottom, startle amplitude in arbitrary units. *P < .05 versus vehicle.

Fig. 7. Effects of scopolamine (1.0 mg/kg) on oxotremorine-induced decrease in body temperature. Each point represents the mean value for eight rats. The vertical lines represent ±S.E.M. values and are absent when less than the size of the point. Abscissa, dose of drug in milligrams per kilogram. Ordinates, change in temperature (°C). *P < .05 versus vehicle.
activity for particular receptor subtypes in vivo than observed in vitro, which might account for their different effects on PPI. Second, it has been demonstrated that some muscarinic receptor antagonists are inverse agonists. Specifically, scopolamine has been demonstrated to be an inverse agonist in rat cardiomyocytes expressing the M2 receptor and in Chinese hamster ovary cells transfected with human M2 or M4 receptors (Jakubik et al., 1995). Although the intrinsic efficacy for each of the antagonists evaluated herein at all of the muscarinic receptor subtypes remains unknown, the possibility must be considered that differences in intrinsic efficacy rather than or in addition to receptor subtype selectivity may also be an important determinant of how muscarinic antagonists modulate PPI. Further studies are needed to elucidate the in vivo selectivity, as well as the intrinsic efficacy, of muscarinic receptor antagonists, including those evaluated in the present studies, to more fully understand the differential effects of muscarinic antagonists on PPI.

Another possible explanation for the differences among the muscarinic antagonists tested in the present study may involve different direct or indirect interactions with other neurotransmitter systems known to affect PPI, including the glutamate, serotonin, and dopamine systems (see Swerdlow and Geyer, 1998, for review). For example, benzotropine has been reported to directly stimulate dopamine release and to block dopamine reuptake, thereby increasing overall central dopaminergic activity (Horn et al., 1971; Model et al., 1989). Dopamine agonists administered systemically or directly into the nucleus accumbens produce significant decreases in PPI (e.g., Swerdlow et al., 1992; Wan and Swerdlow, 1993). Therefore, it cannot be excluded that benzotropine-induced increases in dopamine activity might account for the observed disruption of PPI. It is currently unknown whether the other muscarinic antagonists tested in the present study similarly stimulate dopamine release and block dopamine reuptake through nonmuscarinic receptor mechanisms. Nevertheless, it would be of importance to more fully investigate interactions between muscarinic cholinergic receptors and the dopamine system, as well as possible interactions between muscarinic receptors and other neurotransmitter systems known to affect PPI.

In contrast to the substantial disruption of PPI produced by the muscarinic receptor antagonists scopolamine, trihexyphenidyl, and benzotropine, the noncompetitive nicotinic receptor antagonist mecamylamine had no significant effect on PPI in the present study. Previously, Curzon et al. (1994) reported that mecamylamine produced a significant decrease in PPI. The reasons for the apparent discrepancies between our findings and those of Curzon et al. (1994) are not entirely apparent but may be due to procedural differences such as differences in the prepulse intensities used in the two studies. Curzon et al. (1994) used three different prepulse intensities varying from 5 to 15 dB above a background of 60 dB, whereas a prepulse intensity of 27 dB above a background of 50 dB was used in the present study. Although the present findings support a role for the muscarinic cholinergic system in the mechanisms of PPI, there was insufficient evidence to clearly establish whether mecamylamine-sensitive nicotinic receptors are involved in the mechanisms of PPI.

The lack of effect on PPI by the muscarinic agonists and cholinesterase inhibitors tested in the present study indicates that increases above a certain threshold of muscarinic receptor activation do not enhance PPI in normal animals. However, the reversal of the scopolamine-induced disruption of PPI by the muscarinic agonist oxotremorine suggests that deficits in PPI, as observed in clinical conditions such as schizophrenia, could be due in part to deficits in the muscarinic cholinergic system and might be reversed by treatment with muscarinic agonists. In addition, if the balance between the muscarinic cholinergic and dopamine systems is critical for normal PPI, as suggested above, then it might be expected that muscarinic agonists would also reverse the disruption of PPI produced by dopaminergic agonists. Ongoing studies in our laboratory are focused on evaluating dopaminergic-cholinergic interactions in modulating PPI.

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