Muscarinic Receptor Agonists, Like Dopamine Receptor Antagonist Antipsychotics, Inhibit Conditioned Avoidance Response in Rats

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ABSTRACT
The purpose of our studies was to determine the effects of muscarinic receptor agonists on conditioned avoidance responding in the rat. Rats were trained to avoid or escape an electric shock delivered to the feet in a discrete trial procedure. The muscarinic receptor agonists pilocarpine and [2-ethyl-8-methyl-2,8-diazaspiro(4.5)decane-1,3-dione] hydrochloride (RS86) and the cholinesterase inhibitor physostigmine all decreased the percentage of avoidance responses at doses that produced less than approximately 30% response failures. Similar results were obtained with the antipsychotic drugs haloperidol, trifluoperazine, chlorpromazine, and clozapine. However, the benzodiazepine anxiolytic diazepam did not decrease avoidance responding up to doses that produced ataxia. On the other hand, oxotremorine and arecoline decreased avoidance responding only by producing response failures, whereas aceclidine produced intermediate changes. The muscarinic receptor antagonists scopolamine, trihexyphenidyl, and benzotropine were without effect when administered alone but antagonized the decreases in avoidance responding produced by pilocarpine and RS86. Scopolamine had little effect on the decreases in avoidance responding produced by haloperidol. The newer muscarinic receptor partial agonists or agonist/antagonists [R-(Z)-(1)-a-(methoxyimino)-1-azabicyclo[2.2.2]octane-3-acetonitrile] hydrochloride, talsaclidine, milameline, and xanomeline also produced dose-related decreases in avoidance responding. Our results demonstrate that muscarinic receptor agonists can decrease avoidance responding in a manner similar to dopamine-receptor antipsychotic drugs, suggesting that muscarinic receptor agonists may provide an alternative approach to the treatment of psychosis.

Several investigators have suggested that the muscarinic cholinergic system might be involved in the pathophysiology of schizophrenia. In an early clinical study, Pfeiffer and Jenny (1957) reported that the administration of muscarinic agonists to patients with catatonic schizophrenia produced “lucid intervals” and suggested that muscarinic agonists might be therapeutically useful in treating schizophrenia; however, these studies were not well controlled and amounted to little more than anecdotal reports. Edelstein et al. (1981) reported that a subgroup of patients with schizophrenia responded to physostigmine and confirmed, although other investigators (e.g., Davis and Berger, 1978) did not find positive results with physostigmine. On the other hand, Tandon et al. (Tandon and Greden, 1989; Tandon et al., 1991) proposed that cholinergic hyperactivity underlies at least the negative symptoms of schizophrenia and that muscarinic antagonists might be therapeutically useful in treating the negative symptoms of schizophrenia. Furthermore, the overlap in the psychotic symptoms between schizophrenia and Alzheimer’s disease has led some authors (e.g., White and Cummings, 1996) to propose that deficits in the muscarinic cholinergic system may underlie the psychotic symptoms in both disorders. Consistent with this hypothesis, acetylcholinesterase inhibitors have been reported to reduce psychotic symptoms and other behavioral disturbances in patients with Alzheimer’s disease (Cummings et al., 1993; Gorman et al., 1993; Kaufer et al., 1996). Furthermore, muscarinic receptor antagonists can produce psychotic-like symptoms including auditory hallucinations, hyperactivity, and cognitive disruption (e.g., reviews by Abod and Biel, 1962; Yeomans, 1995). More recently, the muscarinic receptor agonist xanomeline (Bymaster et al., 1994; Shannon et al., 1994) was demonstrated to significantly reduce psychotic behaviors in patients with Alzheimer’s disease (Bodick et al., 1997). In the

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ABBREVIATIONS: RS86, [2-ethyl-8-methyl-2,8-diazaspiro(4.5)decane-1,3-dione] hydrochloride; SB 202026, [R-(Z)-(1)-a-(methoxyimino)-1-azabicyclo[2.2.2]octane-3-acetonitrile] hydrochloride.
latter study, xanomeline was particularly effective in treating and/or preventing hallucinations and delusions (Bodick et al., 1997).

We recently reported that the muscarinic partial agonist PTAC \([5R,6R-(3-propylthio-1,2,5-thiadiazole-4-yl)-1-azacyclo-[3.2.1]octane], which has no or very low affinity for dopamine receptors, has a pharmacological profile similar to that of atypical antipsychotic drugs, including inhibition of dopamine cell firing in the limbic ventral tegmental area (A10) at doses that did not inhibit the substantia nigra (A9), inhibition of \(\alpha\)-amphetamine-induced Fos expression in the nucleus accumbens, and inhibition of conditioned avoidance responding (Bymaster et al., 1998b; Sauerberg et al., 1998). Moreover, PTAC did not produce parasympathomimetic effects at doses that inhibited dopamine cells and conditioned avoidance responding. The inhibition of conditioned avoidance responding by PTAC was consistent with findings that all currently clinically useful typical and atypical antipsychotic drugs inhibit conditioned avoidance response (e.g., Niemegeers et al., 1969; Davidson and Weidley, 1976; Moore et al., 1992), and the potency of antipsychotic drugs in inhibiting conditioned avoidance response correlates with dopamine-receptor blockade and clinical dose (e.g., Arnt, 1982). However, relatively little is known about the effects of muscarinic receptor agonists as a class on conditioned avoidance responding. Previous investigators have demonstrated that the muscarinic agonists arecoline, pilocarpine, and tremorine and the cholinesterase inhibitor physostigmine inhibited conditioned avoidance responding, and these effects were blocked by the muscarinic receptor antagonist atropine but not by quaternary muscarinic receptor antagonists that cross the blood-brain barrier only poorly (Pfeiffer and Jenny, 1957; Chalmers and Erickson, 1964). Similar results with the physostigmine have been obtained in gerbils, mice, and guinea pigs (Kuribara and Tadokoro, 1985; Philippens et al., 1992). Further data are needed to more completely characterize the effects of muscarinic agonists on conditioned avoidance responding.

The purpose of our studies was to investigate the effects of a broad range of muscarinic agonists on conditioned avoidance responding in the rat. Dose-response curves were determined for the classic muscarinic receptor agonists oxotremorine, arecoline, pilocarpine, [2-ethyl-8-methyl-2,8-diazaspiro (4.5)decane-1,3-dione] hydrochloride (RS86), and acetylcholine in rats trained to avoid the presentation of electric shock to the feet in a discrete trial procedure. In addition, dose-response curves were determined for the newer muscarinic receptor partial agonist or mixed agonist/antagonist ligands \([R-(Z)](-)-\alpha-(methoxyimino)-1-azabicyclo[2.2.2]octane-3-acetonitrile] hydrochloride (SB 202026; Bromidge et al., 1997; Loudon et al., 1997), talsaladine (Ensinger et al., 1993), milameline (Schwarz et al., 1993; Sedman et al., 1995), and xanomeline (Sauerberg et al., 1992; Bymaster et al., 1994; Shannon et al., 1994, 1997, 1998a). The effects of physostigmine were also determined. For comparison, dose-response curves for pilocarpine, RS86, and haloperidol were determined alone and in the presence of the muscarinic receptor antagonists scopolamine and/or trihexyphenidyl.

**Materials and Methods**

**Subjects.** Male Fischer-derived F344 rats (Harlan Sprague-Dawley, Indianapolis, IN) were housed individually with constant access to food and water in a large colony room that was illuminated from 6:00 AM to 6:00 PM.

**Apparatus.** The apparatus consisted of operant conditioning chambers enclosed in ventilated, sound-attenuating enclosures (model E10–10; Coulbourn Instruments, Lehigh Valley, PA). Each chamber was equipped with a wheel manipulandum (Verhave et al., 1957) that was 4.2 cm wide and 3.8 cm in diameter with eight spokes (3 mm diameter) spaced equally around the wheel. Each spoke could produce closure of a microswitch, and each closure was recorded as a response. A distributed electric shock could be delivered to the grid floor by a constant-current shock generator (model E13–08, Coulbourn Instruments). Schedule contingencies were controlled and data recorded with the SKED-11 language (State Systems, Kalama-zoo, MI) with a DFP11/73 computer (Digital Equipment Corp., Maynard, MA).

**Procedure.** Rats were required to respond on the wheel manipulandum to avoid or escape foot shock. A trial began with the onset of the conditioned stimulus (house light plus a tone). If the rat responded within 10 s (an avoidance response), the conditioned stimulus was terminated, the presentation of foot shock was avoided, and an intertrial interval was initiated. In the absence of a response within 10 s, foot shock (2 mA) was presented. A response within 10 s after shock onset terminated the house light, tone, and shock stimuli (an escape response) and initiated an intertrial interval. A trial terminated automatically if the rat failed to respond within 10 s after shock onset (a response failure). Each session terminated after 50 trials or after a cumulative 20 trials where the rat failed to respond.

**Drugs.** Oxtremorine sesquifumarate, arecoline hydrobromide, pilocarpine hydrochloride, physostigmine hemisulfate, scopolamine hydrobromide, trihexyphenidyl hydrochloride, benzotropine hydrochloride, trifluoperazine dihydrochloride, chlorpromazine hydrochloride, clozapine free base, haloperidol free base, and diazepam were purchased from Sigma Chemical Co. (St. Louis, MO); RS86, SB 202026, aceclidine hydrochloride, talsaladine fumarate (WAL 2014), milameline oxalate, and xanomeline tartrate were obtained from Lilly Research Laboratories (Indianapolis, IN) or Novo Nordisk Health Care Discovery (Måløv, Denmark). All drugs were dissolved in deionized water, except for clozapine and haloperidol, which were dissolved in deionized water to which a few drops of 8.5% lactic acid was added, and diazepam, which was dissolved in 20% propylene glycol. Doses refer to the form of the drug listed. Drugs were administered s.c. or i.p. (diazepam) in a volume of 1.0 to 3.0 ml/kg, 30 min before the start of an experimental session.

**Data Analysis.** Data are expressed as the mean \(\pm S.E.\) of the percentage of trials that were terminated by avoidance responses or response failures; the remaining percentage of trials were terminated by escape responses. The magnitude of the shifts in the dose-response curves of muscarinic agonists by muscarinic antagonists were compared by calculating ED\(_{50}\) values and 95% confidence limits via curve-fitting techniques with JMP v3.2 software (SAS Institute, Cary, NC). If respective pairs of 95% confidence limits did not overlap, the pair of dose-response curves was considered to be significantly different at \(p < .05\).

**Results**

**Dopamine Antagonists.** The dopamine antagonists haloperidol, trifluoperazine, chlorpromazine, and clozapine (in order of potency) produced dose-related decreases in the percentage of avoidance responses (Fig. 1, top). At intermediate
doses, avoidance responses were replaced primarily by escape responses (trials during which responses occurred during shock presentation). At higher doses, the dopamine antagonists produced increases in the percentage of response failures, i.e., trials during which no response was emitted (Fig. 1, bottom). The largest percentage of increase in response failures, approximately 55%, was produced by haloperidol (Fig. 1). The smallest increase in response failures, approximately 5%, was produced by clozapine. The percentage of response failures correlated with visually observed catalepsy produced by the dopamine antagonists, which interfered with the ability of the animals to respond.

**Diazepam.** Diazepam, over the dose range of 2.5 to 20 mg/kg, produced only an approximately 10% decrease in avoidance responses (Fig. 1). Diazepam did not produce response failures, even though the 10- and 20-mg/kg doses produced readily observable marked ataxia.

**Muscarinic Agonists.** The muscarinic agonists RS86 and pilocarpine produced dose-related decreases in the percentage of avoidance responses while producing less than approximately 30% response failures (Fig. 2, left). Oxotremorine and arecoline also decreased avoidance responding (Fig. 2, top right); however, oxotremorine and arecoline decreased avoidance responding primarily by producing response failures rather than by increasing escape responses (Fig. 2, bottom right). Acetylcholine produced only an approximately 40% reduction in avoidance responses while producing approximately 10% response failures over the dose range tested (Fig. 2, right). The percentage of response failures correlated with visual observations of motor tremor, which interfered with the ability of the animals to respond.

The muscarinic receptor ligands SB 202026, talsaclidine, milameline, and xanomeline also produced dose-related decreases in avoidance responding (Fig. 3, top). These four compounds reduced avoidance responses primarily by increasing escape responses without producing an appreciable percentage of response failures. The maximal percentage of response failures produced by SB 202026, milameline, talsaclidine, and xanomeline were approximately 10, 25 (primarily due to one animal), 2, and 10%, respectively (Fig. 3, bottom). Over the dose ranges tested, visual observations indicated that SB 202026, talsaclidine, and xanomeline produced minimal, if any, motor tremor or salivation, whereas milameline produced modest tremor and salivation.

**Cholinesterase Inhibitor.** Phystostigmine decreased avoidance responses to approximately 35% without producing response failures at the highest dose tested (0.1 mg/kg; Fig. 4). Doses of phystostigmine higher than 0.1 mg/kg were lethal in pilot experiments and were not tested here.

**Muscarinic Cholinergic Receptor Antagonists.** The muscarinic receptor antagonists scopolamine (0.03–3.00 mg/kg), trihexyphenidyl (0.3–10.0 mg/kg), and benztropine (0.3–10.0 mg/kg) had no substantial effect on avoidance responding over the dose ranges tested (Fig. 5).

**Antagonism of Pilocarpine and RS86.** As in previous experiments, pilocarpine and RS86 produced dose-related
decreases in avoidance responses while primarily increasing escape responses and producing modest increases in response failures (Fig. 6). Scopolamine (0.03–0.10 mg/kg) produced dose-related shifts to the right in the dose-response curve for pilocarpine for both reduction in avoidance responses (Fig. 6, top left) and response failures (Fig. 6, bottom left). The ED50 values (95% confidence limits) were 10.4 (8.7–12.4), 16.4 (14.9–18.0), 35.7 (31.6–39.7), and >80 mg/kg for pilocarpine alone and in the presence of 0.01, 0.03, and 0.1 mg/kg, respectively, of scopolamine for avoidance responses. Similarly, trihexyphenidyl (0.3 mg/kg) shifted the dose-response curve for pilocarpine to the right for both reduction in avoidance responses and increases in response failures (Fig. 6, middle). The ED50 values for pilocarpine alone and in the presence of 0.3 mg/kg of trihexyphenidyl were 12.8 (11.3–14.5) and 34.7 (27.6–45.6), respectively, for avoidance responses. Moreover, scopolamine (0.03 mg/kg) shifted to the right the dose-response curve for RS86 for both reduction in avoidance responses and response failures (Fig. 6, right). The ED50 values for RS86 alone and in the presence of 0.03 mg/kg of scopolamine were 4.3 (3.3–5.4) and 9.1 (8.1–10.1), respectively, for avoidance responses. All of these shifts were statistically significant in that the 95% confidence limits in the presence of the antagonists did not overlap with those for the agonist administered alone.

Fig. 3. Dose-related decreases in avoidance responses and increases in response failures produced by muscarinic receptor agonists in rats where behavior was maintained under a discrete-trial avoidance schedule. Each point represents the mean ± S.E. of one observation in each of four to six rats. Veh, vehicle.

Fig. 4. Dose-response curves for the cholinesterase inhibitor physostigmine in rats where behavior was maintained under a discrete-trial avoidance schedule. Each point represents the mean ± S.E. of one observation in each of four rats. Veh, vehicle.

Fig. 5. Lack of effects of the muscarinic receptor antagonists scopolamine, trihexyphenidyl, and benztpine on behavior maintained under an avoidance schedule in rats. Each point represents the mean ± S.E. of one observation in each of five rats. Avoid, avoidance responses; RF, response failure; Veh, vehicle.
Scopolamine-Haloperidol Interactions. To determine whether a muscarinic receptor antagonist blocked the effects of a dopamine antagonist, a dose-response curve for haloperidol was determined alone and in the presence of 0.1 mg/kg scopolamine. Scopolamine produced a nonparallel shift to the right in the dose-response curves for both haloperidol-induced reduction in avoidance responses and increases in response failures (Fig. 7). The primary effect of scopolamine was to reduce response failures and increase escape responses produced by the combination relative to haloperidol alone. Visual observations indicated that scopolamine antagonized the catalepsy produced by haloperidol, which otherwise interfered with the ability of the animals to respond, thereby permitting the animals to emit an escape response rather than fail to respond.

Discussion

The major finding of our studies was that muscarinic cholinergic receptor agonists from several chemical classes inhibited conditioned avoidance responding at doses that, in most cases, did not produce substantial response failures. Moreover, the effects of muscarinic cholinergic receptor agonists on conditioned avoidance responding were qualitatively similar to the effects of dopamine receptor antagonists, including the atypical antipsychotic clozapine. Together with the clinical data that the muscarinic cholinergic receptor agonist xanomeline reduces psychotic behavior in patients with Alzheimer’s disease (Bodick et al., 1997), the data presented herein, as well as our previous findings with the muscarinic receptor ligand PTAC (Bymaster et al., 1998b; Sauerberg et al., 1998), suggest that muscarinic receptor agonists may provide an alternative approach to the treatment of psychosis and schizophrenia.

Several of the compounds tested in the present studies, e.g., pilocarpine, RS86, and SB 202026, are known to be partial agonists at muscarinic receptors (e.g., Richards and van Giersbergen, 1995; Loudon et al., 1997). Thus, the possibility existed that the inhibition of avoidance response could be the result of either agonist or antagonist actions of these compounds at muscarinic receptors. The reductions in avoidance responses produced by pilocarpine and RS86, how-
ever, were antagonized in a dose-dependent manner by the muscarinic receptor antagonists scopolamine and trihexyphenidyl. Moreover, scopolamine and trihexyphenidyl shifted the dose-response curves for the agonists to the right for the most part in a parallel manner, suggesting that the antagonists were acting in a competitive manner. The present findings are consistent with our previous findings that scopolamine antagonized the inhibition of avoidance responding produced by PTAC (Bymaster et al., 1998b). Thus, together with previous findings, the data demonstrate that the inhibition of avoidance responding produced by muscarinic receptor ligands is mediated by agonist actions at muscarinic cholinergic receptors.

In addition to the direct-acting receptor agonists, the cholinesterase inhibitor physostigmine also reduced conditioned avoidance responding. Our findings with physostigmine replicate and extend previous findings (Pfeiffer and Jenny, 1957; Kuribara and Tadokoro, 1985; Philippens et al., 1992). The demonstration that physostigmine can inhibit conditioned avoidance responding in a manner similar to that of direct receptor agonists indicates that cholinergic neurons are part of or directly affect neuronal circuits involved in avoidance responding and that acetylcholine is tonically released in this circuit. Overactivity of the mesocorticolimbic dopamine pathway is widely held to be involved in the pathophysiology of schizophrenia (e.g., Creese et al., 1976). Cholinergic neurons of the pedunculopontine nucleus (Ch5) and laterodorsal tegmental nucleus (Ch6) monosynaptically activate dopamine neurons of the ventral tegmental area (A10) (Bolam et al., 1991). Moreover, muscarinic cholinergic agonists have recently been demonstrated electrophysiologically to directly activate ventral tegmental neurons (Gronier and Rasmussen, 1998). On the other hand, Ch5 and Ch6 cells are inhibited by local injections of muscarinic agonists, presumably by actions at autoreceptors on cholinergic cell bodies (e.g., Yeomans et al., 1993). Thus, it has been suggested (Garcia-Rill et al., 1995; Yeomans, 1995) that the Ch5 and Ch6 cholinergic nuclei may play an important role in modulating mesocorticolimbic dopaminergic pathways and thereby play a role in the pathophysiology of schizophrenia. If the brain stem cholinergic neurons tonically activate mesocorticolimbic dopaminergic pathways, it is possible that the muscarinic receptor agonists evaluated in our studies acted at autoreceptors on the cell bodies of Ch5 and/or Ch6 neurons to inhibit them, thereby decreasing tonic activation of mesocorticolimbic dopaminergic pathways. However, further studies are needed to support or refute this hypothesis.

Although all of the muscarinic receptor agonists used herein decreased conditioned avoidance responding, there were differences among the agonists in the degree of separation between reduction in avoidance responses and increases in response failures. Among the classic muscarinic agonists, RS86 and pilocarpine produced the greatest reduction in avoidance responses (≥80%) with the smallest percentage (<30%) of response failures. In contrast, oxotremorine and arecoline primarily decreased the percentage of avoidance responses by producing response failures, whereas xanomeline produced intermediate changes. The present findings replicate and extend those of previous investigators who demonstrated that the muscarinic receptor agonists tremorine, arecoline, and pilocarpine reduce conditioned avoidance behavior (Pfeiffer and Jenny, 1957; Chalmers and Erickson, 1964). In addition, the newer muscarinic receptor ligands SB 202026, talsaclidine, and xanomeline decreased avoidance responding without producing appreciable response failures, whereas milameline reduced avoidance responses and, like pilocarpine, produced approximately 30% response failures. One possible explanation for the observed differences among the compounds tested may be differences in muscarinic receptor subtype selectivity.

We have previously reported (Bymaster et al., 1998b) that PTAC, which inhibits avoidance responding, is a partial agonist at M2 and M4 receptors but an antagonist at M1, M3, and M5 receptors. Moreover, SB 202026, milameline, and talsaclidine were antagonists of M2-receptor-mediated increases in phosphoinositide levels produced by pilocarpine in vivo, whereas xanomeline and RS86, like pilocarpine, functioned as agonists to increase phosphoinositide levels (Bymaster et al., 1998a). Because the inhibition of avoidance responding is antagonized by muscarinic receptor antagonists, the selectivity profile for PTAC and other compounds suggests a primary role for agonist activity at M2 and/or M4 receptors in mediating inhibition of avoidance responding by muscarinic receptor agonists. Unfortunately, relatively little information is available directly comparing the relative M2 and M4 efficacies and potencies of the compounds tested in our studies. However, pilocarpine, arecoline, oxotremorine, and milameline have been demonstrated to be agonists at human M4 receptors expressed in Chinese hamster ovary (CHO) cells (Schwarz et al., 1993; Sedman et al., 1995). Although similar data have not been published for the other compounds, SB 202026 is a partial M2-receptor agonist in that it produces a partial inhibition of release of acetylcholine (presumably by agonist actions at M2 autoreceptors) (Loudon et al., 1997), whereas talsaclidine (Ensinger et al., 1993) and xanomeline (Shannon et al., 1994) increase rather than decrease heart rate, as would be expected for an M4 agonist. On the other hand, pilocarpine, RS86, arecoline, and oxotremorine are high-efficacy agonists at human M4 receptors expressed in CHO cells (Richards and van Giersbergen, 1995), as are milameline (Sedman et al., 1995) and xanomeline (Bymaster et al., 1997, 1998a); the efficacy of SB 202026 and talsaclidine at M4 receptors has not been reported (Ensinger et al., 1993; Loudon et al., 1997). Thus, although the data available to date suggest that agonist actions at M4 and possibly M2 receptors may be involved in mediating the inhibition of avoidance responding, a role for a combination of agonist and/or partial agonist actions at multiple receptor subtypes cannot be ruled out.

Although all of the available clinically useful antipsychotic drugs are believed to produce their therapeutic effects through influences on the mesocorticolimbic dopaminergic pathway (e.g., Creese et al., 1976), schizophrenia is unlikely a one-neurotransmitter disorder, and roles for multiple neurotransmitters interacting in complex neuronal circuits have been proposed (e.g., Carlsson et al., 1997). Interactions between dopaminergic and cholinergic systems are well known, and several investigators have demonstrated that the brain stem muscarinic cholinergic nuclei (Ch5 and Ch6) synapse onto, and are involved in the modulation of, the mesocorticolimbic dopaminergic system, suggesting a role for the brain stem cholinergic system in the pathophysiology of schizophrenia (Garcia-Rill et al., 1995; Yeomans, 1995). Our findings that muscarinic receptor ligands, perhaps by agonist
actions at $M_2$ and/or $M_4$ receptors in neuronal circuits modulating mesocorticolimbic pathways, inhibit conditioned avoidance responding in a manner similar to that of dopaminergic antagonists are consistent with this hypothesis. Moreover, the data presented herein, together with our previous clinical findings that xanomeline reduces psychotic behavior in patients with Alzheimer's disease and that the muscarinic receptor ligand PTAC has a pharmacological profile similar to that of atypical antipsychotic drugs, suggest that muscarinic receptor subtype-selective agonists may provide a new approach to the treatment of schizophrenia.

References


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