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Effects of Some Antimuscarinics Alone and in Combination With Chlordiazepoxide on Punished and Nonpunished Behavior of Rats

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WITKIN, J. M. AND K. M. WITKIN. *Effects of some antimuscarinics alone and in combination with chlordiazepoxide on punished and nonpunished behavior of rats.* PHARMACOL BIOCHEM BEHAV **39**(2) 453–456, 1991.—Since both diphenyl-substituted antimuscarinics and benzodiazepine anxiolytic drugs have been reported to increase responding under fixed-ratio schedules of food presentation, these antimuscarinics may also have anxiolytic activity. The effects of aprophen and benactyzine on punished responding of rats, a preclinical anxiolytic drug screen, were compared with those of atropine and chlordiazepoxide. None of the antimuscarinics produced consistent overall increases in behavior suppressed by punishment, in contrast to the dose-dependent increases obtained with chlordiazepoxide. Aprophen did not potentiate the anxiolytic activity of chlordiazepoxide. However, a high dose of atropine potentiated the effects of chlordiazepoxide on punished responding. Thus the diphenyl-substituted antimuscarinics, aprophen and benactyzine, do not possess consistent or robust anxiolytic activity in this preclinical screen. The previously reported behavioral excitatory effects of these compounds may therefore be unrelated to this pharmacological action.

Atropine Benactyzine Aprophen Punished behavior Behavioral effects Rats

COMPARISON of structurally diverse antimuscarinics indicated that certain aromatic esters of diethylaminoethanol can induce behavioral stimulatory effects not observed with tropate antimuscarinics such as atropine [cf. (13)]. Whereas the diphenyl-substituted antimuscarinics, aprophen and benactyzine, increased response rates of rats responding under fixed-ratio schedules in which every tenth response produced food, atropine only decreased responding (1,15). The pharmacological basis for the qualitative differences in behavioral activity have not been determined.

Since the increases in fixed-ratio responding observed with benactyzine and aprophen are also observed with sedative hypnotic and classical anxiolytic agents (11,12), we compared the effects of antimuscarinics on punished behavior, a preclinical anxiolytic drug screen. Antimuscarinic compounds have occasionally been reported to produce positive effects under some of these preclinical anxiolytic tests (2,7), and consistent positive findings have been reported when atropine is delivered into the ventromedial hypothalamus (6,9). In addition, increases in punished responding with some drugs have been attributed to their antimuscarinic activities (4). Interactions of antimuscarinics with benzodiazepines were also tested to evaluate the possibility of muscarinic antagonist-induced potentiation of the effects of chlordiazepoxide. In studies of locomotor activity, scopolamine has been reported to potentiate the stimulatory effects of chlordiazepoxide (10), and antimuscarinics have been used in combination with anxiolytics in the clinical management of anxiety [cf. (3)].

METHOD

Subjects

Adult, male Sprague-Dawley rats (Zivic Miller, Allison Park, PA) were maintained at 350 g by postsession feeding in separate living cages. All rats were experimentally naive prior to this study and were housed within a temperature-controlled room with unrestricted access to water. The rats were housed under a 12-h light/dark cycle and tested during the light phase.

Apparatus

Rats were studied in standard operant conditioning test chambers (Coulbourn Instruments, Lehigh Valley, PA) which con-

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tained a response lever. Chambers were contained within sound- and light-attenuating enclosures supplied with white noise to further mask extraneous sounds. Scrambled electric shock could be delivered to the grid floor by a constant current AC source. Experimental events were scheduled and data were collected with a PDP 11/73 computer operating SKED-11 software (State Systems, Kalamazoo, MI).

Punished Responding

After initial training to eat food pellets (45 mg, BioServe, Frenchtown, NJ) delivered to a centrally located receptacle, the rats were trained to depress the lever by requiring these responses for food presentation. Pressing the lever with a minimal downward force of 0.3 g through 1 mm produced food. All responses produced the audible click of a relay. The final schedule under which drug effects were assessed was a multiple fixed-ratio 30 (food) fixed-ratio 10 (food+shock) schedule of food presentation (12). In the presence of green lights, every 30th lever press produced food; when red lights were on, every 10th lever press produced both food and shock (0.2 s). The fixed-ratio requirement was counted from component onset. Shock intensities (0.5–2 mA) were adjusted for each animal to suppress responding to less than 10% of nonpunished response rates. Punishment and nonpunishment schedule components alternated every 3 min and were separated by a 30-s timeout period during which the lights were extinguished and responding had no scheduled consequences. Sessions began with the green lights and consisted of 5 presentations of each schedule component.

Drugs

Aprophen hydrochloride (Walter Reed Army Institute of Research), atropine sulfate (Sigma Chemical Co.), benactyzine hydrochloride (Aldrich Chemical Co.), and chlordiazepoxide hydrochloride (Sigma) were dissolved in isotonic saline and administered by IP injection in a volume of 1 ml/kg. Drug doses are expressed as the salts. All drugs were given 30 min prior to the experimental sessions. Drugs and drug doses were studied in a mixed order, with dose-effect functions for one compound generally being completed prior to investigation of another drug. Each dose or dose combination was generally studied on two separate occasions in each animal. For each dose response curve, at least six rats were used. Since both aprophen and benactyzine have been reported to increase responding under fixed-ratio schedules in the absence of punishment (15), only aprophen was studied in combination with chlordiazepoxide in the present study; these results were compared to drug interaction experiments with atropine.

Data Analysis

Drug effects on response rates were expressed as a percentage of saline and nondrug control values in individual subjects. Dose-effect functions were established by averaging these values across subjects. Drug effects were considered to differ significantly from baseline values in individual animals when they differed by ±2 S.D. from control performances.

RESULTS

Control performance was consistent with previous work under this baseline (12). Rates of punished responding ranged from 0.02–0.28 and nonpunished responding from 0.99–2.07 responses/s across subjects.

Atropine did not significantly alter punished responding when given up to doses that markedly suppressed nonpunished response rates (Fig. 1, circles). Aprophen decreased both punished and nonpunished responding at the highest dose tested (Fig. 1, squares). At 10 mg/kg aprophen, nonpunished responding was significantly increased in only 1 of 6 rats. Benactyzine increased punished responding in only 1 of 6 rats at 10 mg/kg. No other effects of benactyzine differed significantly from control. Nonpunished responding was not increased at any dose of benactyzine and was markedly decreased at the higher doses (Fig. 1, triangles).

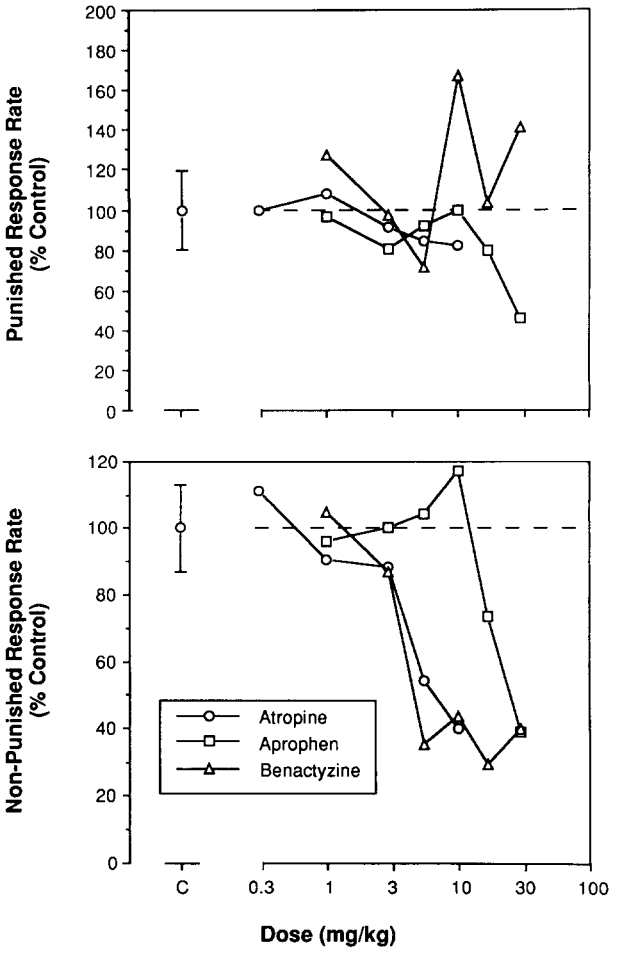

FIG. 1. Effects of atropine, aprophen and benactyzine on punished (upper panel) and nonpunished responding (lower panel). Each point represents the mean effect in 6 rats. Points above C represent control variability ±S.E.M.

In contrast to the antimuscarinics, chlordiazepoxide produced dose-dependent increases in responding suppressed by punishment at doses that had little systematic effect on nonpunished response rates (Fig. 2, unfilled squares). Aprophen at 5.6 mg/kg, which did not significantly alter either punished or nonpunished responding, did not modify the rate-increasing effects of chlordiazepoxide on punished behavior. Nonpunished responding was also not systematically changed in aprophen-treated rats (Fig. 2, filled triangles).

When given in combination with 3 mg/kg chlordiazepoxide, 10 mg/kg atropine potentiated the rate-increasing effects of chlordiazepoxide on punished responding (Fig. 3, top panel). Rates of punished behavior after 3 mg/kg chlordiazepoxide in this experiment were 152.0 and 386.2% of control levels when given in combination with either saline or 10 mg/kg atropine, respec-

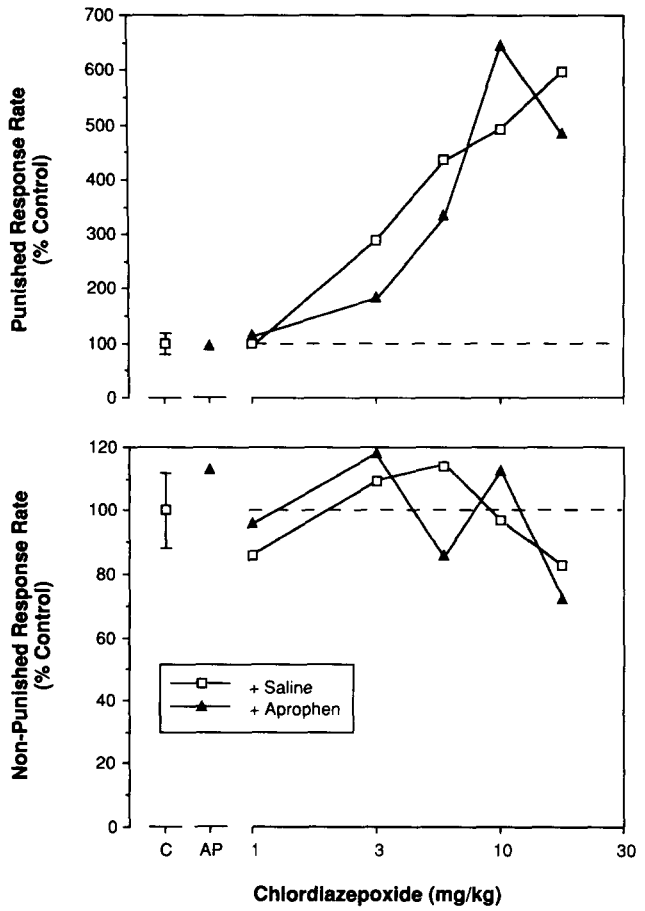

FIG. 2. Effects of chlordiazepoxide alone (squares) and in combination with 5.6 mg/kg aprophen (triangles) on punished (upper panel) and nonpunished responding (lower panel). Points above C represent control variability ±S.E.M. Points above AP represent effects of aprophen alone.

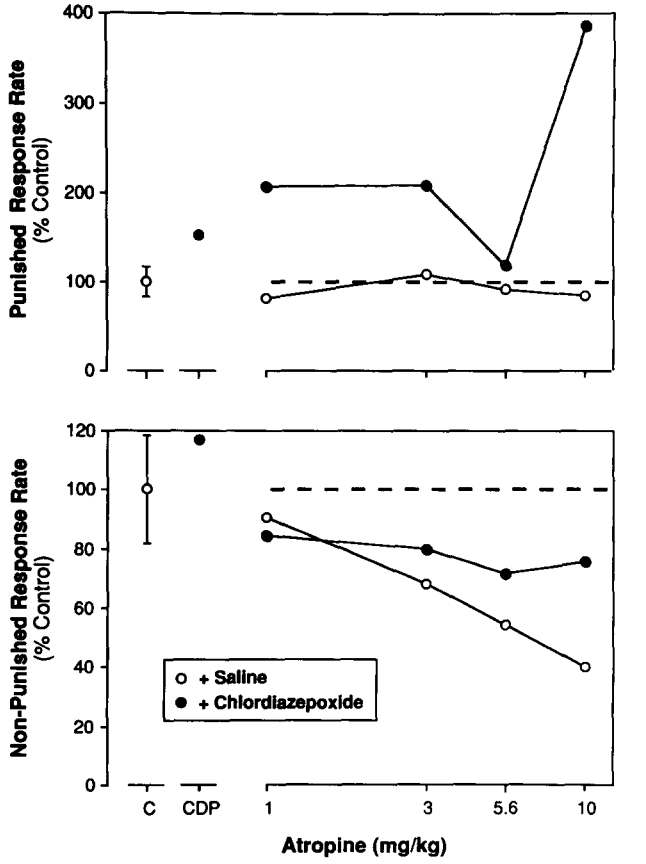

FIG. 3. Effects of atropine alone (open circles) and in combination with 3 mg/kg chlordiazepoxide (filled circles) on punished (upper panel) and nonpunished responding (lower panel). Points above C represent control variability ±S.E.M. Points above CDP represent effects of 3 mg/kg chlordiazepoxide alone.

tively $t(5)=3.08$, $p<0.05$. However, lower doses of atropine (1–5.6 mg/kg) did not enhance the effects of chlordiazepoxide. In addition, decreases in responding produced by atropine were attenuated in the presence of chlordiazepoxide (Fig. 3, bottom panel).

DISCUSSION

Benactyzine in combined form with meprobamate has found previous clinical use in the treatment of depression and anxiety (3). Although one previous report has indicated that benactyzine can increase punished behavior (2), neither benactyzine nor aprophen consistently increased punished responding in the present study. Atropine did not increase punished responding in the present study, confirming previous data obtained with systemic administration (5,8). Nonetheless, under other conditions, benactyzine but not atropine can produce profound behavioral stimulation that can be greater than that observed with cocaine (14).

The lack of anxiolytic activity of the diphenyl-substituted compounds was further documented by the lack of significant interaction of aprophen with chlordiazepoxide (Fig. 2). The fact that atropine under limited dose conditions can potentiate the effects of chlordiazepoxide (Fig. 3) is interesting, albeit enigmatic; nonetheless, this result again emphasizes the qualitative differences that can be observed between atropine and aprophen-like antimuscarinics that have been reported earlier [cf. (13–15)].

Previous studies have demonstrated that certain diphenyl-substituted muscarinic antagonists such as benactyzine or aprophen can produce behavioral excitatory effects that are not observed with atropine (13–15). The results of the present study suggest that the stimulatory effects of benactyzine or aprophen may not be related to anxiolytic activity. However, it is also possible that the peripheral muscarinic antagonist actions of the antimuscarinics interfere with the expression of a central anxiolytic profile. This may have been the case with atropine, which increases punished responding if delivered into the ventromedial hypothalamus (6,9) but not if given systemically.

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