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Differential Effects of Anticholinergic Drugs on Paired Discrimination Performance

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GRAUER, E. AND J. KAPON. *Differential effects of anticholinergic drugs on paired discrimination performance.* PHARMACOL BIOCHEM BEHAV 53(2) 463–467, 1996.—Working and reference memory processes were simultaneously evaluated during the performance of a paired discrimination (PD) task in which visual and spatial discrimination trials were combined within the same session. Atropine (1 and 5 mg/kg), scopolamine (0.02–0.20 mg/kg), benactyzine (1–4 mg/kg), trihexyphenidyl (1–10 mg/kg), and aprophen (5–20 mg/kg) were all found to increase the number of errors performed by overtrained rats during the spatial but not during the visual trials. Although all the anticholinergic drugs tested induced specific working memory impairment at low doses, they differentially affected other, simultaneously recorded, behavioral parameters. Thus, while atropine affected most of the recorded parameters, aprophen induced only a mild effect. Benactyzine was found to have the most specific effect on working memory, with only minimal side effects, a combination that supports its use as the preferred psychopharmacological model of working memory impairment.

Working memory Reference memory Cholinergic antagonist Atropine Aprophen Scopolamine Benactyzine Trihexyphenidyl Maze Rat

COGNITIVE deficits are commonly induced in experimental animals through the administration of various cholinergic antagonists. This cholinergic hypofunction is often accompanied by additional, nonspecific alterations in behaviors that may affect the interpretation of the data. A behavioral paradigm was recently introduced in which the effects of an acute drug administration were evaluated in both visual and spatial discriminations paired within a single session (11). This procedure enabled the analysis of drug effects in terms of working and reference memory processes as empirically defined (19). Other behavioral parameters simultaneously recorded during the performance of this task enabled additional comparisons among the different drugs, based on the extent of their effects on behavior.

A wide range of anticholinergic drugs can be used for the induction of cholinergic hypofunction. Atropine and scopolamine are the most common anticholinergic drugs used to simulate memory impairments. High doses of these drugs have been found to affect spatial and nonspatial discrimination (12,23) associated with both working and reference memory processes in overtrained rats (3). Lower doses of scopolamine were found to affect only spatially oriented, working memory processes (1,2,13).

Trihexyphenidyl (THP, Artane) is an anticholinergic drug with a widespread use in psychiatric and neurological disorders. Recently, its possible involvement in augmenting memory impairment came under investigation (22). Trihexyphenidyl is also known for its euphoric properties and is abused by both normal (6) and psychiatric patients (21), producing strong physical and psychological dependence (14).

Benactyzine and aprophen are structurally similar anticholinergic drugs. Benactyzine was used in the past as a relaxant (7), while aprophen was used as an antispasmodic drug (18). Although both are potent anticholinergics, they are used only sporadically in either research or treatment.

The present study was a comparative evaluation of the behavioral effects of the various anticholinergic drugs at the minimal dose range necessary to produce any significant behavioral changes. The evaluation was based on the behavioral profile obtained for each drug during the performance of the paired discrimination task by well-trained rats. This comparison enabled the selection of the anticholinergic drugs best fitted to be used for an experimental induction of specific and differential deficits in working vs. reference memory processes. In addition, because anticholinergic drugs have wide therapeutic use, the comparison can be used to select drugs with minimal range of side effects.

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METHOD

Animals

Male albino Sprague-Dawley rats (Charles River, England), approximately 300 g at the beginning of the experiments, were used. The animals were individually housed in stainless steel cages on a 12 L : 12 D cycle, in a temperature-controlled environment. Food (Altromin 1324) was available ad lib, but access to water was restricted to 30 min a day, available at about 1600 h. Animals were tested daily between 0800 and 1600 h.

Apparatus

Rats were tested in one of two identical mazes previously described in detail (11). The maze was a rectangular, 30 cm L × 25 cm W × 45 cm H clear Plexiglas box. Two levers, 16 cm apart, were located 5 cm above the floor on one of the narrow walls. A cue light was placed immediately above each lever. Through a hole at the base of the opposite wall, a motorized liquid dispenser could introduce a small cup containing 0.2 cc of liquid (10% sugar in tap water) as reinforcement. A house light was located above the reinforcement hole. An opaque partition, extending two-thirds the length of the box, was placed between the two levers. Two other partitions, made of clear Plexiglas and placed perpendicularly to the end of the middle partition, divided the box into three compartments with a narrow path (the choice point) between them.

Behavioral Task

The paired discrimination procedure was previously described in detail (11). In short, the session was divided into pairs of trials. Each pair consisted of a visual discrimination trial followed by a spatial discrimination trial. In the visual trial, one of the cue lights above the levers was turned on in a random order, and the correct lever was signaled by the light-off cue. In the following spatial trial, no cue lights were available and the correct lever was the one that was incorrect in the previous, visual trial. Each pair of trials was followed by a 10 s time-out in which all lights, including the house light, were off. A press on the correct lever was reinforced with a 6-s presentation of the reinforcement at the opposite wall. This ensured that the rat was positioned at the choice point, away from the levers, at the beginning of the next trial. A correct response also turned off the cue lights.

Procedure

Rats were first trained by successive approximation to press either one of the two levers and receive the reinforcement. The animals were then trained on the visual discrimination trials only (50 trials per daily session) for four to six sessions, after which paired discrimination training began. The daily session included 50 pairs of trials (100 reinforcements) and the maximum time allowed was 2 h per session. Total time to complete the session, as well as responses during time-out periods were recorded and calculated for all 100 trials of the daily session. The number of initial errors (the first response on an incorrect lever for each trial), and that of repetitive errors (repeated responses on an incorrect lever for each trial) were recorded and calculated separately for the visual trials (50 trials) and for the spatial trials (50 trials). Based on the empirical definitions offered by Olton et al. (19), errors on the visual trials were also termed reference memory errors, while errors on the spatial trials were termed working memory errors (11). Animals were trained daily, 5 days a week, until stable performance was reached (a total of about 25 sessions).

Drug Administrations

All rats were trained to stable performance levels prior to the onset of drug administration schedule. Drug injections were spaced at least 1 week apart to minimize behavioral tolerance (15). If performance in the two successive daily sessions prior to drug day were not stable, drug treatment was postponed. Atropine sulfate (Sigma), scopolamine HBr (Sigma), and benactyzine HCl (Aldrich), were dissolved in saline. Aprophen HCl (18) (prepared at the department of Organic Chemistry, IIBR) was dissolved in 0.05 M citrate buffer pH 5.6. Trihexyphenidyl (Artane—Sigma) was dissolved in distilled water. All drugs were administered SC 30 min prior to the onset of the behavioral testing. Drugs were tested in groups of 5 to 12 rats.

Statistical Analysis

Data for each behavioral parameter were analyzed by analysis of variance (ANOVA). Significant main effects were further analyzed by the Dunnett's test enabling comparisons between baseline control and each of the doses of drug treatment. When needed, the Scheffe test was used for a comparison between the different doses.

RESULTS

Because the same period of 2 h used during baseline sessions was imposed as a time limit on drug test sessions, some animals did not complete all 100 trials required. Thus, for each anticholinergic drug, the numbers of trials completed for each of the doses tested was also included in the summary of the data presented in Figs. 1 through 5. In addition, the data were calculated per trial. The scales on the Y-axis were kept constant through all five figures to enable an overall impression of the comparison between compounds. The drugs are described so that the compound with the widest range of ef-

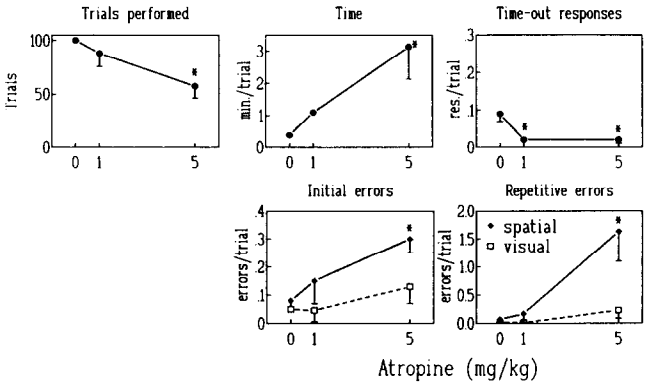

FIG. 1. Effects of atropine (1 and 5 mg/kg, SC) on paired discrimination (PD) performance. Trials performed: total number of trials completed in 2 h (out of 100). Time: total performance time (100 trials). Time-out responses: Responses during time-out periods (total of 100 time-outs, each of 10 s duration). Initial and repeated errors are presented separately for visual (open symbols) and spatial trials (close symbols). Each type of trial was presented 50 trials/session. *$p < 0.03$ compared to baseline (see text for detailed analysis).

fects (i.e., atropine) is described first, and the one with the mildest behavioral effect (i.e., aprophen) is described last.

The effects of atropine, 1 and 5 mg/kg, on the various behavioral parameters obtained during the performance of the PD task is depicted in Fig. 1. As can be seen, an over 40% decrease in the number of trials performed was recorded at 5 mg/kg. This general slow down in performance was also seen in the increase in the time the trials were completed and in the decrease in the number of responses performed during time-outs. At the same time, an increase was seen in the number of both initial and repetitive errors, and this was specific to spatial trials only. No increase in errors was seen during the performance of the visual trials. These observations were supported by the statistical analysis. A one-way ANOVA on each of the parameters showed a significant difference in the total numbers of trials performed, $F(2, 17) = 7.26$, $p = 0.005$. Further analysis showed that the 5 mg/kg dose was significantly different from baseline (Dunnett test, $p = 0.03$). Similarly, 5 mg/kg was significantly different from baseline in the time of performance [ANOVA main effect: $F(2, 17) = 5.24$, $p = 0.017$, Dunnett test: $p = 0.011$], and both the 1 and 5 mg/kg doses of atropine resulted in a decrease in the number of responses emitted during time-outs [ANOVA main effect: $F(2, 17) = 6.7$, $p = 0.007$, Dunnett test, 1 mg/kg: $p = 0.014$, 5 mg/kg: $p = 0.012$]. A significant difference from baseline was obtained following 5 mg/kg in both initial [ANOVA main effect: $F(2, 17) = 5.6$, $p = 0.013$, Dunnett test, $p = 0.008$] and repetitive errors [ANOVA main effect: $F(2, 17) = 7.3$, $p = 0.005$, Dunnett test, $p = 0.003$].

The effects of scopolamine, 0.02 to 0.2 mg/kg on performance of the PD task is depicted in Fig. 2. The numbers of trials completed was unaffected by all doses tested. The differences from baselines can be seen in the time of performance and in the number of errors emitted during the performance of the spatial, but not visual, trials. ANOVA revealed a significant main effect in the time of performance, $F(4, 24) = 12.5$, $p < 0.001$, attributed to the difference between 0.1 and 0.2 mg/kg and baseline (Dunnett, $p < 0.001$). A significant main effect was obtained in the number of initial errors performed during spatial trials, $F(4, 24) = 11.7$, $p < 0.001$, and, again, this was attributed to the difference between the higher doses and baseline performance (0.1 mg/kg: $p = 0.025$, 0.2 mg/kg: $p < 0.001$). The significant main effect seen in the number of repetitive errors of the spatial trials, $F(4, 24) = 5.1$, $p = 0.004$, was attributed to the effect of 0.1 mg/kg only ($p = 0.002$).

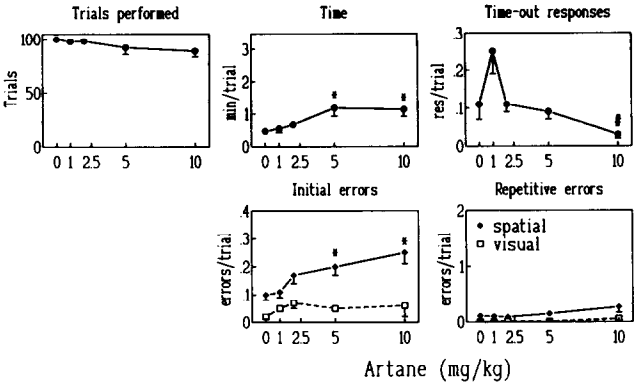

FIG. 3. Effects of trihexyphenidyl (1–10 mg/kg) on paired discrimination task. $*p < 0.02$ compared to baseline, $\#p < 0.02$ compared to 1 mg/kg (see text for detailed analysis). For further details see Fig. 1.

The effects of trihexyphenidyl (1–10 mg/kg) on the performance of the PD task are summarized in Fig. 3. Following the administration of either 5 or 10 mg/kg, an increase can be seen in the time to complete the session and in the number of initial errors emitted during the performance of the spatial trials. Two out of the eight animals injected with 10 mg/kg completed only 3 out of the 100 trials required, and their data were excluded from the analysis. Significant main effects were seen in the time, $F(4, 36) = 4.7$, $p = 0.004$, and in the initial errors of the spatial trials, $F(4, 36) = 5.4$, $p = 0.002$, parameters. In both parameters the post hoc analysis indicated significant differences in the effects of 5 and 10 mg/kg compared to baseline ($p < 0.02$). A significant effect was also obtained in the number of responses performed during time-outs, $F(4, 36) = 3.97$, $p = 0.009$, but this was attributed to a difference between the effect of 1 mg/kg and that of 10 mg/kg (Scheffe test, $p = 0.019$).

The effects of benactyzine (1–4 mg/kg) were described previously (11), and are presented here for comparison with the data recalculated per trial (Fig. 4). A clear, dose-dependent increase can be seen in the number of initial errors obtained

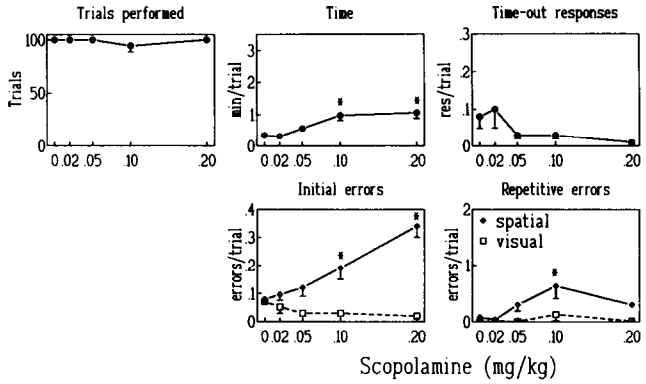

FIG. 2. Effects of Scopolamine (0.02–0.2 mg/kg) on paired discrimination task. $*p < 0.025$ compared to baseline (see text for detailed analysis). For further details, see Fig. 1.

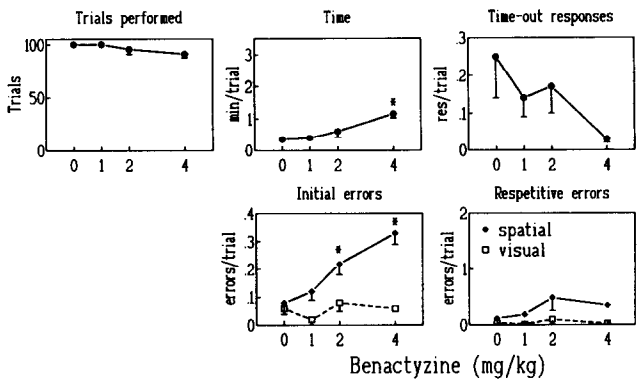

FIG. 4. Effects of benactyzine (1–4 mg/kg) on paired discrimination task. $*p < 0.02$ compared to baseline (see text for detailed analysis). For details see Fig. 1.

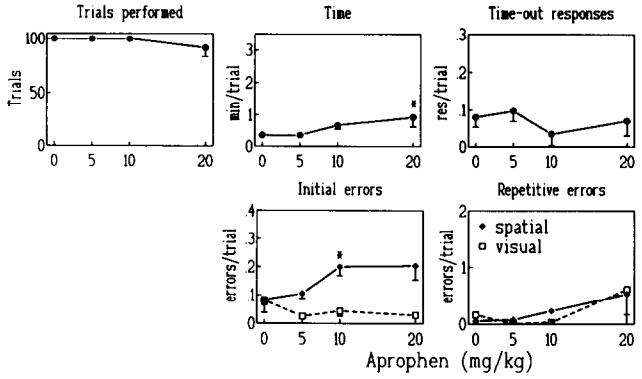

FIG. 5. Effects of Aprophen (5-20 mg/kg) on paired discrimination task. *$p < 0.03$ compared to baseline (see text for detailed analysis). For details see Fig. 1.

during the performance of the spatial trial with a milder increase in performance time. In this group of animals, the number of responses performed during time-outs was relatively high and it tended to decrease with the increase in the dose of benactyzine. Statistical analysis showed a significant effect of initial errors during spatial trials, $F(3, 24) = 10.2$, $p < 0.001$, and in the post hoc analysis both 2 and 4 mg/kg were significantly different from baseline (Dunnett, $p < 0.02$). The significant effect of time, $F(3, 24) = 9.6$, $p < 0.001$, was attributed to the effect of the highest dose tested only (4 mg/kg, Dunnett, $p < 0.001$). The tendency to decrease responses during time-outs with the increase in dose was not statistically significant.

The effects of aprophen (5-20 mg/kg) are depicted in Fig. 5. The effects of aprophen on the parameters recorded during the performance of the PD task were relatively mild. Again, they can be seen as an increase in the time of performance and in the number of initial errors of spatial trials. A one-way ANOVA on performance time showed a significant effect, $F(3, 19) = 3.64$, $p = 0.03$, and further Dunnett analysis showed a significant difference between 20 mg/kg and baseline. The ANOVA on the number of initial errors during spatial trials also revealed significant effect, $F(3, 19) = 4.45$, $p = 0.016$, and further Dunnett test showed both 2 and 4 mg/kg to be different from baseline.

DISCUSSION

Five different anticholinergic drugs were tested at low doses for their effects on PD task. They all caused an increase in the number of initial errors during performance of spatial trials, while none had any effect on performance of visual trials. Correct responding on both types of trials in the PD task required an equal motivational level, similar proprioceptive/motor functions, and intact reference memory process. However, the performance on the spatial task required the additional use of working memory process (11,19). Thus, common to all anticholinergic drugs was the specific impairment in working memory. This is in accord with the prevailing hypothesis of a correlation between cholinergic hypofunction and impaired working memory [e.g., (2,24)]. It should be noted, however, that the specific effect on working memory seen during performance of the PD task, was obtained in overtrained rats at relatively low doses. These doses, although often shown to be effective in disrupting acquisition, were rarely effective in altering performance in well-trained animals (3,20). Moreover, the increase in errors must be attributed to the central effect of the drugs because scopolamine methyl bromide, a quaternary compound that does not readily enter the brain, had no effect on errors in this task (results not shown). Thus, the PD task was demonstrated as an effective and efficient method in detecting specific changes in working memory processes.

Among the five drugs tested, scopolamine and benactyzine produced the specific increase in working memory errors in a dose-dependent manner, with minimal effect on the other parameters tested. Benactyzine, at 2 mg/kg, impaired working memory with no additional changes in behavior. Because benactyzine also seemed to lack the aversive properties associated with scopolamine (17), it should be considered as the drug of choice for a psychopharmacological model of working memory impairment.

Comparison among the different anticholinergic drugs clearly demonstrated a difference in the range of behaviors affected by each of the drugs. Thus, atropine affected almost all the behavioral parameters tested and was the only drug that significantly suppressed responding. In contrast, aprophen induced minimal effects on performance, with only mild effect on memory. Based on such comparisons aprophen, with its mild side effects [see also (10)], should be considered as an alternative or an addition to existing anticholinergic therapy.

Variability in the effects of anticholinergic drugs was previously demonstrated in diverse experimental procedures. Drugs were rank ordered according to their potency in suppressing performance on schedule-controlled behaviors (9,25), or based on their anticonvulsant activity in soman poisoning (5). In vitro affinities to muscarinic receptors could not account for the differences among the compounds (25). Their different affinities for nicotinic sites did not seem to explain the data either (8,16). The identification of several muscarinic receptor subtypes initiated recent attempts to correlate the differential effects of various anticholinergic drugs with specific affinity to one or more of the receptor subtypes (5). These experiments, although promising (4), are still inconclusive. The future development of agents targeting the receptor subtypes may benefit from the present behavioral paradigm, which can help in the understanding of the functional selectivity of these subtypes and/or their neuroanatomical location. Because all the drugs tested in this study were nonspecific anticholinergic drugs, their differential effects may be attributed to any one or more of the mechanisms referred to above.

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