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Alternating Lever Cyclic-Ratio Schedule Analysis of the Effects of Atropine Sulfate

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WELDON, D. T., E. O'HARE, M. A. KUSKOWSKI, J. CLEARY AND J. R. MACH, JR. *Alternating lever cyclic-ratio schedule analysis of the effects of atropine sulfate.* PHARMACOL BIOCHEM BEHAV **54**(4) 753–757, 1996.—Cholinergic dysfunction has been implicated in the behavioral and memory impairment that is the hallmark of conditions such as delirium and Alzheimer's Disease. Anticholinergic drugs have been widely used in procedures designed to mimic aspects of the pathology of these conditions in rats. Procedures in use vary widely in sensitivity and behavioral specificity and may be confounded by administration of high drug doses that may not be physiologically relevant. The current study proposes the use of rats responding on an alternating lever cyclic-ratio schedule to study the effects of the anticholinergic compound atropine sulfate. This procedure enables simultaneous measurement of anticipatory ratio tracking (postreinforcement pause durations), perseverations (lever switching errors), and nonspecific peripheral drug effects (running response rates). Results of this study suggest that the schedule is sensitive to low drug doses (0.1–1.0 mg/kg atropine), measures the ability to track changing ratio conditions and to execute lever alternation, and allows for monitoring of peripheral drug effects during behavioral testing. The procedure's sensitivity and low effective dose range may make it useful in the study of behaviors related to anticholinergic effects.

Delirium Cyclic-ratio Atropine sulfate Anticholinergic Postreinforcement pause (PRP) Perseveration Rat Schedule-controlled behavior

CHOLINERGIC dysfunction has been implicated in several diseases involving behavioral abnormalities (14). Most prominent of these diseases are delirium and Alzheimer's disease (AD). Delirium is an acute confusional state characterized by impairment in attention with behavioral change or perceptual disturbance (1). An anticholinergic hypothesis for delirium has been proposed, based on evidence of acetylcholine synthesis impairment produced by hypoxia, behavioral and EEG manifestations of delirium in anticholinergic intoxication, and elevated serum anticholinergic activity in delirious patients (11). In addition, blind-alley maze running and EEG measurement after high-dose atropine administration in rats has been proposed as a model of human delirium (15,31). Cholinergic dysfunction in Alzheimer's disease has been the focus of pathologic and therapeutic investigation [e.g., (12,13,19,32)]. Senile plaque formation is most common in areas of high cholinergic neuron density, and the hallmark clinical symptom of the disease, memory loss, is thought to be largely due to cholinergic neuron loss. The only approved pharmacological treatment for AD employs a cholinesterase inhibitor.

The intense interest in cholinergic function in delirium and AD has generated several experimental paradigms for evaluation of anticholinergic effects. For example, there have been several reports suggesting that memory and learning deficits associated with AD are attributable to degeneration of the cholinergic magnocellular neurons of the nucleus basalis of Meynert, and lesion-induced damage to the cholinergic projections from this area to the neocortex has been utilized as an animal model of dementia. Also, ibotenic acid lesions of the basal forebrain have been shown to produce deficits in a wide variety of tasks proposed to involve learning and memory [see (19) for review]. Scopolomine and HP 029, a novel anticholinesterase, have also been suggested to produce a useful model of the attentional and memory deficits seen in AD (32).

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More traditionally, retention of the spatial task assessed using the Morris water maze has been shown to deteriorate following intraperitoneal (IP) administration of 5, 25, 50, 75, or 100 mg/kg atropine sulfate in the rat (24). The sensitivity of many of these paradigms is of concern, because they require relatively high doses of anticholinergic drug treatment or rather extensive neuropathology to differentiate between treatment and control conditions. High dose treatment may cause broad generalized dysfunction that is nonspecific to the types of cognitive impairment representative of these diseases.

The current study involves rats responding on a cyclic-ratio reinforcement schedule to evaluate the effects of an anticholinergic drug at relatively low doses. Typically, studies investigating atropine sulfate effects in rats have employed doses ranging from 3.44 to 55.0 mg/kg (15,16,22,25,28–31), and one study, referred to above, used doses as high as 100 mg/kg (24). Many of these experiments used blind alley maze procedures, which may be less sensitive than the operant procedure employed in the current study. We suggest that the drug doses used in the current study (0.1–1.0 mg/kg atropine) should be sufficient for detection of a behavioral effect utilizing an appropriate and sensitive analytical instrument.

The cyclic-ratio schedule was originally proposed as a method to differentiate motivational and palatability effects of drugs on feeding regulation (7,8). The original procedure employed a geometric schedule ranging in ratio values from 2–64, with all responses completed at one lever in the operant apparatus. The current study employs a quadratic schedule ranging in values from 2–56, with each discrete component of the schedule completed at the alternate lever of a two-lever operant chamber. In the current study, we employed the modified form of this schedule in an attempt to observe the effects of the anticholinergic compound atropine sulfate. Modification of the original cyclic-ratio schedule enabled simultaneous measurement of three variables sensitive to deleterious drug effects: postreinforcement pause (PRP) durations, lever switching errors (perseverations), and running response rates. Analysis of PRP durations reflects the ability of animals to track changing parameters of a schedule. Perseverations, or failures to respond at the appropriate lever following reinforcement, may reflect effects on spatial learning. Deviations from baseline running response rates could be argued to suggest peripheral or motoric effects of the drug.

METHOD

Subjects

Six experimentally naive male Sprague–Dawley rats (Harlan: Madison, WI), 90 days old at the beginning of the experiment, were used. They were maintained at 95% of their free-feeding body weights and housed individually with water continuously available in the home cage. The temperature was kept at 23°C under a 12 L:12 D cycle (lights on at 0700 h) in the vivaria.

Apparatus

Six two-lever Coulbourn Instrument Rat Test Chambers (Model E10-10), enclosed in sound attenuating compartments, were employed. The reinforcer was one 45 mg casein-based food pellet (F0021, Bioserv Holton Industries), which was delivered into a tray situated midway between the levers. A Zeos-486 (Zeos, St. Paul, MN) computer programmed in MED-PC (Med Associates, Fairfield, NJ) controlled the experiment and collected data.

Procedure

The original cyclic-ratio schedule, employed by Ettinger and Staddon (7), exposed animals to an ascending, followed by a descending sequence of the geometric ratio values 2, 4, 8, 16, 32, and 64, to make up a cycle of ratio conditions repeated over six cycles. Employing this method, Ettinger and Staddon (7), and later Ettinger, Thompson and Staddon (8), found that over a range of schedule values the response functions were well described by a straight line of negative slope. A subsequent study, using the original geometric schedule, found ratio strain at the higher schedule values (21), and a more recent study demonstrated that the ratio strain could be eliminated by employing a quadratic schedule (20). Thus, two procedural modifications were incorporated into this study. First, a quadratic cyclic-ratio schedule was used. Second, the current application had the added requirement that animals respond in the two-lever operant chamber on the alternate lever following any given reinforcer. For example, if an animal completed one response requirement at the left lever, then the following response requirement in the cycle would be completed at the right lever.

Tests were conducted once daily between the 9th and 11th hour of the 12-h light cycle, and the daily allocation of food was given approximately 30 min after the test session. The subjects were initially trained to press both levers, then reinforced according to an alternating lever fixed-ratio (FR-5) schedule for 5 days. When subjects responded at both levers under FR-5, they were trained on an alternating lever arithmetic cyclic-ratio schedule comprised of an ascending followed by a descending sequence of the ratio values 3, 6, 9, 12, 15, and 18 over a maximum of six cycles. After 14 days on this intermediate training schedule, all animals were tested under the terminal quadratic cyclic-ratio schedule, comprised of the ratio values 2, 6, 12, 20, 30, 42, and 56. Sessions on the terminal schedule were stopped after the delivery of 84 reinforcers (six cycles), within 40 min of the start of the session.

When performance on the terminal schedule stabilized (after approximately 10 sessions), IP injections of saline (0.9% sodium chloride) or atropine sulfate (0.03, 0.1, 0.3, 1.0, and 3.0 mg/kg) were administered 20 min prior to testing. Each animal received one injection of each dose of drug. Data from animals that completed the six cycles in the schedule under the given atropine doses were analyzed. At least 5 days elapsed between drug or saline injections, during which baseline training was maintained. Atropine sulfate doses were measured as concentrations of the total salt dissolved in saline.

RESULTS

Mean overall response rates over the last three cycles of each test session were found to increase as a direct function of ratio value (Fig. 1). Atropine produced a dose-dependent decrease in overall response rates at all ratio values and generally shifted the ratio response rate function to the right. Regression lines were fitted to the data from each dosage level and a two-tailed Dunnett's test was used to compare the elevation of the saline regression line to the other dosage lines (33). The elevation of the saline line was significantly different from each of the other dosage lines ($p < 0.01$; saline vs. 0.03 atropine, $p < 0.05$). No animals were able to complete the session at the 3.0 mg/kg atropine dose.

Figure 2 illustrates the effect of atropine administration on median PRP duration at each schedule value over the last

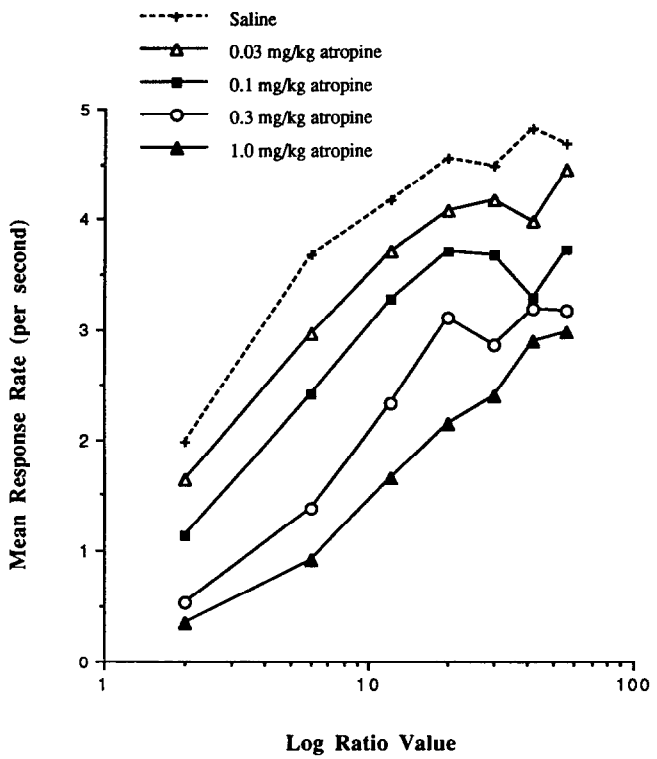

FIG. 1. Mean overall response rates (per second) at each ratio value under saline and atropine doses.

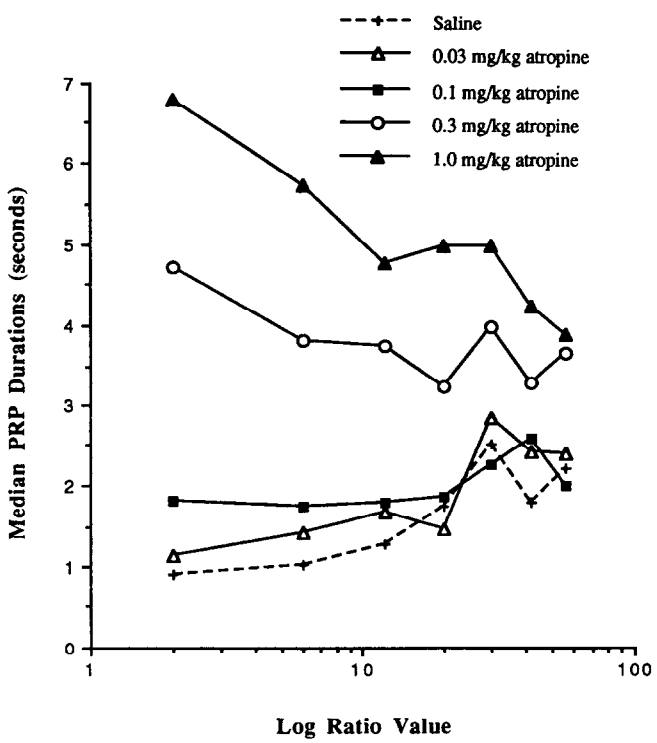

FIG. 2. Median PRP durations (s) at each ratio value under saline and atropine doses.

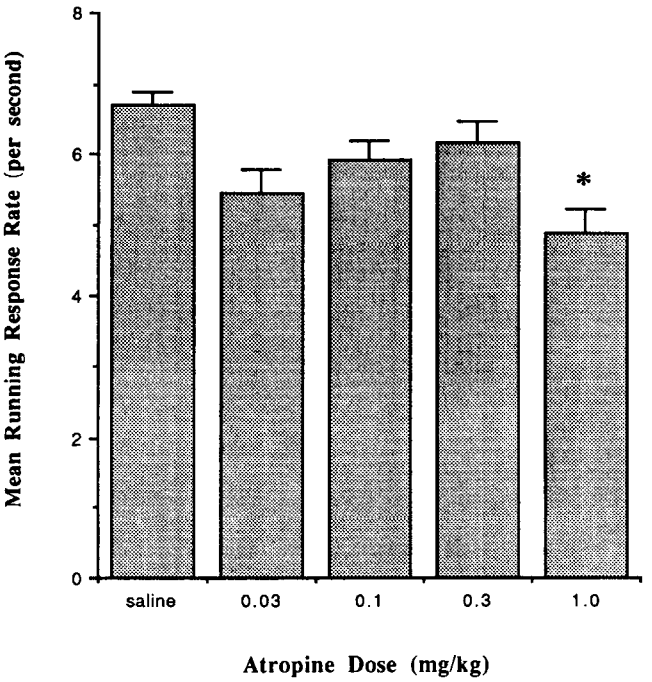

FIG. 3. Mean running response rates (per second) under saline and atropine doses (*$p < 0.05$ vs. saline, t-test).

three cycles of each test session. Median PRPs were analyzed because of the tendency for mean PRPs to be skewed. Median PRPs were regressed on log ratio value for each dose to test for the existence of an increasing trend in PRP duration across ratio values. Typically, PRP durations increase as ratio values increase (17,26) and increases are primarily a function of the upcoming ratio value. A significant positive slope was found for the saline ($p < 0.002$) and 0.03 mg/kg atropine ($p < 0.002$) PRP lines. The PRP trend was disrupted under atropine doses of 0.1, 0.3, and 1.0 mg/kg.

Mean running response rates were not significantly affected by atropine doses of 0.03, 0.1, and 0.3 and mg/kg (Fig. 3). A significant reduction in running response rate was found at the 1.0 mg/kg dose when compared to rates under saline (t test, $p < 0.05$).

Figure 4 shows the effect of increasing doses of atropine on the ability to accurately alternate levers on successive ratios during all six cycles of the test sessions. The mean number of incorrect lever responses made by each animal after saline was 2.5, and after atropine administrations of 0.03, 0.1, 0.3, and 1.0 mg/kg the means were 2.3, 3.2, 4.3, and 8.2, respectively. Analysis by repeated measures ANOVA showed a significant main effect of dose, $F(4, 20) = 3.62$, $p < 0.05$. Atropine (1.0 mg/kg) significantly increased the number of incorrect lever choices made compared to the saline condition ($p < 0.05$, Fisher t-LSD).

DISCUSSION

Deleterious effects of atropine were seen in this study on PRP duration and on lever switching behavior. PRPs have been shown to vary systematically with the upcoming ratio value, increasing as the ratio value increases, once an animal has learned to track a given schedule (9,23). As shown in Fig.

2, the trend for PRPs to increase with increasing ratio values was not seen at doses of 0.1, 0.3, and 1.0 mg/kg, suggesting that the animals were no longer tracking the schedule parameters at these doses.

Figure 4 shows the significant increase in incorrect lever responses following administration of 1.0 mg/kg atropine. Animals responded at the incorrect lever an average of 9.7% of the time after the 1.0 mg/kg dose as opposed to 2.9% of the time following saline administration. This finding is consistent with previous studies in which animals failed to respond at the correct lever in an alternating lever paradigm approximately 10% of the time at this dose (2). This effect of atropine has been attributed to disruption of cholinergic inhibition, which causes behavioral responses rarely observed under normal conditions to be increased under administration of an anticholinergic compound (3,4). Also, disruption of the cholinergic system, especially in the hippocampus and neocortex, has long been suggested to adversely affect memory and other related processes in both animals and humans (5,6). Tasks used to assess these disruptions range from learning of word lists in humans (27), to learning 16-arm radial mazes in rats (10). The alternating lever data are consistent with this hypothesis.

The dose-dependent reductions in overall response rates were predictable, as it is well documented that atropine reduces overall response rates on fixed-ratio schedules (2,3). Analysis of the components of PRP duration, together with analysis of running response rates (response rate minus PRP), suggests that the reduction in overall response rate with increasing concentration of atropine was due to changes in PRP duration. No significant differences in running response rates were found between atropine doses up to and including 0.3 mg/kg when compared to rates under saline, so it is unlikely that the behavioral effects of atropine at these doses were attributable to peripheral or motoric effects of the drug. If the atropine-induced response rates were a product of peripheral or motoric effects, a reduction in running response rates would have been expected.

There is no clear method to determine whether the action of a drug is central or peripheral in cases of depressed responding (3), so interpreting complete failure to respond, as was seen with the 3.0 mg/kg dose, as a solely central drug effect may be erroneous. However, analysis of low doses of atropine, especially 0.1, 0.3, and 1.0 mg/kg, showed changes on two indices of behavior (PRP durations and perseverations) with evidence of only a minimal peripheral effect at the 1.0 mg/kg dose (Fig. 3). These data suggest that future behavioral analyses of anticholinergic drug-induced CNS effects using sensitive operant schedules would be best conducted in the atropine dose range of 0.1–1.0 mg/kg.

Doses of atropine used in a putative animal model of human delirium previously referred to range from 3.0–55 mg/kg (15,31). Data from the current study suggest that the procedures employed in that model may utilize atropine doses that potentially confound behavioral and nonspecific peripheral effects. Estimation of proper doses for small animal studies that attempt to model some aspect of human biology or pathology is difficult. Use of the lowest effective dose range enhances the probability that observed effects are physiologically and pharmacologically relevant, as opposed to behaviorally toxic. Appropriate dose ranges also require behavioral procedures that are sensitive and relevant to some aspects of the human condition that is being modeled.

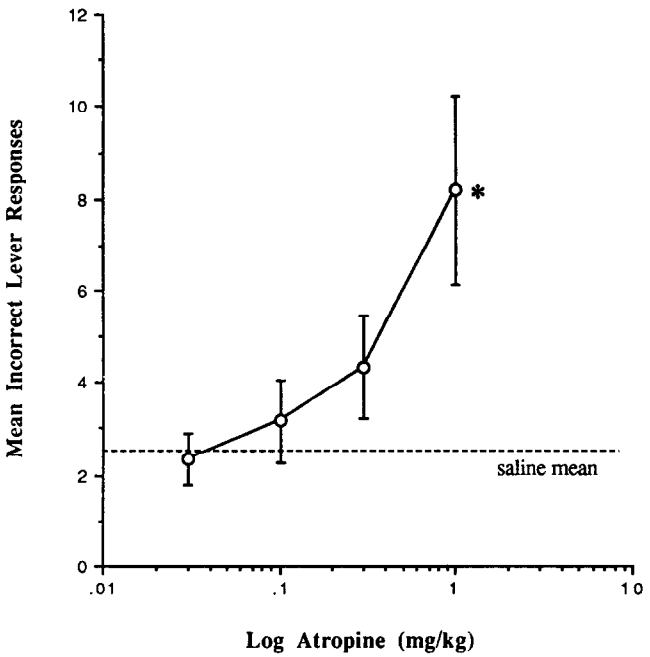

FIG. 4. Mean incorrect lever responses under 0.03, 0.1, 0.3, and 1.0 mg/kg atropine doses (*$p < 0.05$ vs. saline, Fisher t-LSD).

Results from the alternating lever cyclic-ratio schedule suggest that the technique is sensitive to drug effects at low doses, can simultaneously measure drug-induced changes in responding on two behavioral indices, and enables monitoring of peripheral drug effects during behavioral testing. More importantly, the technique allows testing at doses considerably lower than previously reported procedures proposed as models of anticholinergic-induced delirium. Therefore, this operant procedure may be of utility as a measure of anticholinergic effects and useful in the study of behaviors related to these effects.

It is difficult to speculate as to whether a decreased capacity to track schedule contingencies is reflective of a memory deficit, or attentional and related to decreased ability to discriminate stimulus cues. It is similarly difficult to speculate as to whether incorrect lever errors are related to memory errors, or to an increased role of uncontrolled biasing factors. However, it may be the case that working memory deficits are reflected in incorrect lever errors, and reference memory deficits are reflected in a decreased ability to track the schedule contingencies. A possible way to evaluate this contention would be to use hippocampal lesions [after Morris (18)] and assess behavior on the alternating lever cyclic-ratio schedule. A detremental effect on correct lever selection, not reflected in the tracking of schedule contingencies, would add support to the validity of this procedure.

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