Pharmacology Biochemistry and Behavior, Vol. 56, No. 1, pp. 151–154, 1997

0091-3057/97 $17.00 + .00

PII S0091-3057(96)00172-4

Serum Anticholinergic Activity and Behavior Following Atropine Sulfate Administration in the Rat

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Received 22 September 1995; Accepted 27 February 1996

O'HARE, E., D. T. WELDON, K. BETTIN, J. CLEARY AND J. R. MACH, JR. *Serum anticholinergic activity and behavior following atropine sulfate administration in the rat.* PHARMACOL BIOCHEM BEHAV **56**(1) 151–154, 1997.—Anticholinergic agents such as atropine and scopolamine have long been suggested to produce delirium-like states in humans and experimental animals. Evidence for an anticholinergic mechanism in the pathogenesis of human delirium has accumulated, leading to studies of the behavioral effects of the anticholinergic drug atropine in animals. The current study addresses the adequacy of animal models of delirium in terms of sensitivity, specificity and pharmacological relevance. A multiple fixed-ratio fixed-interval reinforcement schedule was used to test the effects of relatively low doses of atropine on behavior in rats. Additionally, total serum anticholinergic activity (SAA) was measured under dose and time course conditions identical to those used in the behavioral study. Atropine reduced high and low rates of responding in a dose-dependent manner, and SAA increased in a dose dependent manner. SAA at atropine doses of 0.1 mg/kg to 1.0 mg/kg was similar to that found in delirious humans. These behavioral and serum level data suggest that relatively low doses of atropine, substantially below those used in previous attempts to model delirium using rats, may be more pharmacologically relevant to delirium and may minimize non-specific peripheral effects of this drug.

Delirium Atropine sulfate Multiple schedule (FR FI) Serum anticholinergic activity (SAA)

DIMINISHED functioning of central cholinergic systems are known to play an important role in several diseases whose main symptoms are known to include cognitive impairment. One of the most prominent of these diseases is delirium. Delirium is a confusional state accompanied by attention deficits, behavioral change or perceptual disturbance (1). Studies of this syndrome have suggested a direct role for cholinergic dysfunction in the etiology of symptoms. Numerous drugs have direct anticholinergic actions and many common medications have recently been discovered to have significant anticholinergic effects (13). For hospitalized elderly patients, it has been estimated that at least 40% of delirium may be attributed to medication (5). A recent study revealed that elderly hospitalized patients with delirium have significantly higher total serum anticholinergic activity (SAA) than non-delirious, age-matched hospitalized controls (9).

Impeding the normal functioning of the cholinergic system with anticholinergic agents, such as atropine and scopolamine, can produce disorientation, hallucinations or delirium in humans (2,6). Atropine-induced symptoms of disorientation, sleep-wake cycle disruption and EEG slowing in experimental animals have long been described as resembling a delirious state (8). These behavioral and neuroelectrical effects are apparent under atropine (1.0–4.0 mg/kg) across a variety of species (16). Evaluation of the effects of anticholinergic agents on tests of cognitive function in animals offers a potentially important tool for studying drug-induced delirium. Indeed, an animal model of delirium has recently been proposed that employs blind-alley maze running, locomotion, EEG changes and subjective descriptions of behavior (7,15). In those studies, 3–55 mg/kg atropine slowed brain wave activity, impaired maze running, increased motor activity and produced other

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behaviors in rats that the authors deemed consistent with delirium (7,15).

While an animal model of delirium would be an extremely valuable tool, proposed models may have several potential weaknesses. The current study addresses issues of procedural sensitivity, behavioral specificity, and pharmacologically suitable doses for an experimental tool employed in understanding anticholinergic drug-induced CNS effects in laboratory animals. The effects of relatively low doses of atropine (0.03–3.0 mg/kg) were tested under a multiple fixed-ratio fixed-interval (FR FI) schedule that has proven sensitive to low dose drug effects (4,12). This schedule was employed because it is a commonly used behavioral assay which has been utilized in the evaluation of a variety of psychoactive compounds over a wide range of doses. Under this reinforcement schedule, both high and low rates of operant responding are evaluated, and an indication of potentially debilitating nonspecific peripheral effects is available. Total serum anticholinergic activity, under the atropine doses used in the behavioral study, was assessed 30 min after drug administration. The relationship between atropine dose, behavioral effect and SAA level is discussed in light of human anticholinergic levels.

MATERIALS AND METHODS

Behavioral Assessment

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

Experimental sessions were conducted using six two-lever Coulbourn Instrument Rat Test Chambers (Model E10-10) enclosed in sound attenuating compartments. 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 bank of three colored stimulus lights was positioned directly above each lever on the chamber wall. A Zeos-486 computer (Zeos, St. Paul, MN) programmed in MED-PC (Med Associates, Fairfield, VT) controlled the experiment and collected data.

Animals were trained to press both levers, then reinforced under a fixed-ratio 5 (FR 5) schedule alternating between the two levers. When the animals responded on both levers under FR 5, they were switched to a multiple fixed-ratio 30, fixed-interval 15-s (FR 30, FI 15-s) schedule. Under FR 30, the house light and right lever lights were illuminated and the right lever produced reinforcement after 30 lever presses. Under FI 15-s, the house light was extinguished, the left lever lights were illuminated and the left lever produced reinforcement for the first response occurring at least 15 seconds after delivery of the last reinforcer. FR and FI conditions alternated every 5 min over the entire 30 minute session. Responses on the incorrect lever at any time during the session had no consequences.

After 50 training sessions, when visual inspection of the data indicated stable performance on the FR FI schedule, the animals were tested for 10 days following intraperitoneal (IP) saline administration given 15 min prior to the test session. IP injections of saline (0.9% sodium chloride) or atropine sulfate (0.03, 0.1, 0.3, 1.0 and 3.0 mg/kg; Sigma, St. Louis, MO) were then administered 15 min prior to testing. Injected volumes were held constant at 1.0 ml/kg. At least five days elapsed between drug injections. Atropine sulfate was dissolved in saline (0.9%) and measured as a concentration of the total salt. Saline injection data were collected as the baseline against which to measure effects of atropine injections. Baseline response rates were means of the rates on the three saline injection days prior to each drug administration.

Serum Anticholinergic Activity

Subjects were 25 experimentally naive male Sprague–Dawley rats (Harlan: Madison, WI), 90 days old at the beginning of the experiment. They were housed individually with water and food continuously available in the home cage. The temperature in the vivaria was kept at 23°C under a 12-h light/12-h dark cycle (lights on 0700).

The twenty-five subjects were randomly assigned to five groups of five animals per group. Each group of animals received one IP injection of either 0.1, 0.3, 1.0 or 3.0 mg/kg atropine sulfate or 0.3 ml saline (0.9% sodium chloride). Injection volumes were held constant at 1.0 ml/kg. Atropine sulfate was dissolved in saline (0.9%) and measured as a concentration of the total salt. Thirty minutes after injection animals were sacrificed by decapitation and blood was collected for serum extraction. The blood was allowed to clot on ice for 30 min, then centrifuged at 3,000 g for 15 min. The serum was removed and stored at −70°C.

For determination of anticholinergic activity, samples of 0.2 ml serum from each subject was tested in duplicate, using an established radioreceptor assay technique (14). Briefly, the 0.2 ml serum samples were incubated in a 2.0 ml volume of phosphate buffer containing the muscarinic antagonist ^{3}H-quinuclidinyl benzilate (^{3}H-QNB) and muscarinic receptor-enriched membranes prepared from separate rat forebrain. The receptor-ligand complexes were isolated by filtration over glass fiber filters and counted by scintillation spectrometry. A standard curve (0.1-50 nM atropine) was prepared for the assay. Sample SAA levels were expressed in terms of the amount of atropine that produced the same degree of competitive inhibition of the specific binding of ^{3}H-QNB to the receptors (nM atropine equivalents/0.2 ml sample).

RESULTS

Atropine generally reduced mean overall response rates under both the FR and FI schedules (Fig. 1). Mean baseline response rates under FR were 150.0 resp/min (SE = 5.3) and under FI were 74.9 resp/min (SE = 3.6). There was a significant main effect for dose under the FR component [RMANOVA, $F(5, 25) = 17.4$, $p < 0.01$] and multiple comparisons (tLSD) revealed all doses except the lowest (0.03 mg/kg) produced response rates significantly different from those under saline ($p < 0.05$). Under the FI reinforcement schedule, there was a significant main effect of dose [$F(5, 25) = 16.8$, $p < 0.01$] and significant differences in overall response rates between saline and all doses of atropine tested (tLSD, $p < 0.05$).

Atropine generally increased mean post-reinforcement pause (PRP) durations, that is, time from reinforcement to the first response, under both reinforcement schedules (FR PRPs are presented in Fig. 2). Mean baseline PRP under FR was 3.3 sec (SE = 0.3). Analysis of the FR component revealed a significant main effect of dose [RMANOVA $F(5, 25) = 4.6$, $p < 0.01$] and multiple comparison tests (tLSD) showed significant differences between mean PRP under saline and under atropine doses of 0.3 and 3.0 mg/kg ($p < 0.05$).

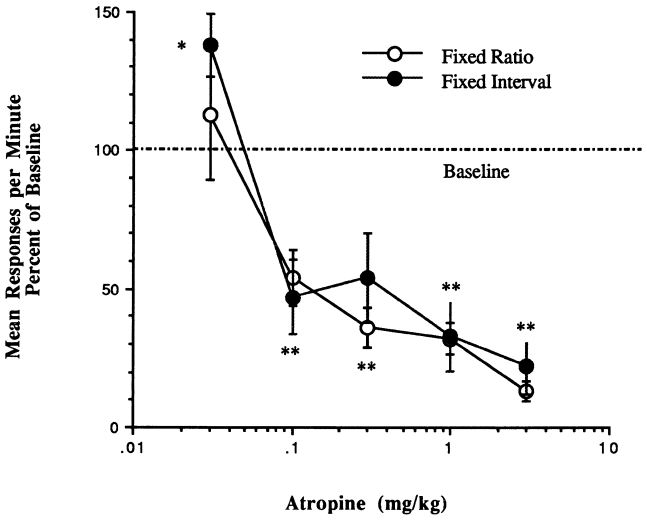

FIG. 1. Atropine effects on mean overall response rates (responses/minute), as percentage of the mean non-drug baseline rate of response, under FR and FI. Asterisks indicate significant differences from baseline. Brackets indicate one SEM.

Figure 3 depicts the mean running response rate for the FR component, expressed as percentage of baseline running rates from the three sessions prior to each drug administration. Running response rates were calculated by dividing total responses by time spent responding under FR (total time minus PRP). Mean baseline running response rate under FR was 151.0 resp/min (SE = 5.3) and under FI was 76.6 resp/min (SE = 3.7). Atropine dose had a significant effect on running response rates (RMANOVA $F(5, 25) = 13.6$, $p < 0.01$), and multiple comparison tests (tLSD, $p < 0.05$) demonstrated significant atropine effects at doses of 0.1, 0.3, 1.0 and 3.0 mg/kg.

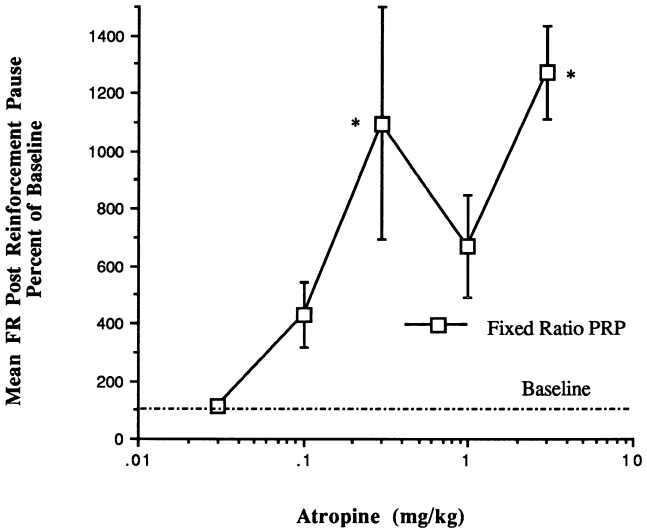

FIG. 2. Atropine effects on mean FR post-reinforcement pause duration as a percentage of the mean non-drug baseline post-reinforcement pause. Asterisks indicate significant differences from baseline. Brackets indicate one SEM.

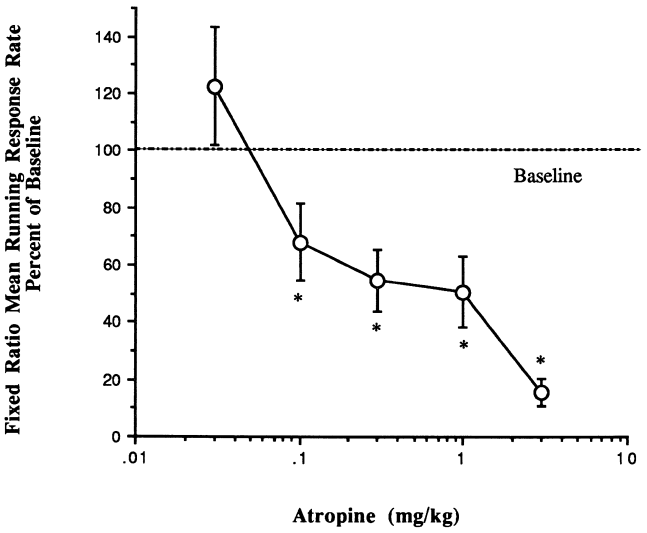

FIG. 3. Atropine effects on FR running response rates as a percentage of the mean non-drug baseline running response rate under FR. Running response rates were calculated by dividing total responses by time spent responding (total time minus PRP). Asterisks indicate significant differences from baseline. Brackets indicate one SEM.

Total SAA levels from the QNB anticholinergic assay increased as atropine dose increased (Fig. 4). Mean SAA, in atropine equivalents, were 0.32 nM (SE = 0.16) 30 min after saline administration. After atropine doses of 0.1, 0.3, 1.0 and 3.0 mg/kg, total respective SAA atropine equivalents were 1.2, 7.6 17.9 and 28.2 nM (SE = 0.4, 2.8, 5.4 and 3.1 nM). The two highest atropine doses of 1.0 and 3.0 mg/kg were significantly elevated over saline (tLSD, $p < 0.05$) with a significant overall effect for dose [ANOVA $F(4, 20) = 15.01$, $p < 0.01$]. Assay reliability for individual samples and their duplicates was high, producing a Spearman Rank Correlation rho = 0.98.

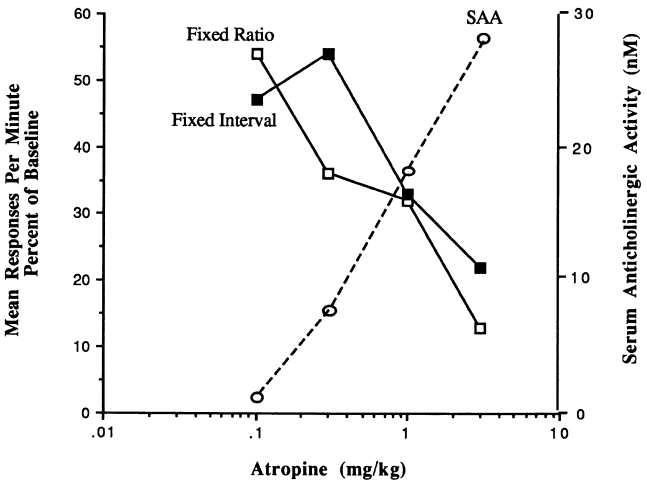

FIG. 4. Total serum anticholinergic activity (right *Y*-axis) and mean responses per minute as a percentage of the mean baseline response rate (left *Y*-axis) under equivalent atropine doses. Serum anticholinergic activity data and mean response rate data were derived from different subject groups.

DISCUSSION

In the current study, atropine generally reduced response rates under FR and FI over the dose range 0.03–3.0 mg/kg. These results are consistent with previously published findings of atropine effects under several operant procedures (11). Recent studies incorporating atropine in a proposed animal model of delirium have also reported atropine-induced performance decrements, but at significantly higher doses, ranging from 3.4 to 55.0 mg/kg (7,15). These studies have typically used blind alley maze procedures which may be less sensitive than operant procedures. Indeed, reports of atropine-induced delirium-like symptoms, including EEG changes, disorientation, and sleep-wake cycle disturbance have historically been observed at doses below 4.0 mg/kg (8,16). The issue of physiologically relevant dose range is important because high doses of anticholinergic drugs, including atropine, can have significant non-cognitive side effects that may occlude behavioral and physiological measures.

Increased PRP durations and decreases in running response rates suggest that non-CNS effects of atropine cannot be ruled out, even at the relatively low doses used in the current study. Decreases in overall response rates, reduced running response rates, and increased PRP durations do not suggest a specific peripheral or cognitive mechanism for the drug effect. The results obtained under the low doses used in the current study indicate that appropriate dose range is an important and relevant consideration if atropine-induced cognitive deficits reminiscent of delirium are to be inferred. Procedural issues must focus on sensitive measures of behavior [such as memory (3) or learning (10)] under atropine doses that are least likely to affect physical ability to respond.

Figure 4 depicts the inverse relationship between SAA and overall response rates on FR and FI. These data suggest an initial reference point from which to begin to estimate the pharmacologically appropriate dose range for anticholinergic effects in the rat. A previous clinical study (9) reported a mean SAA in a group of delirious patients of 6.1 nM atropine equivalents, with a range of approximately 2.0–11.0 nM. For rats in the current study, SAA varied from 1.2–17.9 nM atropine equivalents over an atropine dose range that was behaviorally disruptive (0.1–1.0 mg/kg). As mentioned earlier, previous studies have used atropine doses up to 50 times higher than those tested under the assay and behavioral procedures employed in the current study. It would be premature, however, to assume that the overlapping SAA levels in delirious patients and in rats given low doses of atropine confirms the suitability of administration of atropine as an appropriate analogy of human delirium. Variables such as the source of human serum anticholinergic agents and differences in metabolism, receptor distribution and chronicity of exposure make it difficult to assess the relevance of atropine effects on behavior and SAA to the etiology of human delirium.

Our data suggest that low atropine doses, and their associated behaviors, are potentially appropriate to the study of delirium. In recent studies proposing an animal model of delirium, the authors used objective measures including EEG, behavior, attention, sleep-wake cycle reversal and locomotor activity to substantiate the animal model of delirium (7,15). Unfortunately, the doses used in those studies may be too high to relate to the SAA levels and behavioral disruption observed using more sensitive techniques. Once the appropriate pharmacologically relevant range for anticholinergic drugs is established and supported by objective behavioral and physical measures, animal models of delirium may be proposed that further understanding of this important behavioral phenomenon.

ACKNOWLEDGMENTS

This study was conducted at the animal research facility of the VA Medical Center, Minneapolis, MN, and supported by grants from the Department of Veterans Affairs (JM, JC) and the Alzheimer's Association (JC).

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