Effects of atropine and azaprophen on matching and detection in rhesus monkeys.

Azaprophen (6-methyl-5-diazabicyclo[3.2.1]octan-3-ol, 2,2-diphenylpropionate) (6) is a conformationally restricted analog of atropine. The antimuscarinic characteristics of azaprophen have been investigated in a number of in vitro preparations. For example, azaprophen has been found to be substantially more potent than atropine for inhibiting carbachol-induced amylose release (6, 7, 13) and for inhibiting acetylcholine-induced contractions in guinea pig ileum (6, 7). In contrast, azaprophen has been reported to be slightly less potent than atropine for attenuating carbachol-induced inhibition of prolactin (6, 7). It has been found to be more potent (7) and less potent (13) than atropine for inhibiting [3H]N-methylscopolamine binding, depending on the cell line studied. In a single behavioral assay, azaprophen was slightly less potent than atropine for producing response suppression under a simple schedule of reinforcement in rats and, unlike other benzilate, failed to produce response rate increases (13). It has been suggested that, because of differences in the pharmacological profile (e.g., potency relationships in vitro), azaprophen may interact with muscarinic receptors in a novel manner (13).
Effects of Atropine and Azaprophen on Matching and Detection in Rhesus Monkeys

RAYMOND F. GENOVESE AND TIMOTHY F. ELSMORE

Department of Medical Neurosciences, Division of Neuropsychiatry
Walter Reed Army Institute of Research, Washington, DC 20307-5100

Received 16 June 1988

GENOVESE, R. F. AND T. F. ELSMORE. Effects of atropine and azaprophen on matching and detection in rhesus monkeys. PHARMACOL BIOCHEM BEHAV 32(2) 495-498, 1989.—The effects of the anticholinergic atropine and azaprophen, a novel, conformationally restricted analog of atropine, were examined in rhesus monkeys using delayed match-to-sample and detection tasks. Both compounds (0.01-0.32 mg/kg) produced dose-dependent decreases in the rate of responding under both tasks. Drug effects on the match-to-sample task correlated with drug effects on the detection task. Both compounds produced decreases in the percentage of correct responses on the match-to-sample task when choice trials occurred 4 or 16 sec. but not 0.01 sec. following sample presentation. Doses of atropine and azaprophen decreasing accuracy on the match-to-sample task also decreased the number of responses on the task. In general, atropine was slightly more potent than azaprophen on both tasks. These results further characterize azaprophen's anticholinergic effects.

Atropine Azaprophen Cholinergic Learning Memory Operant conditioning Primate

AZAPROPHEN (6-methyl-6-azabicyclo[3.2.1]octan-3-ol, 2,2-diphenylpropionate) (6) is a conformationally restricted analog of atropine. The antimuscarinic characteristics of azaprophen have been investigated in a number of in vitro preparations. For example, azaprophen has been found to be substantially more potent than atropine for inhibiting carbachol-induced enzyme activities (6, 7, 13) and for inhibiting acetylcholine-induced contractions in guinea pig ileum (6,7). In contrast, azaprophen has been reported to be slightly less potent than atropine for attenuating carbachol-induced inhibition of prolactin (5) and has been found to be both more potent (7) and less potent (13) than atropine for inhibiting 

Subjects

Four adult male rhesus monkeys (Macaca mulatta) weighing between 8.5-11.0 kg were used. Monkeys were individually housed in aluminum primate cages housed in a temperature-controlled environmental room under a 12-hr light-dark cycle. A water bottle was attached to each chamber and was filled regularly. All food, with the exception of daily fruit and vitamin supplements, was presented during the behavioral tasks. To insure the stability of body weights, monkeys were weighed regularly throughout the experiment. All four monkeys had previously been trained

In conducting the research described in this report, the investigators adhere to the "Guide for the Care and Use of Laboratory Animals," as promulgated by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council. The views of the authors do not purport to reflect the position of the Department of the Army or the Department of Defense (para 4-3, AR 360-5).
FIG. 1. Average effects of atropine (squares) and azaprophen (triangles) on session duration of the match-to-sample task in four rhesus monkeys. Ordinate: Session length in minutes. Abscissa: Drug dose in mg/kg. Points above V represent data from vehicle injections. Points above C represent the mean of four noninjection control sessions.

FIG. 2. Average effects of atropine (squares) and azaprophen (triangles) on the number of choice responses on the match-to-sample (top) and detection (bottom) tasks in four rhesus monkeys. Data are from the first 60 min of the sessions. Ordinate: Number of responses. Abscissa: Drug dose in mg/kg. Points above V represent data from vehicle injections. Points above C represent the mean of four noninjection control sessions.

on the concurrent match-to-sample and detection tasks and had pharmacological experience with scopolamine, ap- rophen and physostigmine.

Apparatus

The rear wall of each primate chamber was equipped with an intelligence panel connected to solid-state controlling equipment and a PDP-11/73 computer located in an adjacent room. Each panel contained five press keys (Coulbourn model E36-15), three keys were mounted horizontally 60 cm above the cage floor (upper keys) and two keys (lower keys) were mounted horizontally 45 cm above the cage floor. Each upper press key could be transilluminated with three stimulus colors and each lower press key could be transilluminated with one stimulus color. A food dispenser, equipped with a stimulus light and capable of dispensing 750 mg banana flavored food pellets (BioServe), was mounted in the center of the panel. 30 cm from the cage floor. Experimental events were controlled and monitored using the SKED-11 operating system (State Systems, Kalamazoo, MI).

Behavioral Procedure

Monkeys responded on concurrent delayed match-to-sample and detection tasks. Trials on the matching task were initiated when monkeys pressed the center upper key (initiating response) within 30 sec after it was illuminated white. Following the initiating response, the center upper key was illuminated either red or green (sample stimulus) for up to 30 sec. Monkeys were required to make eight presses (FR8, on the center upper key while the sample stimulus was present). When the FR8 response requirement was met the center upper key went dark and, following a delay of 0.01, 4, or 16 sec, the upper left and upper right keys were illuminated either red or green (choice stimuli) for a maximum of 30 sec. A choice response occurred when monkeys made a single press on either the left or right upper keys while they were illuminated. If the choice response was on the key illuminated with the same color stimulus (red or green) as the previous sample stimulus, a correct response was considered to occur and a food pellet was presented and the food hopper was illuminated for 1 sec.

When a correct choice was made, the next trial started after 5 sec. When an incorrect choice was made, or the response requirements for initiating a trial, selecting the sample stimulus, or making a choice, was not met, the next trial started after 30 sec. The delay interval, sample stimulus color, and position of correct choice stimulus, was randomly determined for each trial. Sessions lasted for 180 min or until 150 food pellets were earned on the match-to-sample task. Sessions were conducted daily and started at 1300 hr.

A simple detection task was presented concurrently with the match-to-sample task. With an average frequency of one in twenty sec, one of the two lower press keys was illuminated white for up to 2 sec. A single press on the illuminated key produced a food pellet with a probability of 0.25. When food pellets were presented on the detection task the food hopper was illuminated for 1 sec. The position of the illuminated key (either right or left) was randomly determined.

Data Analysis

When a response or an experimental event occurred, the elapsed time during the session was recorded. From these data the following measures for the first 60 min of each session were calculated: 1) total choice responses on the
TABLE I

<p>|</p>
<table>
<thead>
<tr>
<th>Monkey</th>
<th>Atropine</th>
<th>Azaprophos</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Match-to-Sample</td>
<td>Detection</td>
</tr>
<tr>
<td>7</td>
<td>0.496</td>
<td>0.509</td>
</tr>
<tr>
<td>8</td>
<td>0.127</td>
<td>0.268</td>
</tr>
<tr>
<td>9</td>
<td>0.286</td>
<td>0.186</td>
</tr>
<tr>
<td>10</td>
<td>0.293</td>
<td>0.306</td>
</tr>
<tr>
<td>Mean</td>
<td>0.301</td>
<td>0.317</td>
</tr>
</tbody>
</table>

Data are ED₅₀ values in μg/kg based on the weight of the base form of the drugs.

match-to-sample task; 2) total responses on the detection task; 3) percent correct choice responses under each delay condition of the match-to-sample task. The first 60 min of the session was chosen for analysis in order to facilitate comparisons of drug effects by minimizing the contribution of potential differences in the duration of action of atropine and azaprophos as well as individual subject differences in the time required to complete the task. The total session time was also calculated. In order to quantify the comparison of the two compounds, ED₅₀ values for response suppression under both tasks were interpolated from dose-effect functions, fitted by least-squares estimation procedures, obtained from data from individual monkeys. Thus, ED₅₀ values represent the dose of drug producing response rates of 50% of control rates.

Pharmacological Procedure

Doses of atropine SO₄ (mol. wt. = 676.8) and azaprophos HCl (mol. wt. = 391.9) (United States Army Medical Research Institute of Chemical Defense) were dissolved in distilled water and distilled water was used for vehicle injections. Injections were IM. about the leg muscles, in a volume of 0.05 ml/kg body wt., 45 minutes before the start of the sessions. Drugs were administered on Tuesdays and Fridays, and data from Thursdays were treated as noninjection control. Drug doses were administered in a mixed order and azaprophos was examined before atropine.

RESULTS

Figure 1 presents the average effects of azaprophos and atropine on the length of time required to complete the match-to-sample task. Both compounds produced dose-dependent increases in session length and a dose of 0.32 mg/kg of either drug increased session length to the maximum allowable duration. Although certain doses of azaprophos (i.e., 0.01 and 0.018 mg/kg) produced small increases in the average session length, whereas equivalent doses of atropine did not, in general, both compounds had similar potencies on this measure.

Atropine and azaprophos produced dose-dependent decreases in the number of responses occurring on the match-to-sample and detection tasks (see Fig. 2). A dose of 0.32 mg/kg of either drug produced a complete or nearly complete suppression of responses under both procedures in all four monkeys. Table 1 presents the ED₅₀ values for response suppression under the match-to-sample and detection tasks, in individual monkeys. In general, atropine was slightly more potent than azaprophos for producing response suppression on both of these measures. Additionally, drug effects on the match-to-sample task were correlated with drug effects under the detection task. That is, each drug suppressed responding on the match-to-sample task to approximately the same extent as on the detection task.

Figure 3 presents the effects of atropine and azaprophos on the average percentage of correct choices on the match-to-sample task for each of the three delay intervals. Under baseline conditions, accuracy on the match-to-sample task depended on the delay interval. That is, average percent correct responding was near 100% on 0.01-sec trials, approximately 90% on 4-sec delay trials, and, approximately 77% on 16-sec delay trials. Neither atropine nor azaprophos had any substantial effect on percent correct responding on 0.01-sec
delay trials at any dose. Certain doses (i.e., 0.10, 0.18, 0.32 mg/kg) of both drugs produced decreases in percent correct responding on 4-sec and 16-sec delay trials. Doses producing decreases in percent correct responses also produced substantial decreases in the total number of responses.

**DISCUSSION**

The major effect observed with both azaprophen and atropine in the present study was to suppress responding. Both compounds produced dose-dependent decreases in the rate of responding on the match-to-sample and detection tasks. Additionally, both drugs produced dose-dependent increases in session length. The similarity between the effects of atropine and azaprophen further characterizes azaprophen's anticholinergic properties.

Atropine was slightly more potent than azaprophen for response suppression on both tasks. The relative potency of atropine and azaprophen observed in the present study is consistent with previous results obtained with in vitro tests of carbachol-induced inhibition of prolactin (5) and [3H]N-methylscopolamine binding (13) assays. But in contrast, these results were consistent with results obtained by Penetar and McDougal (12) for atropine in rhesus monkeys, using a similar procedure. It is notable, in the present study, that nonspecific drug effects contributed to the observed decreases in accuracy and thus, in the present study, the match-to-sample task does not appear to be sensitive to drug effects on memory processes. It is clear, however, that atropine and azaprophen have similar effects on the match-to-sample task.

**ACKNOWLEDGEMENTS**

The authors thank Jeffrey Witkin for helpful comments on the manuscript and Donald Conrad and Lisa King for technical assistance with the conduct of the experiments.

**REFERENCES**