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Effect of Eseroline on Schedule-Controlled Behavior in the Rat

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LIU, W.-F. *Effect of eseroline on schedule-controlled behavior in the rat.* PHARMACOL BIOCHEM BEHAV **38**(4) 747–751, 1991.—Male Sprague-Dawley rats were trained to press a lever on a simple-alternation multiple fixed-ratio (FR) 20-response time-out (TO) schedule for water reinforcement. Twelve 5-min periods of FR reinforcement were each followed by a 5-min TO in which responding had no scheduled consequence. Doses ranging from 0.25 to 8 mg/kg of eseroline, the hydrolysis product of eserine and a potent analgesic agent with weak anticholinesterase activity, were administered SC immediately prior to a 120-min test session. Eseroline produced a dose-dependent monotonic decrease in the number of reinforcements, with significant effects at doses of 1, 2, 4 and 8 mg/kg and an ED_{50} of 2.5 (1.6–3.4) mg/kg. This behavioral disruption was characterized by a rapid onset of pausing (i.e., within 5 min postdosing) and a gradual recovery to normal baseline levels of responding over the remaining session time. The duration of the rate decreasing effects was dose-related with the highest dose having a mean duration of more than 60 min, which was longer than that of previous reports on antinociception produced by eseroline (<60 min). The coadministration of behaviorally inactive doses of the opiate antagonist naloxone (1 and 2 mg/kg, IP) with eseroline (2.5 mg/kg, ED_{50}) antagonized the effects of eseroline on the operant behavior. The coadministration of behaviorally inactive doses of the muscarinic antagonist atropine with eseroline (2.5 mg/kg) did not affect eseroline's behavioral effect. These results suggest that the effects of eseroline on operant behavior is consistent with the effects of eseroline-induced antinociception, reported previously, and appears to be associated with the activation of opiate receptors, but not related to the stimulation of muscarinic receptors via its anticholinesterase activity.

Eseroline Schedule-controlled behavior Fixed-ratio responding Time-course Naloxone Atropine

ESEROLINE is the first degradation derivative of eserine, and its chemical structure is similar to the conformation of morphine (6,9). Previous pharmacological studies have documented the receptor binding properties of eseroline (8), its actions on a variety of isolated smooth muscle tissues or organs (1, 2, 6, 8, 10, 12), its electrophysiological activities on nociceptive neurons (3,4), and some general pharmacological effects, including antinociceptive activities, in rodents (1, 2, 6, 7, 10). Evidence from the pharmacodynamic studies, as noted above, consistently confirms that eseroline is a novel analgesic agent with opiate receptor agonist properties, which is not related to its anticholinesterase activity over the doses of 1–10 mg/kg (IP or SC) evaluated in rodents. However, despite the intensive investigation on the antinociceptive properties, there is a void in the literature on the behavioral pharmacology of eseroline. Based upon this evidence and depending on the dose tested, it seemed reasonable to assume that the behavioral effects of eseroline might involve mechanisms in common with its opiate-like analgesic activities.

The purpose of the present investigation was to examine thoroughly the dose- and time-effect relationships of eseroline on the operant behavior of rats maintained on a simple-alternation multiple fixed-ratio (FR) time-out (TO) schedule of water reinforcement, in order to see if effects of eseroline on operant behavior, in potency and in the time-course of effect, are similar to those of its antinociceptive effects as reported previously. In addition, in order to ascertain whether the effects of eseroline on operant behavior are mediated by mechanisms similar to those involved in its antinociception (i.e., a stimulation on opiate receptors and independent of its anticholinesterase activity), the ability of behaviorally inactive doses of both the opiate antagonist naloxone and the muscarinic antagonist atropine to antagonize the operant behavioral disruption produced by eseroline were investigated.

METHOD

Subjects

Six experimentally naive male Sprague-Dawley rats initially weighing 250–300 g were maintained under a 22-h schedule of water deprivation. They were housed individually with food freely available in the home cage unless specified otherwise.

Apparatus

Two sound-attenuating operant rat chambers, containing a response lever, liquid dipper, and fan were used. Programming was

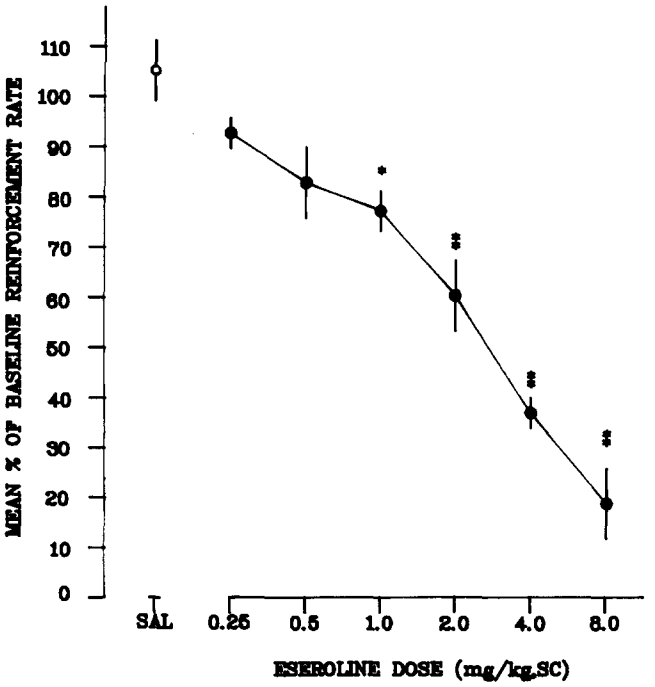

FIG. 1. Behavioral disruption (N = 6) as a function of the dose of eseroline (0.25–8 mg/kg) and vehicle (saline) were SC administered immediately prior to the 120-min FR-TO operant sessions. Data (mean ± SEM) are expressed as the number of reinforcers earned after drug as a percentage of the previous baseline control day. *$p<0.05$, **$p<0.001$, Tukey's test, compared with vehicle.

accomplished by a programmable controller (SYSMAC C-20, Omron, Japan), and responses were monitored by counters and a 5-channel chart recorder (Lafayette Instrument Co.) A 10-W houselight served as the discriminative stimulus. Tap water drops (0.01 ml) served as reinforcers.

Experimental Procedure

Animals were trained to lever press on a simple-alternation multiple fixed-ratio (FR) 20-response time-out (TO) schedule of water reinforcement. Five minutes of the FR and TO components alternated regularly, and were signalled by the on and off of houselight, for a total of twelve of each, resulting in a 120-min operant session. The TO period was added in order to extend the duration of the experimental sessions. During FR periods the number of reinforcers were recorded; during the TO periods the number of responses was recorded, but these responses were not reinforced. Each animal was trained and tested in the same operant chamber except where indicated. Experimental sessions were conducted 5 days per week and terminated after 120 min. The rats were maintained under the multiple FR-TO schedule for 60 daily sessions prior to drug treatments in order to insure a stable baseline of responding. Since under all experimental sessions very little responding occurred during the TO period, only data for the FR schedule were analyzed.

Dose-Effect Determination

After the rates of responding for the 120-min session had stabilized, the effects of saline or eseroline (0.25, 0.5, 1.0, 2.0, 4.0 or 8.0 mg/kg) were tested. The subjects were injected subcutaneously (SC) immediately prior to operant session with a minimum of one week of no treatment preceding each dosing. Each rat was given the six doses on randomly assigned test sessions. Performance on the day prior to dosing was used to determine noninjected control values: data were expressed relative to performance during these sessions (percent of control).

Time-Course Determination

The time-course effects of graded doses of eseroline were determined by analyzing the data obtained from the number of reinforcers received during each of the twelve consecutive 5-min FR periods with TO periods interspersed. In addition, since the behavioral disruption produced by eseroline was resulted mainly from the cessation of lever-pressing, the latencies to onset and offset of pausing were also determined from the chart records.

Interaction of Eseroline and Naloxone

After completion of the dose-effect curve determination, rats were not given any drug injection for 14 consecutive days prior to the start of the interaction study. Rats received eseroline, 2.5 mg/kg (SC) (i.e., ED_{50}), alone, or in combination with naloxone, 1.0 and 2.0 mg/kg (IP), immediately before the operant session. A saline injection (SC plus IP) was also incorporated as the vehicle control. Both doses of naloxone were behaviorally inactive on FR responding on the basis of a previous report (11) and our preliminary determinations.

Interaction of Eseroline and Atropine

Following completion of the above behavioral interaction experiment, the rats were given 14 drug-free sessions before the start of this study. The rats received a single injection of the ED_{50} of eseroline (2.5 mg/kg, SC) alone, or in combination with atropine (0.5 and 1.0 mg/kg, IP), immediately before the operant session. A vehicle control was also incorporated. Both doses of atropine were behaviorally inactive on FR performance on the basis of a previous report (13) and our preliminary experiments.

Drugs

Eseroline salicylate was a gift from Prof. A. Bartolini, Florence, Italy. Naloxone hydrochloride was a gift from Endo Laboratories (New York). Atropine sulfate was purchased from Sigma Chemical Co. (Chicago). All drugs were freshly dissolved and made up to volume with sterile normal saline. All injections were given in a volume of 2 ml/kg.

Data Analysis

In both the dose-effect and time-course determinations, the behavioral measures analyzed were the individual reinforcement rates for each of the FR components, and the overall rate of reinforcement during all the FR components combined. In order to assess the statistical reliability of the results, data from both the dose-effect and time-course determinations, and the drug interaction studies were subjected to a repeated measures analysis of variance (ANOVA). In those instances of significant ($p<0.05$) overall treatment effects, mean contrasts using Tukey's test were subsequently determined.

A linear regression analysis using the method of least square was carried out with the data from the linear portion of the dose-effect function to determine ED_{50} value (the dose producing a decrease in reinforcement rate to 50% of the control rate) and 95% confidence limits.

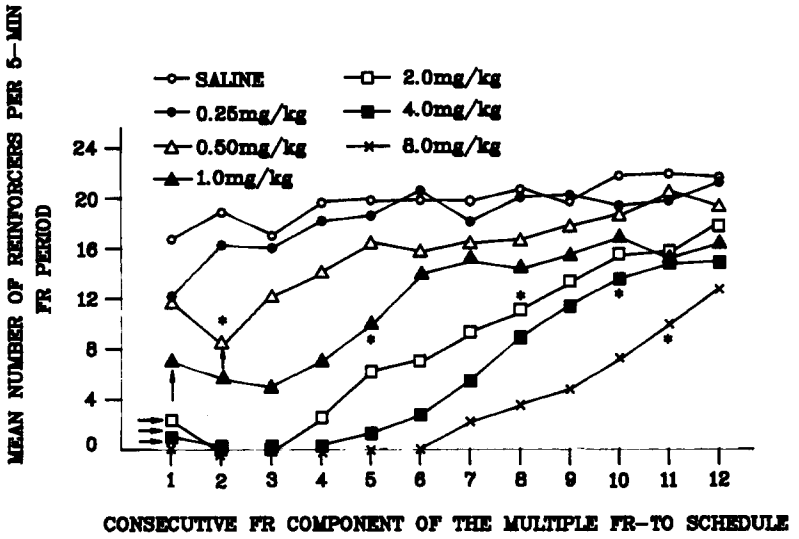

FIG. 2. Time course relationship of eseroline (0.25–8 mg/kg) on fixed-ratio responding (N = 6). Each value represents mean number of reinforcers earned at each 5-min FR period during the 120-min multiple FR-TO sessions. ↑ *Indicates significant below corresponding vehicle controls over the time periods observed. (Two-way repeated measures ANOVA followed by Tukey's test, p's<0.05.)

RESULTS

Control Performance

Overall FR reinforcement rates maintained under the multiple schedule ranged from 10 to 51 reinforcers per 5-min FR period during noninjection control sessions for the six rats, whereas overall TO response rates ranged from 8 to 21 responses per 120-min session. In view of the very little amount of responding which occurred under the TO component, only the data for the FR schedule are presented. Under this schedule, the reinforcement rate for all rats in the absence of drug was relatively stable across the whole session.

Dose-Effect of Eseroline

Eseroline produced a monotonic dose-dependent decrease in the overall reinforcement rate maintained by the multiple schedule (Fig. 1). The estimated ED_{50} value for eseroline was 2.5 (1.6–3.4) mg/kg. There was a significant effect of treatment on reinforcement rate, $F(6,30) = 21.63$, $p<0.001$. Mean contrast analysis indicated a significant difference between the 1.0, 2.0, 4.0 and 8.0 mg/kg groups from the vehicle-treated group (p's<0.05).

Time-Course Effect of Eseroline

The time-course of eseroline effects on operant reinforcement rates is shown in Fig. 2. A repeated measures two-way ANOVA (dosages × time periods) revealed significant effects for dose, $F(6,420) = 4.87$, $p<0.001$, time of observation, $F(11,420) = 45.05$, $p<0.001$, and dose × time interaction, $F(66,420) = 4.19$, $p<0.001$. Planned comparisons by Tukey's procedure revealed that the mean reinforcement rate for the 0.25 mg/kg eseroline group was not significantly ($p>0.05$) below the control level during any exposure to the FR. At 0.5 mg/kg, the rate-decreasing effect was evident 10 min postdosing and lasted for only 10 minutes. At higher doses (≥1.0 mg/kg) of eseroline, the rate-decreasing effects were all evident within 5 min postdosings, and lasted for 40, 70, 90 and 110 minutes with respect to doses of 1, 2, 4 and 8 mg/kg. There was a gradual increase in responding over the remaining time period for each of these doses. These data are also summarized in Table 1. During the course of the experiment, the animals showed no observable overt signs of toxicity.

From visual inspection of the response patterns obtained from the chart records (data not included), it was evident that the rate-decreasing effects of eseroline were due primarily to disruption of ratio run (i.e., cessation of responding) as indexed by the absence of any reinforcer being earned in the FR periods. These time-course data of cessation of responding analyzed from the chart records are presented in Table 1. As with the data for mean reinforcement rate shown in Fig. 2, the onset of cessation of responding observed at doses ≥1 mg/kg was all within 5 min postdosing and a gradual increase in the number of reinforcers obtained over the remaining FR periods followed by offset of pausings was clearly observed (data not shown). This duration of action increased in a dose-dependent manner.

Interaction of Eseroline with Naloxone

The coadministration of behaviorally inactive doses of naloxone (1.0 and 2.0 mg/kg, IP) with eseroline (2.5 mg/kg, SC) revealed significant drug effects on the reinforcement rate during the FR component, $F(3,15) = 25.38$, $p<0.001$. Mean contrast analysis indicated that administration of eseroline immediately before the session significantly suppressed FR responding (Table 2). The overall rate of reinforcement in the presence of 2.5 mg/kg (SC) eseroline alone was 49.6 ± 4.7% of control ($p<0.001$). Both doses of naloxone attenuated this effect of eseroline (p's<0.01), with responding after the high dose of naloxone at a rate of 91.2 ± 4.6% of control ($p<0.001$), which completely antagonized the eseroline's effect.

Interaction of Eseroline With Atropine

The coadministration of behaviorally inactive doses of atro-

TABLE 1

TIME-COURSE EFFECT OF ESEROLINE-INDUCED OVERALL DISRUPTION AND CESSATION OF OPERANT RESPONDING UNDER A SIMPLE ALTERNATION MULTIPLE FR-TO SCHEDULE OF WATER REINFORCEMENT IN RATS (N = 6; TIME IN MINUTES, MEAN ± SEM)

Dose (mg/kg, SC)	Overall Disruption		Cessation of Responding		
	Onset	Duration	No. Rats Affected	Onset	Duration
0	0	0	0	—	—
0.25	0	0	0	—	—
0.5	10	10	1	60	10
1	<5	40	4	≤5	27.5 ± 7.5
2	<5	70	5	≤5	33.3 ± 4.6
4	<5	90	6	≤5	53.3 ± 5.6
8	<5	110	6	≤5	83.3 ± 9.5

pine (1.0 and 2.0 mg/kg, IP) with eseroline (2.5 mg/kg, SC) revealed drug effects during the FR component, $F(3,15) = 11.5$, $p<0.001$; the significant drug effects were due to the reinforcement rates produced by eseroline alone and in combination with both doses of atropine that were all significantly lower than that of the vehicle control (p's<0.01). However, there were no significant differences (p's>0.05) in the reinforcement rate between eseroline alone and eseroline in combination with both doses of atropine (Table 3).

DISCUSSION

The results of the present study demonstrate that acute SC injections of eseroline over a dose range of 0.25 mg/kg to 8 mg/kg in rats resulted in a dose-dependent decrease in operant responding maintained under a simple-alternation multiple FR-TO schedule for water reinforcement, with significant effects at doses ranging from 1 to 8 mg/kg and an ED_{50} of 2.5 (1.6–3.4) mg/kg. The disruptive effects on operant performance produced by eseroline were characterized by a rapid cessation of responding (within 5 min postdosings), as indicated by its occurrence in most of the animals studied (see Table 1), and a gradual return to normal response rate over the remaining time period, as observed from the chart records (data not shown) and Fig. 2. The duration of eseroline's effect was also increased in a dose-related manner. As a whole, eseroline effects showed a dose-related decrease in reinforcements with a reciprocal increase in durations of response cessation.

The coadministration of behaviorally inactive doses of the opiate antagonist naloxone (1 and 2 mg/kg) with eseroline (2.5 mg/kg) antagonized the operant performance disruption of eseroline, whereas coadministration of behaviorally inactive doses of the muscarinic antagonist atropine (1 and 2 mg/kg) did not antagonize eseroline's effects. These results suggest that the operant behavioral effects of eseroline are mediated by an agonistic effect on opiate receptors, but not on muscarinic receptors indirectly.

A comparison of the present results with its antinociceptive actions reported previously reveals that both behavioral disruptive and analgesic effects of eseroline do involve the same mode of pharmacological action. The behaviorally active doses of eseroline in the present experiment were in the same dose range as those of eseroline-induced analgesia in rodents (1 to 10 mg/kg, SC or IP) reported previously (1–4, 6, 7, 10), and with the same mechanism of actions which was antagonized by naloxone (1–4, 6, 7, 10) but not by atropine (1.0 mg/kg) (1). The analgesic effects of eseroline have been shown to be rapid in onset (within 10 min postdosing) and short in duration (no longer than 45 min) after the drug injections, with the maximum effect in about 15 min (1,10). Although the onset and the maximal effective times of behavioral disruption observed in the present study are about the same as those of analgesic effects of eseroline, the duration of behavioral effects, whether measured from the mean reinforcement rates (see Fig. 2) or cessation of responding (see Table 1), seem longer than that of the analgesic effects, which are most apparent with the two high doses of eseroline. This discrepancy

TABLE 2

ANTAGONISM OF ESEROLINE-INDUCED OPERANT BEHAVIORAL DISRUPTION BY NALOXONE (N = 6)

Treatments	Mean (± SEM) % Predrug Baseline Rate of Reinforcement	p Values Versus	
		Vehicle	Eseroline
Saline (SC + IP)	103 ± 3.5	—	—
Eseroline, 2.5 mg/kg (SC)	49.6 ± 4.7	<0.001	—
Eseroline, 2.5 mg/kg (SC), + Naloxone, 1 mg/kg (IP)	76.9 ± 5.2	<0.01	<0.01
Eseroline, 2.5 mg/kg (SC), + Naloxone, 2 mg/kg (IP)	92.2 ± 4.6	NS	<0.001

TABLE 3

LAKE OF ANTAGONISM OF ESEROLINE-INDUCED OPERANT BEHAVIORAL DISRUPTION BY ATROPINE (N = 6)

Treatments	Mean (± SEM) % Predrug Baseline Rate of Reinforcement	p Values Versus	
		Vehicle	Eseroline
Saline (SC + IP)	107.8 ± 5.6	—	—
Eseroline (2.5 mg/kg, SC)	51.8 ± 5.1	<0.001	—
Eseroline (2.5 mg/kg, SC) + Atropine (1 mg/kg, IP)	48.2 ± 7.4	<0.001	NS
Eseroline (2.5 mg/kg, SC) + Atropine (2 mg/kg, IP)	45.9 ± 8.5	<0.001	NS

in duration of action might be due to the higher involvement of motoric function in operant responding than the classical antinociceptive tests (e.g., tail-flick and hot-plate tests), since eseroline has a cataleptogenic effect (6,10) in rats. The ED_{50} value of eseroline-induced catalepsy in rats was reported to be 7.6 (5.4–10.6) mg/kg (SC) (6), which was about two times and three times greater than that of the analgesia and behavioral deficits, respectively. Hence, the gradual recovery to normal levels of responding from the behavioral disruption might also be ascribed to the subtle motor impairments even at subcataleptic doses of eseroline.

In conclusion, the results of the present study indicate that eseroline produces a dose-related rate decreasing effect on operant behavior in the rat. The behavioral disruptive effects in most pharmacodynamic aspects are consistent with the previously defined analgesic effects of eseroline, in that both pharmacological effects are rapid in onset, with an exception of the behavioral effects lasting longer than the analgesic effects, have the same order in potency, and are fully antagonized by naloxone but not by atropine. These findings suggest that eseroline-induced analgesic effects are closely paralleled by behavioral deficits, and opiate receptors, rather than muscarinic receptors, are responsible for the operant behavioral disruption of eseroline.

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