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Antagonism of Etonitazene's Effects in Rats and Pigeons[1]

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DYKSTRA, L. A., W. WHARTON AND D. E. MCMILLAN. *Antagonism of etonitazene's effects in rats and pigeons.* PHARMAC. BIOCHEM. BEHAV. 6(2) 215–219, 1977. – The effects of etonitazene were studied in the pigeon under a mult FR FI schedule of food presentation and in the rat under a continuous avoidance-escape schedule. A low dose of etonitazene increased rates of responding by pigeons under the FI component of the multiple schedule, whereas higher doses produced dose-related decreases in rates of responding under both components of the multiple schedule. These effects were blocked by cyclazocine. Under the continuous avoidance-escape schedule, etonitazene produced only dose-related decreases in rates of responding by rats, and these decreases were blocked by naloxone.

Schedule-controlled behavior Shock avoidance Etonitazene Narcotic antagonists Cyclazocine Naloxone Pigeons Rats

ETONITAZENE is an extremely potent narcotic which has many effects which are similar to those of morphine. For example, the threshold pressure at which rats escape from a graded pressure applied to their tails is increased following both morphine and etonitazene; however, etonitazene is about 1000 times more potent than morphine in producing this effect [8, 16, 26]. Very low doses of etonitazene have also been reported to suppress signs of morphine withdrawal in monkeys [2] and in rats [25]. Moreover, rats will consume quantities of both etonitazene and morphine in drinking water which are sufficient to produce physical dependence as shown by withdrawal symptoms upon challenge with naloxone or by discontinuation of drug [19, 21, 26].

In this study, the effects of etonitazene are examined on responding under a multiple schedule of food presentation in pigeons and under a continuous avoidance-escape schedule in rats. The antagonism of etonitazene's effects by narcotic antagonists is also examined. Responding under multiple schedules of food presentation in pigeons has previously been shown to be sensitive to the actions of a number of narcotic agonists and narcotic antagonists [3, 4, 6, 17, 20, 22]. Moreover, the effects of narcotic agonists under a multiple schedule of food presentation are antagonized by narcotic antagonists, such as cyclazocine and naloxone [4, 17, 22]. Similarly, responding under schedules of shock avoidance in rats has been shown to be sensitive to the actions of narcotic agonists and antagonists [10, 11, 12, 13, 14, 15, 23]. Although the effects of a variety of narcotic agonists and antagonists have been studied using multiple schedules of food presentation in pigeons and shock avoidance schedules in rats, the effects of the very potent narcotics, such as etonitazene, have not been studied using these behavioral baselines. In the present experiments we show that etonitazene exerts typical morphine-like effects on schedule-controlled behavior, which are subject to reversal by narcotic antagonists.

EXPERIMENT 1

METHOD

Animals

Four male White Carneaux pigeons were housed individually and maintained at 80% of their free feeding weights. Water was always available in the experimental chamber and the home cage. All birds had been conditioned to peck a transilluminated key and had previously performed under various schedules of food presentation.

Apparatus

The apparatus was a modification of the pigeon chamber described by Ferster and Skinner [5]. The chamber was sound and light-attenuating. The chamber was illuminated by a 7.5 W bulb (a.c.) and contained a translucent plastic response key, 2 cm in dia. which could be transilluminated by red or blue lights. The minimum force required to operate the key was about 15 g. Below the key was a food magazine where grain could be made available for 3-sec access periods. White noise was present in the chamber at all times.

Procedure

The pigeons performed under a multiple fixed ratio, fixed interval schedule (mult FR FI) of food presentation.

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In the presence of a blue key light, the 30th key peck (FR 30) produced 3-sec access to grain. In the presence of a red key light, the first response after 5 min (FI 5) produced 3-sec access to grain. The FR and FI components alternated after each food presentation. If the bird did not respond within 40 sec after 5 min had elapsed during the FI component, the schedule changed to the FR component. If the bird did not make 30 responses within 40 sec during the FR component, the schedule changed to the FI component. A session terminated at the first scheduled component change after 60 min.

Drugs

Doses of etonitazene hydrochloride were calculated as the salt and dissolved in distilled water. Cyclazocine was used as the free base and dissolved in a minimal volume of lactic acid. Distilled water and a dilute lactic acid solution were used for vehicle injections. Ten minutes before the test session, injections were made into the breast muscle in a volume of 1.0 ml/kg of body weight. When two injections were given, etonitazene was injected in one side of the breast muscle and cyclazocine was injected immediately thereafter into the other side of the breast muscle. Injections were generally given every Tuesday and Friday with Thursday serving as a non-injection control day.

Data Analysis

Average rates of responding were computed from data recorded on digital counters and elapsed-time meters. Quarter life was defined as a percentage of the FI time taken for the first 25% of the FI responses to occur [7]. The quarter life provides a numerical description of the patterning of FI responding. Experiments were conducted Monday through Friday.

RESULTS

Dose-effect curves were first determined for etonitazene alone and then in the presence of various doses of cyclazocine. After determining the dose-effect curve in the presence of various doses of cyclazocine, the dose-effect curve was redetermined for etonitazene alone to show that changes in the etonitazene dose-effect curve in the presence of cyclazocine were not the result of tolerance. It has been shown previously that little tolerance develops to morphine's effects on mult FR FI performance in pigeons when morphine is given twice weekly [20], but tolerance to etonitazene has not been examined under these conditions.

Figure 1 shows the dose-effect curves for the effects of etonitazene on the rates of responding under the mult FR FI schedule of food presentation. Dose-effect curves are shown for etonitazene alone (shaded area) and in the presence of various doses of cyclazocine. A very low dose of etonitazene (0.01 mg/kg) increased the rates of responding under the FI component, but did not alter rates of responding under the FR component. Higher doses (0.018–0.1 mg/kg) of etonitazene produced dose-related decreases in the rates of responding under both schedule components.

All doses of cyclazocine antagonized the increase in rates of responding under the FI component produced by etonitazene; however, the decreases in rates of responding under both schedule components were not blocked by 0.01 mg/kg of cyclazocine and were only partially antagonized

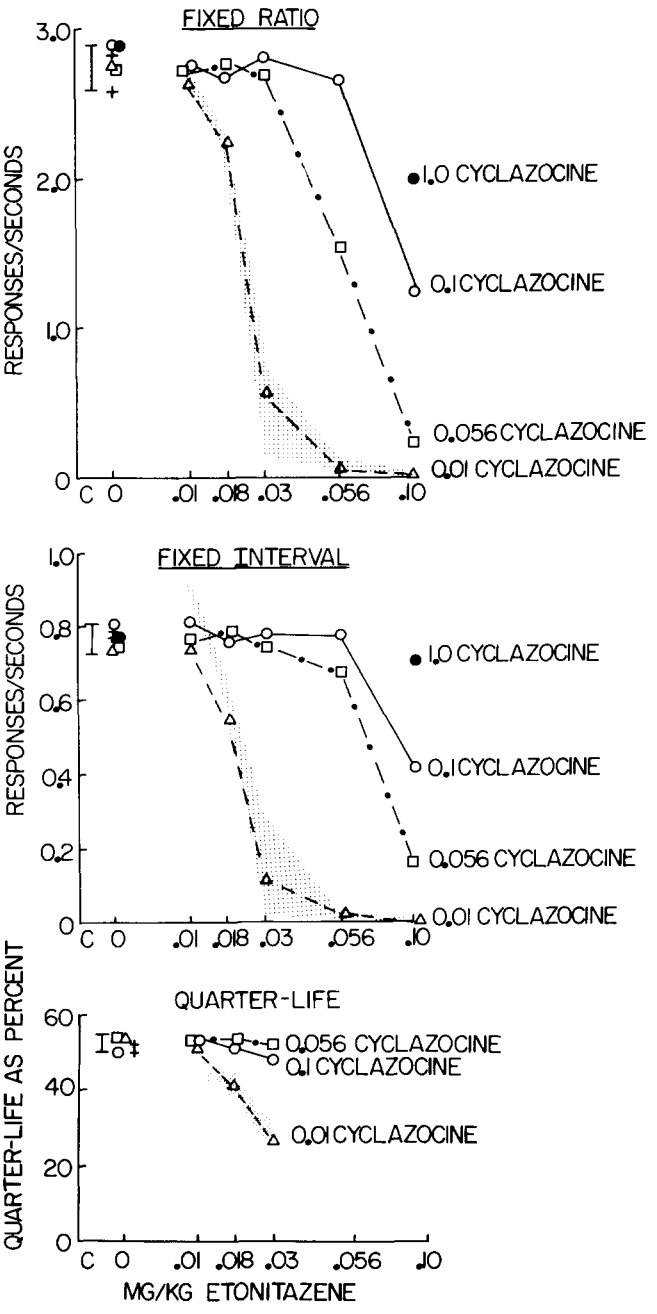

FIG. 1. Effects of etonitazene alone and in the presence of several doses of cyclazocine on rates of responding during the FR and FI components of a multiple FR 30 FI 5 schedule and on quarter life values. Abscissa: dose of etonitazene in mg/kg, log scale. Ordinate: mean rates of responding during the entire session and quarter life as percentage of FI time. The brackets at C represent the range of mean control values (Thursdays). +'s at O show the effects of control injections on the rate of responding and the quarter life. Other points at O show the effects of a dose of cyclazocine alone. The shaded area is the range of values for two determinations of the etonitazene dose-effect curve, with one dose-effect curve determined before and one after the study of antagonisms. ○—○, □– –□, and △— —△ are dose-effect curves for etonitazene in the presence of different doses of cyclazocine and the point ● is for one dose of etonitazene in the presence of 1.0 mg/kg of cyclazocine. The dose of the antagonist is marked beside the end of each curve at the 0.1 mg/kg dose of etonitazene. Each point is the mean of a single injection in each of four birds.

by 0.056 mg/kg of cyclazocine. Larger doses of cyclazocine produced progressively greater degrees of block of the rate-decreasing effects of etonitazene.

Figure 1 also shows the dose-effect curves for the effects of etonitazene and etonitazene in combination with cyclazocine on the pattern of responding in the FI component. On noninjection control days, the mean quarter life values ranged between 50 and 55%. Doses of 0.018 and 0.03 mg/kg of etonitazene decreased the quarter life (proportionally more of the total key pecks occurred at the beginning of the FI interval). Quarter life values were not calculated at the higher doses of etonitazene because of the few number of responses. Doses of 0.1 and 0.056 mg/kg of cyclazocine completely blocked the quarter life decreasing effects of etonitazene.

EXPERIMENT 2

METHOD

Animals

Three experimentally naive Long-Evans hooded male rats, approximately 90 days old at the beginning of the experiment were used. Each rat was housed individually and allowed free access to food and water in his home cage.

Apparatus

The experimental chamber consisted of a standard Lehigh Valley Model 1316 rat test chamber with one lever on the left hand side of the front panel. A lever press response was counted when the rat pressed the bar and subsequently released it. A light over the lever remained on during the session. The entire chamber was illuminated by a 28 V (d.c.) houselight. A Lehigh Valley Constant Current d.c. Shocker, Model 113-04, equipped with a grid scrambler, was the shock source. Shock intensity was approximately 2.0 mA.

All experimental variables were programmed through standard relay circuitry which was located in the room adjacent to the experimental chamber. Responses were recorded both on a cumulative recorder and on digital counters.

Procedure

The rats performed under a variation of the continuous shock-postponement schedule introduced by Sidman [24]. Shocks were scheduled to occur every 10 sec. If a response occurred during the 10 sec before shock delivery, shock onset was delayed by 10 sec. If 10 sec elapsed without a response, a continuous shock was delivered through the grid floor. The shock remained on until a response occurred. Responses which occurred before the shock was presented were designated as avoidance responses. Responses which occurred during shock presentation were designated as escape responses. Each rat was tested in a 4 hr experimental session every other day.

Drugs

Doses of etonitazene hydrochloride and naloxone hydrochloride were calculated as the salt and dissolved in distilled water. Distilled water was used for vehicle injections. Ten minutes before the test session, injections were made into the peritoneal cavity in a volume of 1.0 ml/kg of body weight. When two injections were given, etonitazene was injected in one side of the peritoneal cavity and naloxone was injected immediately thereafter into the other side of the peritoneal cavity. Injections were generally given every other experimental session (once every four days) with the previous experimental session serving as a noninjection control day.

When etonitazene was given alone, it was given in an ascending dosage order in one rat and a descending dosage order in the other two rats. In one rat the etonitazene dose-effect curve first was obtained alone and then in the presence of naloxone. In the other two rats the etonitazene dose-effect curves first were obtained in the presence of naloxone and then for etonitazene alone.

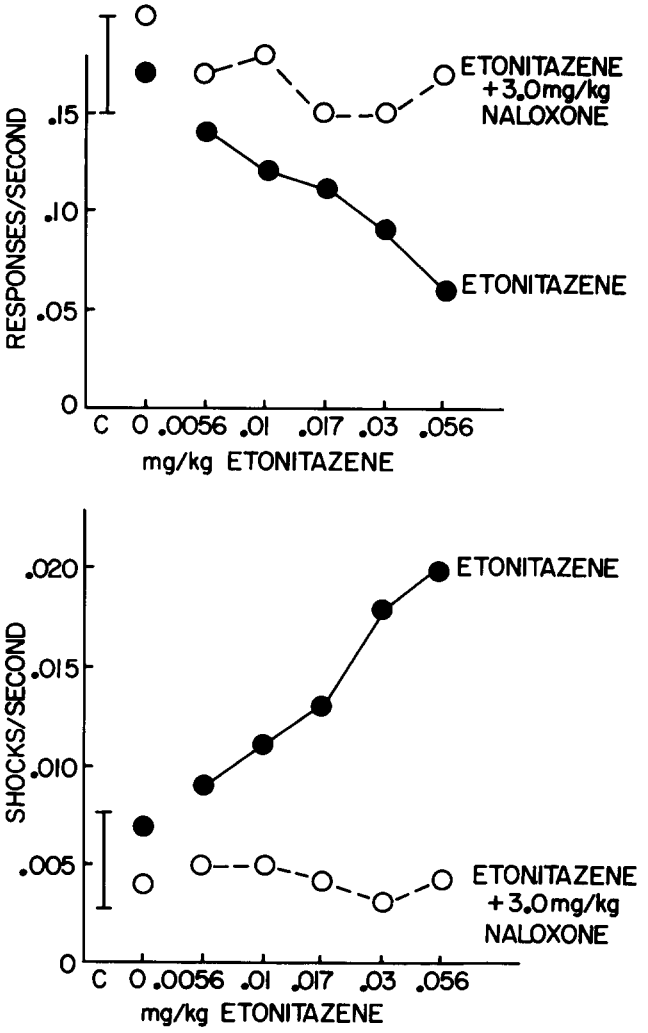

FIG. 2. Effects of etonitazene alone and in the presence of 3.0 mg/kg of naloxone on rate of responding and shock frequency under a schedule of shock avoidance. Abscissa: Dose of etonitazene in mg/kg, log scale. Ordinate, upper graph: mean rates of responding during the first hour of the session; Ordinate, lower graph: mean shock rates during the first hour of the session. The brackets at C represent the range of control values. Points at O show the effects of control injections (filled circles) and of naloxone alone (open circles). •—• is the dose-effect curve for etonitazene alone and ○– – –○ is the dose-effect curve for etonitazene in the presence of 3.0 mg/kg of naloxone. Each point is the mean of a single injection in each of three rats.

RESULTS

Dose-effect curves were determined for etonitazene alone and for etonitazene in the presence of 3.0 mg/kg of naloxone. Figure 2 shows the dose-effect curves for the effects of etonitazene alone on the rates of responding and the number of shocks received during the first hour under the schedule of shock avoidance. Dose-effect curves are presented only for the first hour of the experimental session since etonitazene generally did not alter responding during the last three hours of the session. Etonitazene produced dose-related decreases in rates of responding and increases in the shock rate. Both effects were antagonized by 3.0 mg/kg of naloxone.

DISCUSSION

Low doses of etonitazene increase rates of responding by pigeons under the FI component of a multiple FR FI schedule of food presentation. Higher doses of etonitazene decrease rates of responding under both FI and FR components of the multiple schedule. These effects are blocked by the narcotic antagonist, cyclazocine; however, the rate-increasing effects of etonitazene are blocked by lower doses of cyclazocine than are the rate-decreasing effects. Etonitazene also produces dose-related decreases in rates of responding by rats under a schedule of shock avoidance. This effect of etonitazene also is blocked by a narcotic antagonist, naloxone.

The effects of etonitazene on operant behavior and the antagonism of these effects by narcotic antagonists can be compared to the effects of morphine under similar conditions. In pigeons, the rate-decreasing effects of morphine begin to be observed at 3.0 mg/kg and are nearly complete after 10 to 30 mg/kg [4, 6, 20, 22]. In the present study, similar rate decreases occurred over a range of 0.018 to 0.056 mg/kg of etonitazene, indicating that etonitazene is about 500 to 1000 times more potent than morphine in decreasing rates of responding under a multiple FR FI schedule of food presentation in the pigeon.

The effects of 0.018–0.056 mg/kg of etonitazene on the schedule-controlled behavior of the pigeon were completely blocked by 0.1 mg/kg of cyclazocine but not by 0.01 mg/kg of cyclazocine. McMillan *et al.* [22] showed that the effects of equipotent doses of morphine on the behavior of pigeons under the same schedule of reinforcement were also not blocked by 0.01 mg/kg of cyclazocine, but were blocked by 0.1 mg/kg of cyclazocine, although the block was not complete.

Quantitative comparisons between the effects of etonitazene and morphine under schedules of shock avoidance in rats are more difficult to make because of parametric variation between studies, although it is apparent that morphine and etonitazene have qualitatively similar effects on responding under schedules of continuous avoidance. In the present study, 0.0056 mg/kg of etonitazene decreased rates of responding under the shock avoidance schedule to about 85% of control when the shock was approximately 2.0 mA d.c. and each response postponed shock for 10 sec. Heise and Boff [9] showed that 1.2 mg/kg of morphine decreased rates of responding to about 85% of control under a schedule of shock avoidance in which a 0.6 mA a.c. shock was scheduled to occur every 20 sec and every response postponed shock for 40 sec. Similarly, Holtzman and Jewett [13] demonstrated that 2.0 mg/kg of morphine decreased rates of responding to a similar extent when a 0.8 mA a.c. shock was scheduled to occur every 15 sec and each response postponed shock for 30 sec. Therefore, if schedule parameters are ignored, etonitazene appears to be about 200–350 times more potent than morphine in decreasing rat avoidance behavior. It can be very misleading to ignore such schedule parameters since Holtzman and Jewett [13] also showed that 2.0 mg/kg of morphine increased rates of responding when responses avoided a 1.3 mA a.c. shock rather than a 0.8 mA a.c. shock under the same conditions.

Moreover, Holtzman and Jewett [13] demonstrated that low doses of morphine altered responding under a continuous avoidance schedule for at least two hours, while the duration of morphine's effects was greater than two hours following higher doses. In the present studies, etonitazene only altered responding during the first hour of the session.

In the present study 3.0 mg/kg of naloxone completely blocked the effects of etonitazene on responding by rats under a schedule of continuous shock avoidance. Naloxone has been reported to antagonize the effects of morphine on a discrete trial avoidance schedule in a similar dose range [23]. In addition, naloxone has been reported to antagonize other effects of etonitazene in rats, including muscle rigidity [1] and schedule-induced drinking and lever pressing [18].

Thus etonitazene and morphine produce similar effects on the behavior of pigeons maintained by a multiple schedule of food presentation. These drugs also produce similar effects on the behavior of rats maintained by shock avoidance schedules. In both rats and pigeons, these behavioral effects are blocked by narcotic antagonists. In pigeons, the doses of cyclazocine required to block etonitazene are close to those required to block nearly equipotent doses of morphine. On the basis of these experiments, there is little to suggest that the behavioral effects of morphine and etonitazene differ, except for potency and duration of action.

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