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Establishment of Etonitazene as a Reinforcer for Rats by Use of Schedule-Induced Drinking[1]

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MEISCH, R. A. AND L. J. STARK. *Establishment of etonitazene as a reinforcer for rats by use of schedule-induced drinking.* PHARMAC. BIOCHEM. BEHAV. 7(3) 195–203, 1977. – Drinking of etonitazene HCl by 6 rats was studied during daily 4-hr sessions. Five related experiments were conducted sequentially. In the first experiment schedule-induced polydipsia was established. Subsequently, etonitazene concentrations (1.25, 2.5 and 5.0 μg/ml) were substituted for water, and intake of large quantities of the drug occurred. In the second experiment the concurrent food reinforcement schedule was discontinued and lever presses maintained by etonitazene (5 μg/ml) persisted. In the third experiment the number of lever presses required per dipper presentation of etonitazene (5 μg/ml) was increased, and rate of lever pressing increased directly with the response requirement whereas number of dipper presentations remained constant. In the fourth experiment water was substituted for the 5 μg/ml etonitazene solution. Water responding declined to low rates, but when etonitazene was reintroduced, responding increased to previous levels. Thus, etonitazene (5 μg/ml) was functioning as a positive reinforcer. In the final experiment, progressive increases in the etonitazene concentration (5, 10, 20 and 40 μg/ml) resulted in both systematic decreases in response rate and increases in quantity (μg) consumed.

Rats Etonitazene HCl Schedule-induced drinking Etonitazene reinforcement Fixed-ratio schedules Etonitazene concentration Etonitazene drinking

ETONITAZENE is an opioid approximately 1000 times as potent as morphine with effects qualitatively similar to those of morphine [20]. A number of studies have suggested that etonitazene can act as a positive reinforcer for rats. For drug-naive rats, etonitazene methane sulfonate in concentrations of 5 and 10 μg/ml is probably not aversive, for when these drug concentrations are substituted for water, the drinking patterns and volumes consumed per 24 hr do not differ from water values [20]. Also, during the first 6 hr of drug access, the intake of 5 μg/ml etonitazene is significantly greater than the intake of water [20]. The hydrochloride salt of etonitazene may be more palatable than the methane sulfonate salt, for when rats were restricted to etonitazene HCl concentrations of 3.0 or 10.0 μg/ml, the volumes consumed exceeded water baseline values [12].

Etonitazene drinking is markedly increased if rats are first made physiologically dependent by intraperitoneal injections of increasing doses of morphine. For example, when 5 μg/ml etonitazene was substituted for water during a 24 hr period after the last injection, morphine-dependent rats drank significantly greater volumes of drug solution than did saline-injected control rats [20]. Volumes of drug solution consumed by the dependent rats were also significantly larger than either their water intake or the water intake of control rats when rats received only one liquid at a time [20]. The greater drinking of 5 μg/ml etonitazene than of water suggests that this drug solution can function as a positive reinforcer for morphine-dependent rats.

Additional data are consistent with the notion that 5 μg/ml etonitazene can function as a reinforcer. For example, during a period 24 to 48 hr since the last injection, significant differences in liquid intake occurred between morphine-dependent and saline-injected rats. Relative to saline-injected rats, morphine-dependent rats drank more of a 5 μg/ml etonitazene solution and less water [20].

In several studies, access to etonitazene solutions was contingent upon lever pressing [8,10]. Fixed-ratio responding by rats was maintained by presentations of 0.1 ml of 5 μg/ml etonitazene flavored with small amounts of anise oil and quinine hydrochloride [10]. Maximum ratios maintained were from 40 to 100 lever presses per dipper presentation. Before such responding was established, the rats received morphine intraperitoneally in doses that were gradually increased to 100 mg/kg. In addition, the rats were water deprived when initially exposed to etonitazene.

In another study [8], lever pressing was initiated by restricting rats' access to liquid. For 6 days liquid was available only during daily 8-hr sessions. Each lever press delivered 0.3 ml of 3 μg/ml etonitazene either in water or in quinine sulfate solution of 0.2 mg/ml. Control rats received the vehicle solutions of either water or quinine sulfate. When a water bottle was introduced into the operant-

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conditioning chamber, the rate of lever pressing maintained by vehicle solutions declined to levels below that maintained by etonitazene solutions. These results also suggest that etonitazene was serving as a reinforcer.

The general purpose of our experiments was to further define conditions under which etonitazene-reinforced lever pressing can be established and maintained. These experiments differed from some previous ones in certain respects: The rats were not pretreated by injections of any drug; the etonitazene solutions were not flavored by any additives; daily drug access was limited to at most a single 4-hr session; and the rats served as their own controls. The five experiments were conducted sequentially.

EXPERIMENT 1: SCHEDULE-INDUCED ETONITAZENE DRINKING

When food-deprived rats are intermittently presented with small pellets of food, they drink unusually large volumes of water and certain other liquids, a phenomenon termed schedule-induced polydipsia [3,4]. In the present experiment, etonitazene drinking was obtained by substituting etonitazene solutions for water under conditions of schedule-induced polydipsia. It was anticipated that schedule-induced etonitazene intake would result in the establishment of the drug as a positive reinforcer.

The experimental design used in the present study is similar to a design used in an earlier experiment with ethanol [18]. During an initial 2-hr component, liquid is presented after each lever press. During a second 2-hr component, food pellets are intermittently available, and liquid is again presented after each lever press. In the earlier study with ethanol, water-reinforced performance was characterized by a very low rate of lever pressing during the first component and a high schedule-induced rate of lever pressing during the second component [18]. The schedule-induced drinking insured that sufficient quantities of the drug would be drunk so that the rats would discriminate the drug effects that occur upon absorption.

METHOD

Animals

Six experimentally naive male albino Wistar rats (Bio-Lab Corporation, St. Paul, MN) were housed individually. At the beginning of the experiments the rats were approximately 6 months old and were maintained at 70% of their free-feeding weights as determined at age 4.5 months. The free-feeding weights were: N-1, 483 g; N-2, 492 g; N-3, 515 g; N-4, 477 g; N-5, 500 g and N-6, 490 g. Water was always available in the rats' home cages except during initial training as explained below.

Apparatus

Three identical operant conditioning chambers were used (Lehigh Valley Electronics No. 143–25). Each chamber was contained in a sound-attenuating cubicle (LVE No. 132–02). On one end of the chamber were two levers (LVE No. 121–05), a pellet receptacle, an opening for a liquid dipper cup attached to a solenoid-driven arm (LVE No. 114–02), 6 cue lights (lever lights), a speaker, a Sonalert (2.9 KHz, Mallory and Co.), and a house light. A light was also mounted 3.0 cm above the opening for the dipper cup. The force requirement for the levers was approximately 0.3 N.

With each operation of the pellet dispenser, a single 45-mg Noyes food pellet was delivered to the receptacle. The 0.1 ml dipper cup was constantly available in the up position, except during the 0.8 sec refilling operation, when it was lowered into the reservoir. Liquid was contained in partially covered reservoirs to minimize evaporation. Masking white noise was constantly present, and an exhaust fan provided ventilation. Automatic data recording and programming equipment were located in an adjacent room. The temporal pattern of lever presses and dipper presentations was continuously recorded by a cumulative recorder and by a counter which printed out every 10 min.

Procedure

Each rat received 7 g of Purina Laboratory Chow each day until reduced to 70% of its free-feeding weight. After reaching its 70% weight, each rat was placed in the operant-conditioning chamber for 4 hr a day at a regular starting time. Initially, the rats were deprived of water for 24 hr, and the rats' supplementary feedings of Purina Laboratory Chow were placed in the operant-conditioning chamber before the start of the session. This procedure was used to increase the frequency of water responding, since the rats usually drink after eating. The dipper, containing water, was automatically presented on the average of once each minute, with the time between water presentations varying randomly from 7 to 124 sec. Within one to two sessions the rats reliably approached and drank water from the dipper. Subsequently, automatic water presentations were discontinued, and the rats were trained to press a lever for water. Each press on the right-hand lever resulted in a refilling operation, during which a Sonalert tone sounded and the light above the dipper opening went off. After the rats were trained to press the lever and drink from the dipper, three more sessions were conducted before water bottles were restored to the home cages.

Following the initial sessions of water-reinforced lever pressing, the rats were trained to press the left-hand lever for 45-mg Noyes food pellets. The food pellets were presented on a continuous reinforcement (CRF) schedule (i.e., each press on the left-hand lever produced a food pellet). Food was never available until 2 hr of the session had elapsed. Availability of food was signalled by illumination of the 6 lights above the levers and by the simultaneous offset of the house light. Throughout all 4 hr of every training or control session water was concurrently available on a continuous reinforcement (CRF) schedule. Within one or two sessions of training, food-reinforced lever pressing was established. Following acquisition of food-reinforced lever pressing, the rats obtained food pellets under a continuous reinforcement schedule. Within 4 to 17 sessions food-reinforced lever presses promptly occurred when food availability was signalled by illumination of the lever lights. Number of food pellets per session was limited to 200. After each session, supplementary feedings of Purina Laboratory Chow were given in the home cages to maintain the rats at 70% of their free-feeding weights.

Subsequently, during the last 2 hr of each session, the rats were placed on a *chain* differential reinforcement of other behavior (DRO) *n* CRF schedule of food reinforcement. This schedule of food reinforcement was selected because it engenders a stable low rate of lever pressing that minimally competes with drinking. The DRO *n* component was correlated with illumination of the

TABLE 1

SEQUENCE OF EXPERIMENTAL CONDITIONS AND NUMBER OF 4-HR SESSIONS UNDER EACH CONDITION

Food Schedule DRO (sec)	Liquid Schedule	Drug Conc. μg/ml	Rat N-1	N-2	N-3	N-4	N-5	N-6
CRF	FR 1	0 (water)	17	14	16	4	7	6
chain DRO 5 CRF	FR 1	0	1	1	1	1	1	1
chain DRO 10 CRF	FR 1	0	1	1	1	1	1	1
chain DRO 20 CRF	FR 1	0	1	1	1	1	1	1
chain DRO 40 CRF	FR 1	0	53	94	55	82	54	105
chain DRO 40 CRF	FR 1	1.25	14	12	18	18	15	17
chain DRO 40 CRF	FR 1	2.50	7	17	24	16	10	14
chain DRO 40 CRF	FR 1	5.00	13	10	8	10	12	9
EXT	FR 1	5.00	10	8	7	7	7	10
EXT	FR 2	5.00	6	12	6	32	8	23
EXT	FR 4	5.00	10	7	16	11	20	5
EXT	FR 4	0	11	10	23	20	10	—
EXT	FR 4	5.00	17	8	16	30	12	—
EXT	FR 4	10.00	23	29	21	17	12	—
EXT	FR 4	20.00	26	11	12	14	7	—
EXT	FR 4	40.00	22	7	41	11	8	—
EXT	FR 4	5.00	7	8	6	6	10	—

house light, and under this schedule not pressing the left-hand (or food) lever while the house light was on, minimized the time between the CRF components. In other words, presses on the food lever during the DRO component delayed the onset of the CRF component for *n* sec. After *n* sec elapsed without a press on the food lever, the CRF component was automatically instated. The CRF component was correlated with illumination of the lever lights and offset of the house light, and in this component the first press on the food lever produced delivery of a 45-mg Noyes food pellet and a return to the DRO component. The cumulative duration of the food CRF components was measured each session. Each rat received one session at each of the following DRO values: 5, 10 and 20 sec. Prior to the introduction of etonitazene, the rats were run at DRO 40 sec from 53 to 105 sessions, until there was no trend in either food or water-reinforced lever pressing.

Etonitazene hydrochloride was presented in ascending concentrations of 1.25, 2.5 and 5.0 μg/ml, and each concentration was present for at least five sessions. Changes were made when there was no trend in the values of the dependent variables as determined by visual inspection of the data. Table 1 lists the number of sessions at each concentration. Drug solutions were prepared using tap water, and all liquids were at room temperature when presented. Concentrations are expressed in terms of the salt. The volume consumed was determined by subtracting the volume remaining in the reservoir from the volume added, and corrections were made for volumes lost through evaporation and handling.

RESULTS AND DISCUSSION

Dipper Presentations During Hr 1 and 2

Figure 1 shows that the median number of dipper presentations for 6 rats increased as a function of etonitazene concentration up to 2.5 μg/ml. When the concentration was increased to 5 μg/ml, the number of dipper presentations decreased (Fig. 1). However, at all etonitazene concentrations, the median number of dipper presentations exceeded the water value. Thus, the data suggest that these etonitazene concentrations were serving as reinforcers. These results were obtained during the first 2 hr of the 4-hr sessions. During this component food pellets were never available, and therefore, drinking was not schedule-induced. Under these conditions, lever presses that produced water occurred at the low median rate of 33 per hr.

Dipper Presentations During Hr 3 and 4

During the last 2 hr of each session food was concurrently available on a chain DRO 40 sec CRF schedule, and schedule-induced polydipsia occurred. For example, at 0% (water) the median number of dipper presentations was 415 (Fig. 2), whereas during the first 2 hr the median number was 33 (Fig. 1). Also, at each concentration more

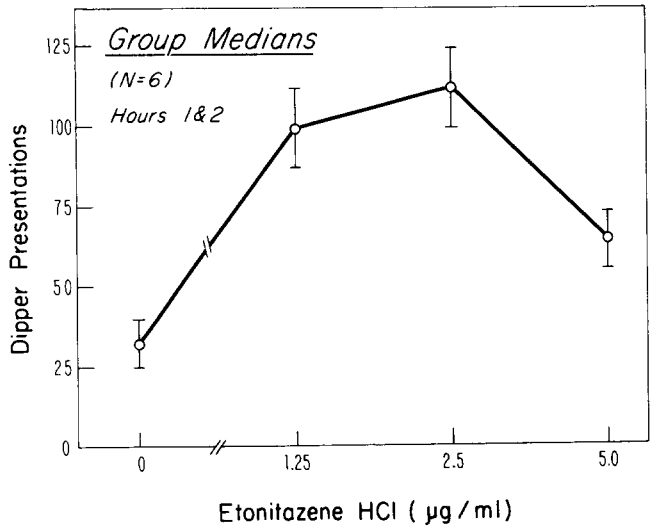

FIG. 1. Median number of dipper presentations during the first 2 hr as a function of etonitazene concentration. Each point is a median of 6 means (6 rats × 1 mean each; each mean is for the last 5 sessions at each concentration). Brackets indicate the median standard error of the mean; each median is based on 6 standard errors (6 rats × 1 S.E. each). Note that during the first 2 hr the rats never received concurrent food.

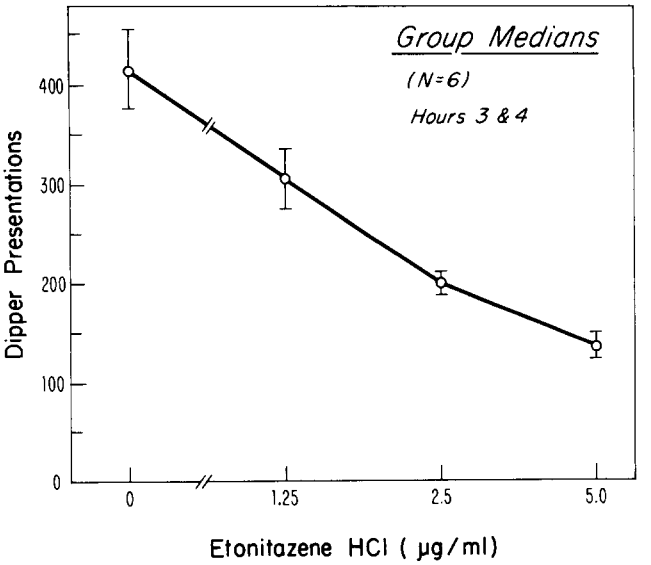

FIG. 2. Median number of dipper presentations during the last 2 hr of 4-hr sessions. Values are plotted as a function of etonitazene concentration. Each point is a median of 6 means (6 rats × 1 mean each; each mean is for the last 5 sessions at a particular concentration). Brackets indicate the median standard error of the mean; each median is based on 6 standard errors (6 rats × 1 S.E. each). Note that during the last 2 hr (i.e., hours 3 and 4) the rats concurrently received food and schedule-induced drinking occurred.

dipper presentations were obtained and more etonitazene was consumed during the last 2 hr than during the first 2 hr (cf., Fig. 2 with Fig. 1).

Figure 2 also shows that during the last 2 hr of the 4-hr sessions the median number of dipper presentations decreased as the etonitazene concentration increased. Studies of schedule-induced pentobarbital and ethanol drinking have similarly demonstrated decreases in number of dipper presentations with increases in drug concentration [13, 17, 18].

Volume Consumed and Quantity (µg/kg/hr) of Etonitazene Intake

The volume consumed during the 4-hr sessions increased over water values when 1.25 µg/ml etonitazene was present (Fig. 3). Further increases in the etonitazene concentration produced successive decreases in the volume consumed. However, the decreases were less than that which cause decreases in the drug quantity consumed (µg of drug/kg of body wt/hr). The amount of etonitazene intake actually increased with increases in drug concentration (Fig. 3). In other studies of schedule-induced drug drinking, increases in the concentration of morphine, methadone, or ethanol resulted in similar systematic decreases in the volume consumed and increases in amount of drug intake [9, 17, 18].

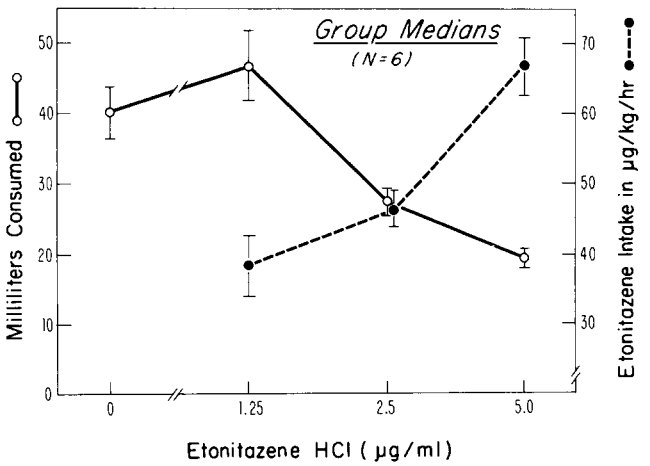

FIG. 3. Median ml and quantity (µg/kg/hr) consumed as a function of etonitazene concentration. Values are for 4-hr sessions. Each point is a median of 6 means (6 rats × 1 mean each; each mean is for the last 5 sessions at a particular concentration). Brackets indicate the median standard error of the mean; each median is based on 6 standard errors (6 rats × 1 S.E. each).

Food-Reinforced Behavior

Figure 4 shows that as the etonitazene concentration was increased to 2.5 µg/ml, the total number of food responses decreased while the cumulative duration in the CRF food component increased. The total number of food responses equals food responses emitted in both the DRO and CRF components. The increased cumulative duration reflects an increase in the latency to press the food lever during the signalled (CRF) food component. A further increase in concentration to 5.0 µg/ml resulted in an increase in the number of responses and a decrease in the cumulative duration spent in the CRF component; that is, when the concentration was increased to 5.0 µg/ml, food performance shifted back toward that obtained when water

was present (Fig. 4). This return of food-reinforced behavior toward water values may reflect the development of tolerance. Number of food pellets obtained per session did not vary in a systematic manner with etonitazene concentration.

The changes in food-reinforced behavior are important, in that they indicate the rats were drinking sufficient etonitazene to alter concurrent behavior. Studies of schedule-induced pentobarbital and ethanol drinking have also found concentration-related changes in food-reinforced performance [13,17].

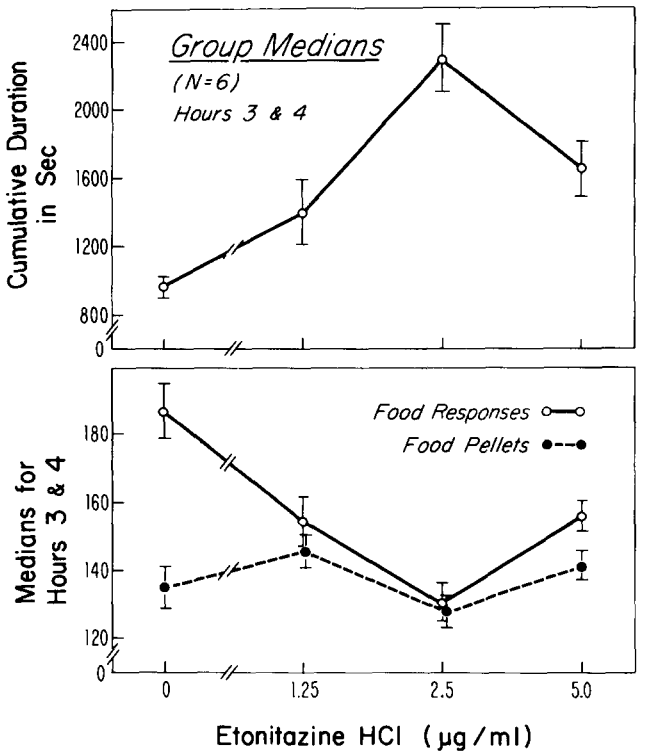

FIG. 4. Measures of food-reinforced behavior at each etonitazene concentration. Values are for hours 3 and 4 of the 4-hr sessions. The upper frame shows median cumulative durations (sec) spent in the CRF component of the chain DRO 40" CRF schedule of food reinforcement. The lower frame shows median presses on the food lever and the median number of food pellets obtained. Note that food responses include those emitted during the DRO component as well as those emitted during the CRF component. Each point is a median of 6 means (6 rats × 1 mean each; each mean is for the last 5 sessions at a particular concentration). Brackets indicate the median standard error of the mean; each median is based on 6 standard errors (6 rats × 1 S.E. each).

EXPERIMENT 2: TERMINATION OF SCHEDULE–INDUCED POLYDIPSIA: EFFECTS ON ETONITAZENE DRINKING

In a number of studies of schedule-induced ethanol drinking, when intermittent access to food pellets within sessions was eliminated, ethanol drinking persisted at levels substantially above those that occurred when water was present [5, 15, 16, 18]. These results indicate that ethanol had been established as a positive reinforcer since ethanol drinking could not be accounted for by the liquid property of the ethanol solutions. The present experiment compared intake of 5 µg/ml etonitazene in the presence and absence of schedule-induced drinking.

METHOD

Animals and Apparatus

Rats and apparatus were the same as in Experiment 1.

Procedure

After a rat's schedule-induced drinking of 5 µg/ml etonitazene was stable for five sessions, the concurrent schedule of food reinforcement was discontinued and presses on the left (food) lever had no scheduled consequences. The lever lights were no longer used, and illumination was provided by the house light. Thus, the stimulus conditions during the last 2 hr were the same as during the first 2 hr. Other aspects of the procedure, such as the session length, were also the same as in Experiment 1. Values of etonitazene intake in the absence of concurrent food are for five consecutive stable sessions.

RESULTS AND DISCUSSION

Table 2 shows that for each of the 6 rats, the volume consumed decreased when concurrent food was no longer available. The median value for the group changed from 19.6 to 13.9 ml (Table 2). For 4 of the rats, number of dipper presentations also decreased. Although drinking did decrease, substantial intake of the 5 µg/ml solution persisted. To further evaluate the reinforcing efficacy of the drug solution, presentation of the dipper was made contingent upon more than one lever press. This experiment is described below.

TABLE 2

MILLILITERS CONSUMED OF 5 µG/ML ETONITAZENE (MEANS OF 5 SESSIONS ± S.E.) DURING 4-HR SESSIONS IN THE PRESENCE AND ABSENCE OF CONCURRENT FOOD

Rats	Volume Consumed (ml)	
	Concurrent Food	No Concurrent Food
N1	14.1 (0.4)	10.7 (1.0)
N2	23.3 (1.6)	14.9 (0.7)
N3	15.8 (0.7)	12.8 (0.7)
N4	34.5 (3.8)	29.6 (1.0)
N5	15.9 (0.9)	6.2 (0.4)
N6	29.0 (5.8)	20.2 (2.2)
Median	19.6 (1.3)	13.9 (0.9)

EXPERIMENT 3: ETONITAZENE–REINFORCED PERFORMANCE AS A FUNCTION OF FIXED–RATIO SIZE

If a drug is functioning as a positive reinforcer, then presentation of the drug should maintain intermittently reinforced responding. In the present experiment access to a 5 µg/ml etonitazene solution was contingent on one, two

or four lever presses per dipper presentation. That is, etonitazene access was scheduled according to fixed ratios (FR's) of 1, 2 and 4.

METHOD

Animals and Apparatus

Animals and apparatus were the same as in Experiment 2.

RESULTS AND DISCUSSION

Figure 5 shows that the number of lever presses per session increased directly as a function of fixed-ratio size while the number of dipper presentations remained constant. These results are consistent with the notion that the etonitazene solution is functioning as a positive reinforcer. However, intake of the etonitazene solution could be due to its liquid character and not due to the presence of the drug. The next experiment was designed to determine if the drinking of the etonitazene solution was due to the presence of etonitazene in the solution.

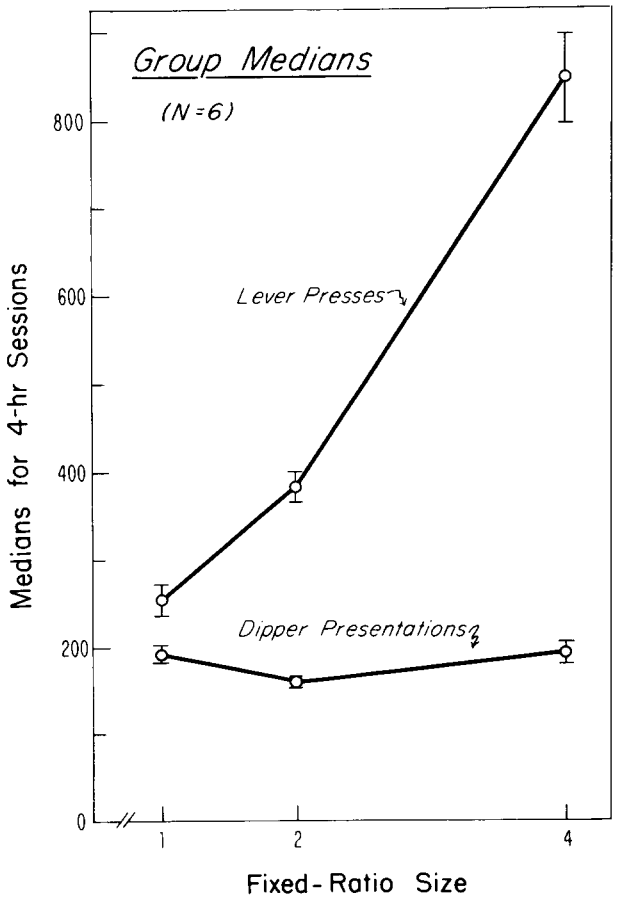

FIG. 5. Median number of lever presses and dipper presentations as a function of fixed-ratio size. Each dipper presentation resulted in access to 0.1 ml of etonitazene at a concentration of 5 μg/ml. Each point is a median of 6 means (6 rats × 1 mean each; each mean is for the last 5 sessions at a particular concentration). Brackets indicate the median standard error of the mean; each median is based on 6 standard errors (6 rats × 1 S.E. each).

EXPERIMENT 4: BEHAVIOR AS A FUNCTION OF LIQUID PRESENT: 5μg/ml ETONITAZENE OR WATER

In this experiment the drug was initially present in the solution, then absent, and, finally, reintroduced. These manipulations were done to determine if behavior was being maintained by the drug.

METHOD

Animals and Apparatus

Animals and apparatus were the same as in Experiment 3.

Procedure

After 5 stable sessions of drug-reinforced performance, water was substituted for etonitazene 5 μg/ml. Etonitazene was reintroduced following 5 stable water sessions. Again, the data reported are from 5 stable sessions. Four lever presses resulted in a dipper presentation (FR 4). Other aspects of the procedure were the same as in Experiment 3.

RESULTS AND DISCUSSION

Figure 6 shows that when water was substituted for the drug solution, the number of dipper presentations greatly decreased. When the drug solution was again available, the number of dipper presentations returned to previous drug values. Thus, the 5 μg/ml etonitazene solution was functioning as a positive reinforcer. When water replaced etonitazene the number of dipper presentations gradually declined. Water was present for a median of 11 sessions before etonitazene was reintroduced (see Table 1). The resumption of lever pressing when etonitazene was reintroduced indicates that rats can discriminate the presence of etonitazene without using taste additives or exteroceptive stimuli. For Rat N–6, the number of dipper presentations declined when water was introduced. However, when the drug solution was reintroduced, the number of dipper presentations did not increase. No explanation is apparent as to why this rat's behavior differed from the others.

Figure 7 shows that when etonitazene was present, the rate of dipper presentations was approximately constant over time. This temporal pattern differs from the negatively accelerated pattern of rats' ethanol intake [6].

EXPERIMENT 5: ETONITAZENE–REINFORCED BEHAVIOR AS A FUNCTION OF ETONITAZENE CONCENTRATION

Rate of intravenous drug self-administration varies in an orderly manner with drug dose [19]. Similarly oral drug intake should vary with drug concentration. In the present experiment changes in etonitazene-reinforced behavior were studied as a function of drug concentration.

METHOD

Animals and Apparatus

Rats and apparatus were the same as in Experiment 4.

Procedure

Etonitazene HCl concentration was progressively in-

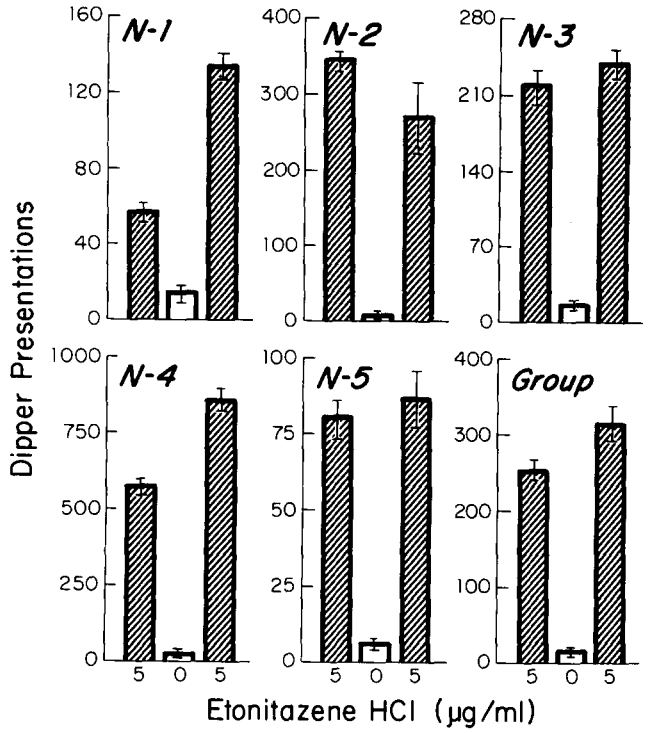

FIG. 6. Dipper presentations as a function of liquid present: 5 μg/ml etonitazene or water. Dipper presentations followed every fourth lever press (FR 4). Hatched bars represent etonitazene values, and open bars represent water values. Each bar is a mean of the last 5 sessions during each phase. Brackets indicate the standard error of the mean. Group bars are means of 25 observations (5 rats X 5 sessions each), and the brackets indicate the mean standard error of the mean (n = 5; 5 rats X 1 S.E. each).

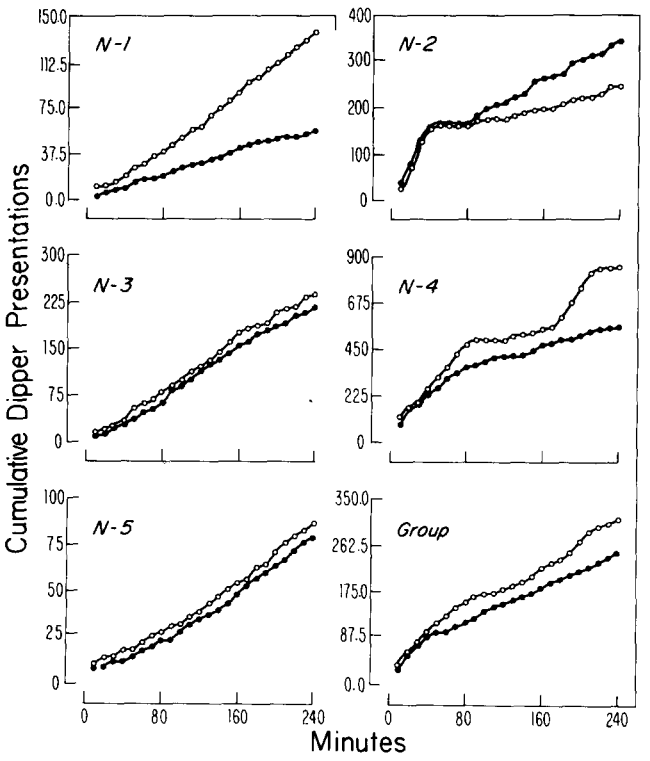

FIG. 7. Dipper presentation of 5 μg/ml etonitazene cumulated at 10-min intervals over 4-hr sessions. These temporal patterns of dipper presentations are for the drug sessions used in calculating the means that are illustrated in Fig. 6. Each point is a mean based on observations from 5 sessions. Filled circles are for etonitazene sessions preceding the series of water sessions, whereas unfilled circles represent etonitazene sessions following the series of water sessions. Group values are means of 25 observations (5 rats X values from 5 sessions each).

creased from 5 to 10 to 20 and, finally, to 40 μg/ml. After the series of sessions at 40 μg/ml, 5 μg/ml was reintroduced (retest). Changes from one concentration to the next were made after the completion of 5 consecutive stable sessions. Table 1 specifies the total number of sessions at each concentration for each rat. Other aspects of the procedure were the same as in Experiment 4 including the requirement of 4 lever presses per dipper presentation.

RESULTS AND DISCUSSION

Figure 8 shows that as the etonitazene concentration increased, both the number of dipper presentations and volume consumed decreased. In contrast drug intake rate (μg/kg of body wt/hr) increased as the concentration increased. With rats and rhesus monkeys similar relationships have been found between ethanol concentration and ethanol-reinforced behavior in that concentration increases result in both decreases in dipper presentations or liquid deliveries and increases in rate of intake (mg/kg of body wt/hr) [7,18]. These results are also similar to those obtained with morphine-dependent rats that drank etonitazene methane sulfonate solutions during a 24-hr period following a morphine injection [20]. With the morphine-dependent rats the volume consumed decreased while the drug intake rate (μg/kg of body wt/24 hr) increased as the concentration was progressively doubled from 5 to 10 to 20 to 40 μg/ml [20].

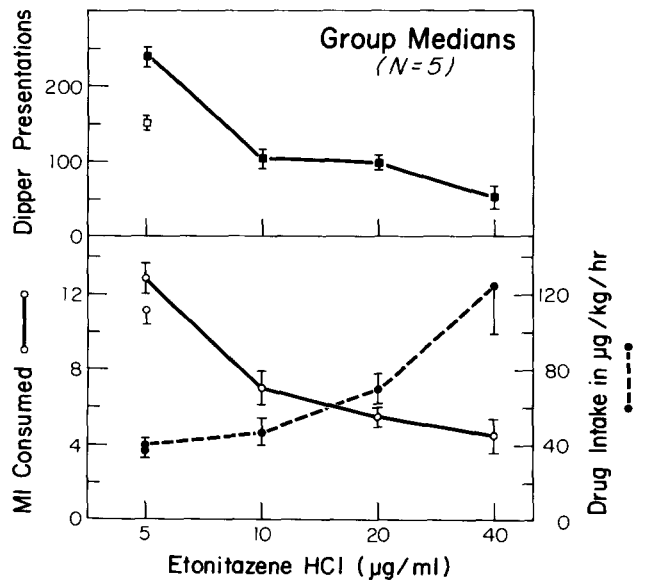

FIG. 8. Etonitazene-reinforced performance as a function of concentration. Each point is a median of 5 means (5 rats X 1 mean each; each mean is for the last 5 sessions at a particular concentration). Brackets indicate the median standard error of the mean; each median is based on 5 standard errors (5 rats X 1 S.E. each). Unconnected points are retest values at 5 μg/ml.

GENERAL DISCUSSION

This series of experiments demonstrates that after a phase of schedule-induced etonitazene drinking, the drug can function as a positive reinforcer for rats since: (1) etonitazene drinking persists when schedule-induced drinking is terminated; (2) access to etonitazene, 5 μg/ml, maintains fixed-ratio responding; and (3) responding is maintained by the presence of the drug and not by the liquid properties of the drug solution. To establish etonitazene as a reinforcer, neither pretreatment with morphine nor the addition of taste cues such as quinine, sodium chloride or anise was necessary. Also, water deprivation was not necessary for the initiation of etonitazene drinking. Additionally, it was found that food-reinforced performance was altered by schedule-induced drug intake, that the time pattern of intake was approximately constant over the 4-hr session, that among rats large differences in drug intake occurred, and that etonitazine-reinforced behavior varied in an orderly manner with drug concentrations. Some of these results extend or confirm the findings of others. However, an interlocking series of experiments that used subjects as their own controls has not been previously reported.

Leander and McMillan [8,11] used schedule-induced polydipsia to obtain drinking of 5 μg/ml etonitazene. They observed changes in behavior, such as the collapse of rats on the cage floor. However, they did not report any changes in food-reinforced behavior. Recording changes in food-reinforced performance provides a quantitative objective measure over time of drug-induced alterations in behavior [13]. Other investigators have observed increased muscle tone [8], hyperactivity [10], gnawing on the floor and other objects [10,20], and autophagia [2]. We also noted from time to time these behaviors occurring during the 4-hr sessions, but our observations were not made systematically. Since these behaviors were not seen in the home cages immediately prior to sessions, they were probably not part of an abstinence syndrome.

Leander and McMillan [8,11] concluded that during schedule-induced polydipsia the 5 μg/ml etonitazene solution came to function as a reinforcer, since the rats preferred this drug in saline to tap water when both liquids were available within the session. During the initial 2 hr preceding the subsequent 2 hr of schedule-induced drinking (Experiment 1), we observed more dipper presentations with etonitazene than with water. Thus, our data are consistent with Leander and McMillan's conclusion that etonitazene came to function as a reinforcer during schedule-induced polydipsia; and more generally, these studies confirm the findings of Wikler and coworkers [20] that etonitazene can serve as a reinforcer for rats.

The maintenance of intermittently reinforced lever pressing is also consistent with etonitazene serving as a reinforcer. Such maintenance of lever pressing was observed in the present series of experiments and has previously been observed by others [8,10]. However, in previous studies the control procedure of substituting vehicle (water) for drug solution was not carried out.

In some studies etonitazene solutions were made more discriminable by adding quinine and/or anise [8,10]. Also, to facilitate etonitazene drinking, the rats were initially water deprived [8,10] and/or made physiologically dependent upon morphine [10, 20, 21]. In the present series of experiments, none of these procedures was necessary to establish etonitazene as a reinforcer.

The effects of a self-administered drug depend in part on the temporal pattern of intake. The temporal pattern observed in the present experiments was a constant rate, and this pattern differs from the negatively accelerated time course seen with ethanol [14].

We found large differences among rats in etonitazene intake, and sometimes we saw the same rats show marked variability from session to session. Other investigators have noted large intrasubject [8] and intersubject [1, 2, 10] variability in etonitazene drinking. The reasons for such variability are not known.

Many questions concerning etonitazene as a reinforcer remain to be answered. These questions concern in part the variables controlling etonitazene drinking and the interaction among these variables. Particular questions of interest include: Under what conditions is etonitazene most rapidly established as a reinforcer? How does intake change as a function of past history of drug self-administration? Will establishing etonitazene as a reinforcer facilitate the establishment of other narcotics as reinforcers? These issues are currently under investigation in our laboratory.

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