Pharmacology Biochemistry and Behavior, Vol. 42, pp. 579-586, 1992
Printed in the U.S.A.

0091-3057/92 $5.00 + .00

Etonitazene Delivered Orally Serves as a Reinforcer for Lewis but Not Fischer 344 Rats

TSUTOMU SUZUKI,* FRANK R. GEORGE† AND RICHARD A. MEISCH‡[1]

**Department of Pharmacology, School of Pharmacy, Hoshi University, Shinagawa-ku, Tokyo 142, Japan*
†Department of Psychology, Center on Alcoholism, Substance Abuse, and Addictions, University of New Mexico, Albuquerque, NM 87131
‡Department of Psychiatry and Behavioral Sciences, Medical School, University of Texas Health Science Center at Houston, Houston, TX 77030-3497

Received 10 December 1990

SUZUKI, T., F. R. GEORGE AND R. A. MEISCH. *Etonitazene delivered orally serves as a reinforcer for Lewis but not Fischer 344 rats.* PHARMACOL BIOCHEM BEHAV 42(4) 579-586, 1992.—Oral etonitazene self-administration was systematically investigated in two inbred strains of rats, Lewis (LEW) and Fischer 344 (F344). For LEW rats, etonitazene maintained higher rates of lever pressing and was consumed in larger volumes than the water vehicle when the reinforcement schedule was fixed ratio (FR) 8. In contrast, with F344 rats responding did not systematically exceed water values at any etonitazene concentration. LEW rats also drank substantially more etonitazene than F344 rats, and at FR 8 only LEW rats showed the typical inverted U-shaped function between etonitazene concentration and number of responses. For the LEW strain, response rate increased as FR size increased from FR 1 to FR 2 and FR 4, but decreased at FR 8. For the F344 strain, as FR size increased response rate showed small increases, but the response rates were far lower than those of the LEW strain. The results support the conclusion that etonitazene was an effective reinforcer for LEW but not F344 rats. These findings demonstrate genetic differences in opioid reinforcement of operant behavior and indicate that genotype can be an important determinant of whether etonitazene serves as a reinforcer.

Etonitazene Etonitazene reinforcement Opioid Oral route Drug concentration Fixed-ratio size Drug self-administration Drug reinforcement Behavioral genetics Genetic differences Lewis rats Fischer 344 rats

MANY studies have examined rodent strain differences in alcohol drinking [for reviews, see (2,16)]. Relatively few studies have examined strain differences in intake of other drugs, although there are several reports of genetic differences in oral opioid intake (2,8,17,21,27,35,37). These studies have employed either drinking bottle procedures similar to that used by Nichols and coworkers (33,34) and Kumar et al. (22) or the food-cup choice technique developed by Yanaura and Suzuki (43). Differences in morphine intake have been found in different rat stocks (27), selectively bred rat strains (35), inbred rats (34), and inbred mice (12,21). Similarly, differences in intake of the potent opioid etonitazene have been observed in different rat stocks (8) and in inbred mouse strains (14,17).

A different way to conceptualize and measure opioid ingestion comes from studies of drug-reinforced behavior that employed operant conditioning procedures (11,40,41). These methods have been quite successful in analyzing drug-seeking behavior and have made possible the study of a broad range of factors affecting that behavior. Most drugs that humans abuse serve as reinforcers for animals (18). For example, opioids maintain responding at higher rates than vehicle and thus have been demonstrated to serve as reinforcers for animals when delivered intravenously (11,40,41) or orally (28,39).

A potent opioid used in many studies is etonitazene; it can serve as a reinforcer orally for rats and rhesus monkeys (4,7,26,29,32). Etonitazene is well absorbed orally and does not appear to be markedly aversive in taste at behaviorally active concentrations (25,26,42).

In pharmacogenetic studies, inbred or selectively bred rodents are usually used. Two inbred rat strains that differ in many respects are the Lewis (LEW) and Fischer 344 (F344) strains (13). A recent finding is that in LEW rats relative to F344 rats ethanol maintained substantially higher response rates across concentrations and fixed-ratio (FR) sizes (36).

[1] Requests for reprints should be addressed to Richard A. Meisch, M.D., Ph.D., Department of Psychiatry and Behavioral Sciences, University of Texas Health Science Center at Houston, 1300 Moursund, Houston, TX 77030-3497.

Thus, ethanol served as a stronger reinforcer for LEW rats. Also, LEW rats exhibit a greater preference for morphine and codeine than do F344 rats (37).

The purpose of the present study was to use operant conditioning procedures to compare the establishment and maintenance of etonitazene-reinforced behavior in LEW and F344 rats.

METHOD

Animals

Adult (11 weeks) LEW(CRL/BL) (LEW) and F344(CRL/BL) (F344) male rats were obtained from Charles River Laboratories (Wilmington, MA). Animals were housed individually in wire-grid-bottom cages in a temperature-controlled room (26°C) with a 12 L:12 D cycle (lights on 7:00 a.m.-7:00 p.m.) and were given free access to Purina Laboratory Chow and tapwater. They were allowed to adapt to this environment for 2 weeks.

Apparatus

The experimental chambers were octagonal with four alternating walls constructed of Plexiglas and the other four of aluminum. Two aluminum walls on opposite sides of the chambers were each equipped with a lever (Coulbourn Instruments Inc., Lehigh Valley, PA) and three colored lights (4.76 W) above the lever. Responses on the right (ineffective) lever were recorded and served as a measure of nonspecific responding but had no programmed consequence. The aluminum wall to the left of the wall holding the effective lever was equipped with a drinking spout, two colored lights above the spout, and a Sonalert® (Mallory, Indianapolis, IN, No. S6628-24DC) above the lights. The spout was used to deliver a minute amount of liquid in response to a lick. In this system, an electronic circuit sensed the small current (resistance adjusted to 5.0 MΩ) traveling from the brass spout through the animal's body to the grounded cage floor. As the rodent's tongue contacted the spout tip, a solenoid valve was opened momentarily to deliver a droplet of liquid (approximately 5.0 μl/lick) directly onto the tongue. This delivery system was adapted from a system developed by Beardsley and Meisch (1). The lights above the lever and the Sonalert above the spout were used as discriminative stimuli for lever pressing. The lights above the spout were used as discriminative stimuli for licking. System control and data acquisition were by solid-state programmable modules (Coulbourn Instruments, Inc.) located in an adjacent room.

Procedure

Four F344 and four LEW rats were used. At the start of training, F344 and LEW rats weighed approximately 200 and 300 g, respectively. Rats were initially food deprived to 80% of their free-feeding weights at 16 weeks of age by rationing their daily food allotment. Sessions were run 7 days a week. Four individual operant chambers were used, and two successive sessions were run each day, with four animals per session. Sessions occurred at a constant time during the light phase of a normal light/dark cycle.

Etonitazene concentrations (expressed in μg/ml) were prepared using etonitazene HCl stock solution (100 μg/ml) in tapwater. The solutions were prepared, sealed, and stored at room temperature for approximately 1 h before use. The volume consumed was measured at the end of each session. All concentrations are in terms of the salt.

Establishment of Licking and Water-Reinforced Lever Pressing

Water bottles were removed from the rats' home cage for 22 h/day, and to increase further the probability of drinking the daily feedings of Purina Laboratory Chow were placed in the operant chamber 30 min after the beginning of the session. During the daily 1.5-h session, water was delivered each time the rat made contact with the spout. Rats had access to water in their home cage for 0.5 h after each session. During sessions, a white houselight was lit continually.

When rats reliably drank from the spout, water deliveries were used to shape presses on the left lever. Water deliveries were made available for five sessions on a heterogeneous chain FR 1 (lever press) 30 (FR 1) (spout contact) schedule of reinforcement: A single lever press was necessary to initiate the second component of the chain, in which 30 reinforced spout contacts were allowed, that is, chain FR 1 30 (FR 1: liquid) (30 spout contacts). Subsequently, five additional sessions were run at each of the following values: chain FR 1 20 (FR 1), chain FR 1 10 (FR 1), and chain FR 1 20 (FR 1). This extended testing with water ensured that rats were experienced with both pressing the lever and drinking from the spout. After rats were run for five sessions under each condition, water bottles were restored to the home cages and food continued to be given in the operant chamber 0.5 h after the beginning of the session.

Induction of Etonitazene Drinking

Within-session feedings of Purina Laboratory Chow continued for a series of 48 daily 2-h sessions. The purpose of the within-session feedings was to induce liquid drinking. During the first 27 sessions, 0 μg/ml etonitazene (i.e., water vehicle) was available, then 0.625, 1.25, 2.5, and 5 μg/ml etonitazene for seven sessions each. The session feedings were then discontinued and food was given to rats in their home cages after each session. There was free access to water in the home cages.

Locomotor-Activity Testing

On the last or next-to-the-last day of each treatment condition, behavioral activation was measured in an open-field apparatus similar to that developed by DeFries and Hegman (10). This is an arena constructed of white Plexiglas 91.44 cm on each side and marked off into 36 equal squares. Movements between squares interrupt photocell beams, activating electronic counters. The recorded score for the activity test was the total number of times a beam was broken within a 3-min period that followed completion of the experimental session. The 0 μg/ml (water) conditions were used as the vehicle control values.

Etonitazene Drinking After Termination of Induction

After the termination of within-session access to food, rats continued to have access to 5 μg/ml etonitazene for eight sessions. The objective was to determine the persistence of etonitazene-maintained responding in the absence of food-induced drinking. Next, rats received seven sessions at 0 μg/ml (water vehicle), and then were retested for seven sessions at 5 μg/ml. The purpose of these manipulations was to determine if etonitazene maintained higher response rates than the water vehicle.

Behavior at FR 1 as a Function of Etonitazene Concentration

At FR 1, a concentration-response function was obtained. A series of etonitazene concentrations was presented in a de-

scending and then an ascending sequence to control for order effects. The test sequence was: 5, 2.5, 1.25, 0.625, 0.313, 0.156, 0.079, 0 (water vehicle), 0.079, 0.156, 0.313, 0.625, 1.25, 2.5, and 5 μg/ml. Each concentration was present for seven sessions.

Behavior as a Function of FR Size

The size of the FR schedule was then increased gradually. Each rat was exposed to 5 μg/ml etonitazene at FRs 1, 2, 4, and then 8. Each FR value was present for seven consecutive sessions. The objective was to determine if intermittent presentations of etonitazene would maintain behavior.

Behavior as a Function of Liquid Present: Drug or Vehicle

At FR 8, rats were initially given access to 0 μg/ml (i.e., water) for five sessions, then etonitazene concentrations of 1.25 μg/ml for two sessions, 2.5 μg/ml for 2 sessions, 5 μg/ml for 14 sessions, 0 μg/ml for 19 sessions, and 5 μg/ml for 5 sessions. Concentrations of 1.25 and 2.5 μg/ml were interpolated between the initial water test and the first test at the 5 μg/ml concentration so the transition between water and 5 μg/ml would not be abrupt. In some previous studies, etonitazene-reinforced behavior extinguished slowly (6,32). Water, therefore, was presented for an extended number of sessions. Drug- and vehicle (water)-maintained behavior were studied at FR 8 rather than at a lower FR size because increasing differences between drug and vehicle are usually observed as FR size is increased (19,20,24).

Behavior at FR 8 as a Function of Etonitazene Concentration

All rats were then run with water available under an FR 8 schedule for 24 sessions. Subsequently, etonitazene concentrations were tested in the following sequence: 1.25 μg/ml for six sessions, 2.5 μg/ml for five sessions, 5 μg/ml for five sessions, and 0 μg/ml for nine sessions.

Statistical Analyses

Data are expressed as the mean ± SEM. Student's *t*-test or repeated- or non-repeated-measures analysis of variance (ANOVA) were used where appropriate for statistical analyses.

TABLE 1

ETONITAZENE INTAKE (μg/kg OF BODY WEIGHT) DURING FOOD-INDUCED DRINKING AS A FUNCTION OF ETONITAZENE CONCENTRATION FOR LEW AND F344 RATS

Etonitazene Concentrations (μg/ml)	Etonitazene Intake (μg/kg body weight)	
	LEW	F344
0.625	18.4 ± 2.1	10.8 ± 2.8
1.25	36.0 ± 3.2	33.9 ± 9.5
2.5	57.7 ± 7.1	58.7 ± 13.6
5.0	91.9 ± 12.5	88.3 ± 17.1

Each value represents the mean ± SEM of four animals.

RESULTS

Induction of Water and Etonitazene Drinking

The left portion of Fig. 1 shows number of responses under an FR 1 schedule of lever pressing as a function of etonitazene concentration for LEW and F344 rats. Results are shown for the 90-min period after the introduction of food (data from the first 30 min of the session are not shown). Consistent with a previous report (36), LEW rats had higher levels of baseline responding, thereby obtaining significantly more water deliveries than did F344 rats ($p < 0.001$). During this and all subsequent phases, the number of lever-press responses on the right (ineffective) lever, which served as a measure of nonspecific lever pressing, was low and did not differ between strains.

For LEW rats, there was a progressive decrease in responding when the etonitazene concentration was increased, F(concentration) = 4.02, $p < 0.01$. In contrast, for F344 rats there was no systematic change in responding across concentrations. At lower concentrations (0.625 and 1.25 μg/ml) LEW rats emitted more responses. However, the differences

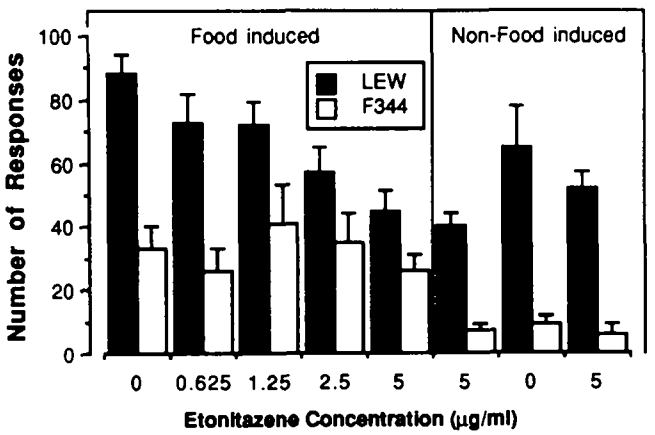

FIG. 1. Number of lever-press responses for the final 90-min portion of the 2-h session (following the introduction of food) as a function of etonitazene concentration for LEW and F344 rats. One reinforcement component represents one lever press activating the spout for 20 licks of 5 μl liquid per lick. Each bar represents the mean of four animals (n = 28; 4 rats × 7 sessions). Brackets indicate the SEM (n = 4; 4 rats × one mean each).

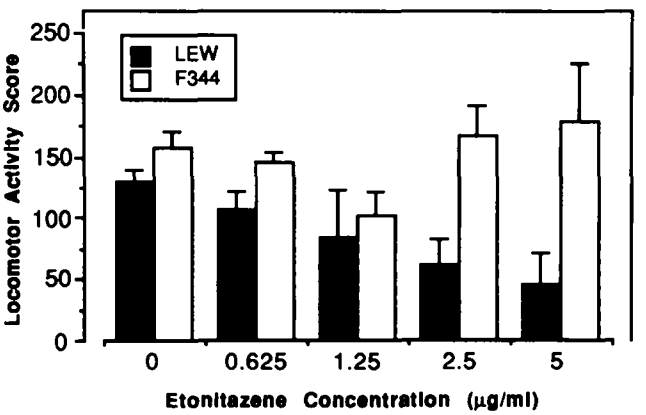

FIG. 2. Open-field activity score after 2 h self-administration session as a function of etonitazene concentration. Each bar represents the mean number of photobeam interruptions during a 3-min period following the 2-h self-administration session (four rats per strain; one score per animal). Brackets indicate the SEM.

between the strains became less as concentration was increased. Etonitazene intake (μg/kg of body weight) was also similar for both strains (Table 1). Similarities in intake occurred despite differences in responding since F344 rats weighed less than LEW rats.

Locomotor Activity

Figure 2 illustrates that LEW rats but not F344 rats showed etonitazene concentration-dependent decreases in open-field activity. LEW rats appeared mildly cataleptic after the sessions at 2.5 and 5 μg/ml etonitazene; two F344 rats displayed a Straub's tail and another had a front paw tremor. The differences between strains in open-field activity were significant, $F(\text{strain}) = 7.56, p < 0.01$.

Etonitazene Drinking After Termination of Induction

When food was no longer available in the operant chamber, the behavior of LEW and F344 rats changed in different ways (Fig. 1, right panel). For F344 rats, both etonitazene (5 μg/ml) and water responding showed marked decreases. In contrast, for LEW rats etonitazene responding was essentially unchanged and water responding was moderately decreased relative to rates of water-maintained responding when food was available in the chamber. For both strains, substitution of the water vehicle for the 5-μg/ml drug solution resulted in response rates that exceeded drug values; however, the magnitude of this increase differed between strains with F344 rats showing only a slight nonsignificant increase. Both drug and water values for LEW rats were substantially higher than for F344 rats.

Behavior at FR 1 as a Function of Etonitazene Concentration

Table 2 shows the number of liquid deliveries as a function of etonitazene concentration at FR 1. Food was not present in the experimental chambers during these tests. For both strains, there was no orderly relation between number of liquid deliveries and drug concentration. However, at all test points the number of deliveries for LEW rats far exceeded those for F344 rats.

TABLE 2

MEAN LIQUID DELIVERIES (n = 7 ± SEM) PER 2-h SESSION AS A FUNCTION OF ETONITAZENE CONCENTRATION FOR LEW AND F344 RATS

Etonitazene (μg/ml)	LEW		F344	
	Mean	SEM	Mean	SEM
5	51.6	(5.1)	5.9	(3.0)
2.5	54.4	(7.7)	5.5	(2.0)
1.25	63.6	(6.1)	7.5	(2.1)
0.625	66.1	(17.6)	8.9	(3.3)
0.313	62.5	(21.0)	8.5	(3.5)
0.156	73.9	(9.2)	3.3	(1.1)
0.078	82.3	(6.3)	3.6	(1.8)
0	68.8	(2.2)	3.4	(1.6)
0.078	70.1	(2.7)	4.8	(2.2)
0.156	65.3	(5.7)	3.4	(1.6)
0.313	46.5	(1.8)	3.4	(2.1)
0.625	56.6	(2.3)	1.6	(0.7)
1.25	72.8	(11.9)	3.0	(1.5)
2.5	62.3	(12.8)	4.5	(2.2)
5	42.9	(4.8)	3.1	(1.7)

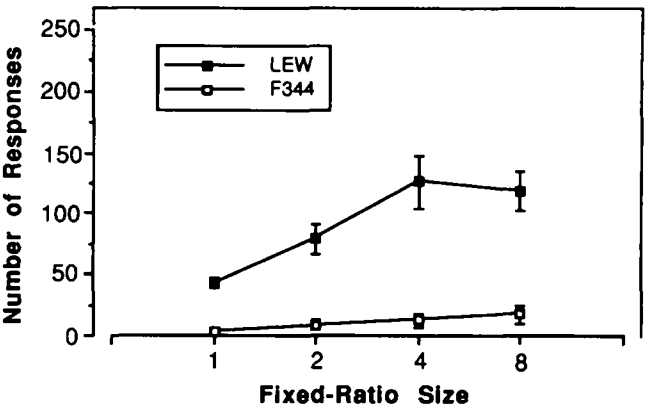

FIG. 3. Mean lever-press responses per session as a function of FR value. Each point represents the mean of four animals (n = 28; 4 rats × 7 sessions). Brackets indicate the SEM (n = 4; 4 rats × one mean each).

Behavior as a Function of FR Size

Figure 3 shows responses as a function of FR size for LEW and F344 rats at 5 μg/ml etonitazene. LEW rats emitted significantly more responses than F344 rats, $F(\text{strain}) = 7.82$, $p < 0.01$. For LEW rats, increases in FR value resulted in increases in response rate at FR 2 and again at FR 4, but a slight decrease in rate at FR 8. For F344 rats, response rate remained low at all FR sizes; however, increases in FR size produced progressive increases in response rate. These increases were not statistically significant.

Behavior as a Function of Liquid Present: Drug or Vehicle

At FR 8, rats were retested with the water vehicle, then with 5 μg/ml, water vehicle again, and back to 5 μg/ml etonitazene. Figure 4 shows that the drug solution maintained higher response rates than water in LEW rats. When water

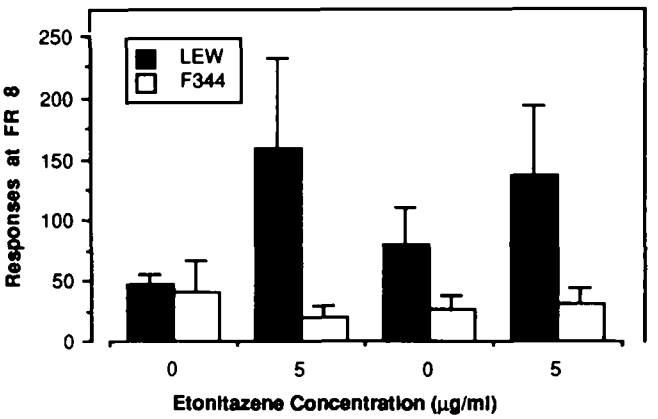

FIG. 4. Number of lever presses per session under an FR 8 schedule as a function of liquid present: drug (5 μg/ml etonitazene) or vehicle (water). Each bar represents the group mean of the last five sessions at each condition (n = 20; 4 rats × 5 sessions). Brackets indicate the SEM (n = 4; 4 rats × one mean each).

was then substituted for drug, response rates of LEW rats gradually decreased over 15 sessions and then stabilized (the transition phase is not shown in Fig. 4). This decrease is probably an extinction effect following removal of the drug reinforcer from the liquid solution. When etonitazene was reintroduced, response rates of LEW rats promptly increased and remained at higher levels than those observed during the last five water sessions of the preceding condition. Rates of drug-maintained responding were much higher in LEW than in F344 rats during both drug tests. Statistical analysis of the results confirmed the presence of a significant genetic difference, $F(1, 24) = 13.87$, $p < 0.001$. There was a significant strain × concentration interaction indicating that responding by the LEW and F344 rats differed as a function of drug or vehicle, $F(3, 24) = 3.19$, $p < 0.05$. This difference is due to differences in drug responding relative to water responding by LEW rats. LEW rats showed significantly higher drug than water responding, $F(1, 12) = 8.25$, $p < 0.02$.

Behavior at FR 8 as a Function of Etonitazene Concentration

Figure 5 shows the mean number of responses as a function of etonitazene concentration under an FR 8 schedule. For LEW rats, response rate appears to be an inverted U-shaped function of drug concentration whereas F344 rats showed no changed rates of responding across drug concentrations. Figure 5 also shows that the response rate of the LEW rats was significantly higher than that of the F344 rats, $F(\text{strain}) = 7.56$, $p < 0.01$. When water replaced the 5-μg/ml concentration, the rate of responding decreased across sessions. Table 3 documents that LEW rats consistently consumed more etonitazene (μg/kg of body weight/session) than did F344 rats, $F(\text{strain}) = 8.29$, $p < 0.01$.

Pattern of responding and time course of intake. Figure 6 shows representative cumulative records at each etonitazene concentration for LEW rat ML5 and for F344 rat MF5. These records illustrate the higher response rates and different response patterns maintained by etonitazene in a LEW rat relative to those maintained in a F344 rat.

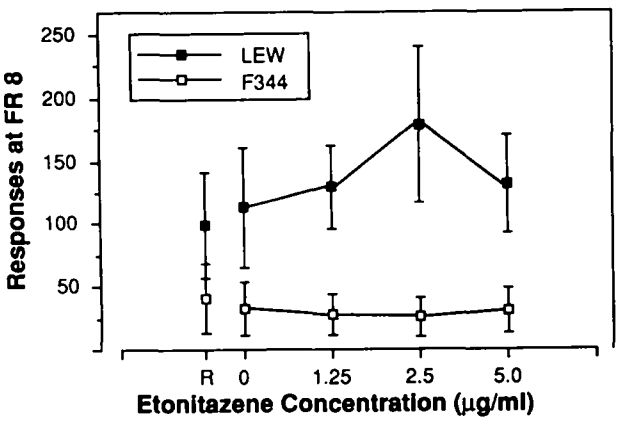

FIG. 5. Number of lever presses per session under an FR 8 schedule as a function of etonitazene concentration. Points plotted above R are the retest values of 0 μg/ml (i.e., water values). These retest values were obtained after completing the block of sessions at 5 μg/ml. Each point represents the mean of four animals ($n = 20$; 4 rats × 5 sessions). Brackets indicate the SEM ($n = 4$; 4 rats × one mean each).

TABLE 3

ETONITAZENE INTAKE (μg/kg OF BODY WEIGHT) PER 2-h SESSION AS A FUNCTION OF ETONITAZENE CONCENTRATION FOR LEW AND F344 RATS

Etonitazene Concentrations (μg/ml)	Etonitazene Intake (μg/kg body weight)	
	LEW	F344
1.25	7.9 ± 2.0	2.3 ± 1.2
2.5	22.3 ± 7.0	4.6 ± 2.2
5.0	33.2 ± 8.9	10.0 ± 5.6

Each value represents the mean ± SEM of four animals.

DISCUSSION

Animals continued to respond actively throughout the test sessions although during the food-induced training phase of this study etonitazene drinking resulted in effects such as catalepsy and Straub's tail. Such effects were noted in rats of both strains. Overt effects of etonitazene have often been observed in etonitazene self-administration studies (5–7,9,32). During the food-induced training phase, both strains also consumed substantial and equivalent amounts of drug. The substantial intake (μg drug per kg body wt per session) and the presence of overt effects are important for they indicate that the F344 rats' lack of etonitazene-reinforced behavior during subsequent conditions cannot be attributed to lower intake than LEW rats, to lack of prior drug contact, or to inability to perform the required operant task as a result of acute drug effects. Although the taste of the drug may be aversive, it did not prevent drinking during the food-induction phase.

The present results reveal quantitative and qualitative differences in etonitazene-maintained behavior in two inbred rat strains, the LEW and F344 strains. Quantitatively, LEW rats consistently consumed more etonitazene than did F344 rats, and this higher consumption occurred across a broad range of drug concentrations and FR sizes. The qualitative difference was that etonitazene came to serve as a reinforcer for LEW but not for F344 rats. With LEW rats it was possible to show that etonitazene at concentrations of 2.5 and 5 μg/ml maintained higher response rates than did vehicle control (water). Two additional findings support the conclusion that etonitazene functioned as a reinforcer for LEW rats: a) Etonitazene deliveries maintained patterns of responding characteristic of behavior reinforced under FR schedules; and b) etonitazene maintained lever-pressing behavior varied in what appears to be a characteristic inverted U-shaped pattern as a function of drug concentration. However, the high variability found with LEW rats when concentration was varied means that this finding needs to be replicated. With F344 rats, drug did not maintain higher response rates than water and responding did not change as a function of drug concentration. However, although the amounts of drug consumed by F344 rats (μg/kg/session) remained quite low response rates did increase when FR size was increased. This finding deserves additional study in future experiments.

The lack of clear reinforcing effects in F344 rats may relate to decreased sensitivity to opioids. ED_{50}s for the analgesic activity of morphine as measured by the hot plate method were 3.86 (2.32–5.69, 95% confidence limits) mg/kg SC for LEW rats and 19.47 (14.24–30.51) mg/kg SC for F344 rats

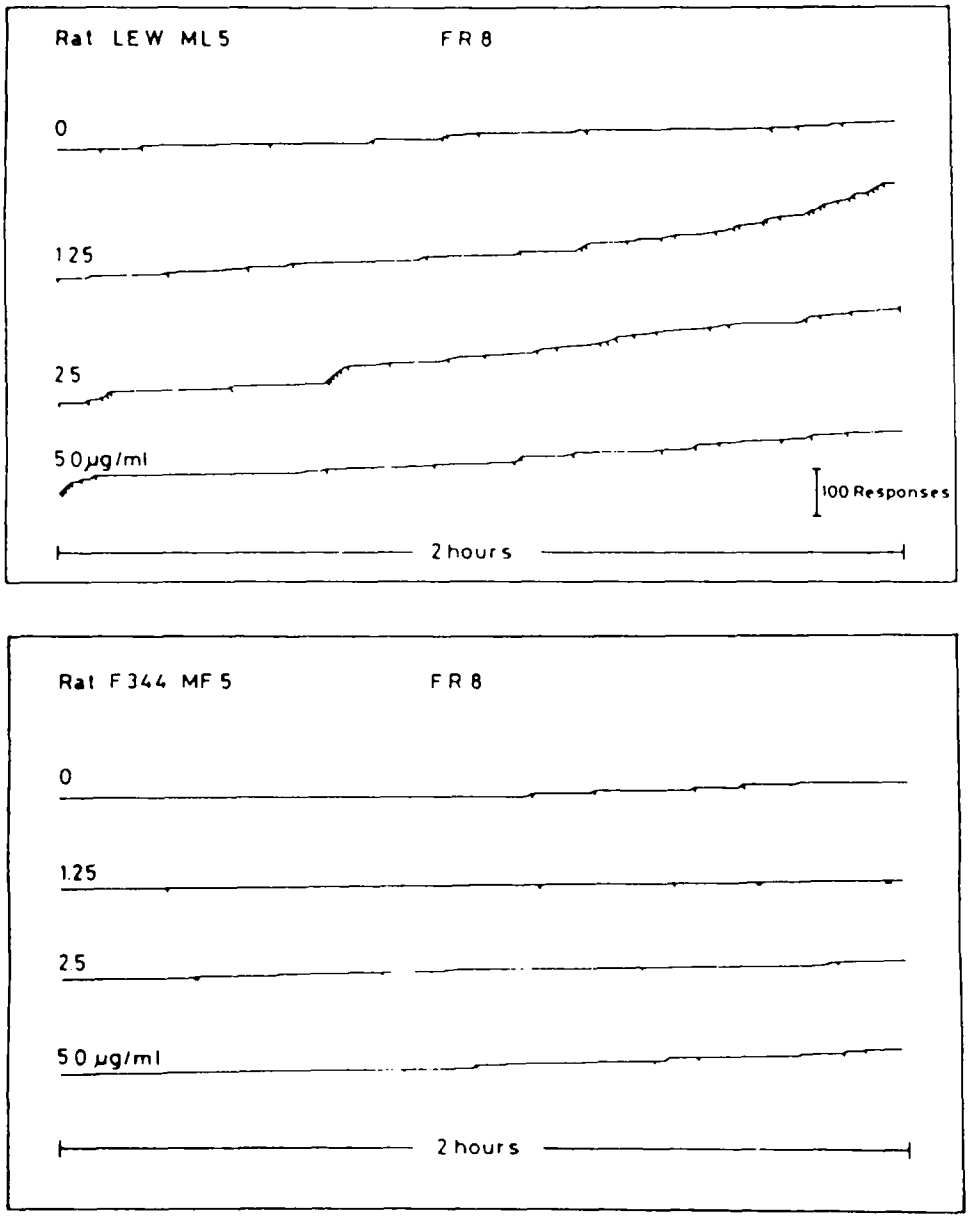

FIG. 6. Representative cumulative records for LEW rat ML5 (upper panel) and F344 rat MF5 (lower panel) showing performance under various etonitazene concentrations. The pen moved upward with each response on the lever. Diagonal marks of the response pen show liquid deliveries after completion of each FR 8 schedule requirement. Each record shows a complete 2-h session. Note that at all concentrations etonitazene-reinforced responding of LEW rat ML5 was greater than responding of F344 rat MF5.

(38). However, further studies are required to determine what drug actions correlate with reinforcing effects.

That etonitazene served as a reinforcer in this study for at least one strain is consistent with a number of past studies showing that orally delivered etonitazene can serve as a reinforcer of lever-pressing behavior and can maintain responding under fixed-ratio schedules (7,23,25,29,32). With Lewis rats, liquid deliveries were an inverted U-shaped function of etonitazene concentration and etonitazene intake (µg/kg/session) increased with increases in drug concentration. Similar findings have been observed in an earlier study of etonitazene-reinforced lever pressing with Wistar rats (6). Differential effects of different drug concentrations and clear separation of drug- and vehicle-maintained responding were seen when the FR value was increased to FR 8 but not at FR 1. These findings are similar to findings in other drug self-administration studies: Increases in FR size resulted in better separation of the differential effects of independent variables (19,20, 24,30,31).

Substantial variability in drinking was noted both between and within rats. Again, such variability in etonitazene drinking has been found in prior studies (5-7,9,25,32). Finally, the

present findings are also congruent with home-cage drinking studies that have shown significant intake of etonitazene (3,5,42), as well as genetic differences in home-cage preference drinking of etonitazene (8,14) and other opioids (2,8,17,21, 27,35,37).

The differences between LEW and F344 rats in etonitazene intake are similar to differences between these strains in ethanol-reinforced behavior (36). However, in the earlier study (36) ethanol did function as a *weak* reinforcer for F344 rats whereas in the present study etonitazene did not clearly serve even as a weak reinforcer for this strain. These findings are congruent with a larger body of data that indicates that across drug classes (e.g., CNS depressants, psychomotor stimulants, opioids) some strains may show high drug-reinforced behavior and other strains low drug-reinforced behavior (15).

The primary importance of the present findings is they indicate that genotype can be an important determinant of drug-reinforced behavior with a drug other than ethanol. Although operant conditioning procedures have been used to demonstrate genetic differences when ethanol serves as a reinforcer (15), such procedures have not been used in studying genetic differences with other drugs. The existence of genetic differences in drug reinforcement should be followed up by studies using experimental approaches developed in pharmacogenetics to examine possible mechanisms involved in drug reinforcement.

ACKNOWLEDGEMENTS

This research was supported in part by a grant from the University of Minnesota's Office of Alcohol and Other Drug Abuse Programming to T. S. and R. A. M; by New Investigator Research Awards AA-06104 and AA-06924 and research grant AA-07754 to F. R. G. from the National Institute on Alcohol Abuse and Alcoholism, and by research grant DA-00944 to R. A. M. from the National Institute on Drug Abuse. Experiments were conducted when the authors were at the University of Minnesota and subsequently at NIDA's Addiction Research Center. The authors thank Dr. Gregory Lemaire for helpful comments on the manuscript.

REFERENCES

1. Beardsley, P. M.; Meisch, R. A. A precision drinking device for rats tested with water, etonitazene, and ethanol. Pharmacol. Biochem. Behav. 14:871-876; 1981.
2. Broadhurst, P. L. Drugs and the inheritance of behavior: A survey of comparative pharmacogenetics. New York: Plenum Press; 1979.
3. Carlson, K. R. Taste vs. CNS effects in voluntary oral opiate intake: Studies with a novel device and technique. Pharmacol. Biochem. Behav. 34:419-423; 1990.
4. Carroll, M. E.; Meisch, R. A. Etonitazene as a reinforcer: Oral intake of etonitazene by rhesus monkeys. Psychopharmacology (Berl.) 59:225-229; 1978.
5. Carroll, M. E.; Meisch, R. A. Effects of food deprivation on etonitazene consumption in rats. Pharmacol. Biochem. Behav. 10:155-159; 1979.
6. Carroll, M. E.; Meisch, R. A. Concurrent etonitazene and water intake in rats: Role of taste, olfaction, and auditory stimuli. Psychopharmacology (Berl.) 64:1-7; 1979.
7. Carroll, M. E.; Meisch, R. A. Determinants of increased drug self-administration due to food deprivation. Psychopharmacology (Berl.) 74:197-200; 1981.
8. Carroll, M. E.; Pederson, M. C.; Harrison, R. G. Food deprivation reveals strain differences in opiate intake of Sprague-Dawley and Wistar rats. Pharmacol. Biochem. Behav. 24:1095-1099; 1986.
9. Chernov, H. I.; Ambrose, F. G.; Plummer, A. J. Patterns of consumption of etonitazene solutions by naive rats. Arch. Int. Pharmacodyn. Ther. 175:309-318; 1968.
10. DeFries, J. C.; Hegman, J. P. Genetic analysis of open-field behavior in inbred mice. In: Lindzey, G.; Thiessen, D. D., eds. The mouse as a prototype. New York: Appleton-Century-Crofts; 1970:23-56.
11. Deneau, G.; Yanagita, T.; Seevers, M. H. Self-administration of psychoactive substances by the monkey. Psychopharmacologia 16:30-48; 1969.
12. Eriksson, K.; Kiianmaa, K. Genetic analysis of susceptibility to morphine addiction in inbred mice. Ann. Med. Exp. Fenn. 49: 73-78; 1971.
13. Festing, M. F. W. Inbred strains. In: Baker, H. J.; Lindsey, J. R.; Weisbroth, S. H., eds. The laboratory rat. Vol. 1. New York: Academic Press; 1979:55-69.
14. Forgie, M. L.; Beyerstein, B. L.; Alexander, B. K. Contributions of taste factors and gender to opioid preference in C57BL and DBA mice. Psychopharmacology (Berl.) 95:237-244; 1988.
15. George, F. R. Genetic approaches to studying drug abuse: Correlates of drug self-administration. Alcohol 7:207-211; 1990.
16. George, F. R.; Goldberg, S. R. Genetic approaches to the analysis of addiction processes. Trends Pharmacol. Sci. 10:78-83; 1989.
17. George, F. R.; Meisch, R. A. Oral narcotic intake as a reinforcer: Genotype and environment interactions. Behav. Genet. 14(abstr.):603; 1984.
18. Griffiths, R. R.; Bigelow, G. E.; Henningfield, J. E. Similarities in animal and human drug-taking behavior. In: Mello, N. K., ed. Advances in substance abuse. Vol. 1. Greenwich, CT: JAI Press; 1980:1-90.
19. Henningfield, J. E.; Meisch, R. A. Ethanol as a positive reinforcer via the oral route for rhesus monkeys: Maintenance of fixed-ratio responding. Pharmacol. Biochem. Behav. 4:473-475; 1976.
20. Holz, W. C.; Gill, C. A. Drug injections as negative reinforcers. Pharmacol. Rev. 27:437-446; 1975.
21. Horowitz, G. P.; Whitney, G.; Smith, J. C.; Stephan, F. K. Morphine ingestion: Genetic control in mice. Psychopharmacology (Berl.) 52:119-122; 1977.
22. Kumar, R.; Steinberg, H.; Stolerman, I. P. Inducing a preference for morphine in rats without premedication. Nature 218:564-565; 1968.
23. Leander, J. D.; McMillan, D. E. Schedule-induced narcotic ingestion. Pharmacol. Rev. 27:475-487; 1975.
24. Lemaire, G. A.; Meisch, R. A. Pentobarbital self-administration in rhesus monkeys: Drug concentration and fixed-ratio size interactions. J. Exp. Anal. Behav. 42:37-49; 1984.
25. Lewis, M. J.; Margules, D. L.; Ward, L. B., Jr. Opioid-reinforced operant behavior: Selective suppression by alpha-methyl-para-tyrosine. J. Comp. Physiol. Psychol. 88:519-527; 1975.
26. McMillan, D. E.; Leander, J. D. Schedule-induced oral self-administration of etonitazene. Pharmacol. Biochem. Behav. 4: 137-141; 1976.
27. Meade, R.; Amit, Z.; Pachter, W.; Corcoran, M. E. Differences in oral intake of morphine by two strains of rats. Res. Comm. Chem. Pathol. Pharmacol. 6:1105-1108; 1973.
28. Meisch, R. A.; Carroll, M. E. Oral drug self-administration: Drugs as reinforcers. In: Bozarth, M. A., ed. Methods of assessing properties of abused drugs. New York: Springer-Verlag; 1987: 143-160.
29. Meisch, R. A.; Kliner, D. J. Etonitazene as a reinforcer for rats: Increased etonitazene-reinforced behavior due to food deprivation. Psychopharmacology (Berl.) 63:97-98; 1979.
30. Meisch, R. A.; Lemaire, G. A. Oral self-administration of pentobarbital by rhesus monkeys: Maintenance of behavior by different concurrently available volumes of drug solution. J. Exp. Anal. Behav. 52:111-126; 1989.

31. Meisch, R. A.; Lemaire, G. A. Reinforcing effects of a pentobarbital-ethanol combination relative to each drug alone. Pharmacol. Biochem. Behav. 35:443-450; 1990.
32. Meisch, R. A.; Stark, L. J. Establishment of etonitazene as a reinforcer for rats by use of schedule-induced drinking. Pharmacol. Biochem. Behav. 7:195-203; 1977.
33. Nichols, J. R.; Headlee, C. P.; Coppock, H. W. Drug addiction I. Addiction by escape training. J. Am. Pharm. Assoc. 45:788-791; 1956.
34. Nichols, J. R.; Hsiao, S. Addiction liability of albino rats: Breeding for quantitative differences in morphine drinking. Science 157:561-563; 1967.
35. Satinder, K. P. Oral intake of morphine in selectively bred rats. Pharmacol. Biochem. Behav. 7:43-49; 1977.
36. Suzuki, T.; George, F. R.; Meisch, R. A. Differential establishment and maintenance of oral ethanol reinforced behavior in Lewis and Fischer 344 inbred rat strains. J. Pharmacol. Exp. Ther. 245:164-170; 1988.
37. Suzuki, T.; Otani, K.; Koike, Y.; Misawa, M. Genetic differences in preferences for morphine and codeine in Lewis and Fischer 344 inbred rat strains. Jpn. J. Pharmacol. 47:425-431; 1988.
38. Suzuki, T.; Otani, K.; Misawa, M. Analgesic actions of, tolerance to and physical dependence on morphine in Lewis and F344 inbred rat strains. Abstract of the 108th Meeting of Pharmaceutical Society of Japan, Hiroshima; 1988:235.
39. Suzuki, T.; Uesugi, J.; Yoshii, T.; Yanaura, S. Preference for and oral self-administration of morphine and codeine in rats. In: Saito, S.; Yanagita, T., eds. Learning and memory: Drugs as reinforcer. Amsterdam: Excerpta Medica; 1982:202-220.
40. Thompson, T.; Schuster, C. R. Morphine self-administration, food-reinforced, and avoidance behaviors in rhesus monkeys. Psychopharmacologia 5:87-94; 1964.
41. Weeks, J. R. Experimental morphine addiction: Method for automatic intravenous injections in unrestrained rats. Science 138: 143-144; 1962.
42. Wikler, A.; Martin, W. R.; Pescor, F. T.; Eades, C. G. Factors regulating oral consumption of an opioid (etonitazene) by morphine-addicted rats. Psychopharmacologia 5:55-76; 1963.
43. Yanaura, S.; Suzuki, T. Preference for morphine and drug-seeking behavior in morphine dependent rats. Jpn. J. Pharmacol. 28:707-717; 1978.