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Reinforcement with Intragastric Infusions of Ethanol: Blocking Effect of FLA 57[1]

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DAVIS, W. M., T. E. WERNER AND S. G. SMITH. *Reinforcement with intragastric infusions of ethanol: Blocking effect of FLA 57.* PHARMAC. BIOCHEM. BEHAV. **11**(5) 545–548, 1979.—Suppression of oral intake of ethanol by FLA 57 has been reported for rats and was attributed to an inhibition of dopamine β-hydroxylase. We have demonstrated the ability of FLA 57 (50 mg/kg, IP) to suppress bar-pressing for intragastric (IG) delivery of doses of ethanol (25 mg/kg). This indicates that the effect on oral intake of ethanol may not be attributed to a taste factor, e.g., a decreased palatability of the ethanol solution. The same dose of FLA 57 did not suppress responding for IG doses of sweet milk. Thus, there was not an impairment of appetitive behavior in general through some nonspecific depressant or toxic action. Furthermore, the primary reinforcing action of ethanol, when used to establish a buzzer as a conditioned reinforcer through repeated pairings, was blocked if FLA 57 was given before pairings. This was evidenced by a failure of such rats to bar-press above the baseline level in a later test of conditioned reinforcement, which contrasted with the increased responding seen for rats receiving saline instead of FLA 57 before ethanol. These data support the previous findings on oral ethanol and confirm that FLA 57 can impair the mechanism by which ethanol produces positive reinforcement in rats.

Ethanol Intragastric infusion FLA 57 blocking effect

A NUMBER of lines of evidence suggest that brain catecholamines (CA), or norepinephrine (NE) in particular, may be critically involved in the reinforcing effect of ethanol. Most cogent to the present study are several works that employed enzyme inhibitors to reduce brain CA levels below normal. Inhibition of tyrosine hydroxylase by means of α-methyltyrosine, which diminishes both dopamine (DA) and NE, was found to suppress the euphoric effects of ethanol in man [1]. The experience of euphoria has been equated with a primary positive reinforcing property of ethanol [9]. Consumption of ethanol by rats was reduced moderately and markedly by disulfiram and FLA 63, respectively; both drugs inhibit dopamine β-hydroxylase (DBH). In contrast, calcium carbimide, an inhibitor of aldehyde dehydrogenase but not DBH, failed to reduce ethanol intake [4]. In a parallel study, another new DBH inhibitor, FLA 57, also suppressed oral intake of ethanol by rats [2]; these findings were attributed to an impairment of the mechanism by which ethanol-associated positive reinforcement occurs. This impairment was inferred to result from the depletion of brain NE by FLA 57. An analogous suppression of intracerebroventricular self-administration of a metabolite of ethanol, acetaldehyde, was also produced by FLA 57 [3].

The present studies were conducted to test the ability of FLA 57 to block the reinforcing action of ethanol under other circumstances, i.e., when the drug was self-administered by intragastric infusion [14], and when it was used to establish a conditioned reinforcer [15]. As a control for specificity of the interaction with ethanol self-administration, the effect of FLA 57 on another appetitive behavior was also determined. This served to provide data parallel to that previously obtained for another DBH inhibition, U-14,624 [8]

METHOD

Animals

Adult male rats (N=8/group) of Sprague-Dawley stock weighing 400–500 g were used. All rats were drug-naive at the start of the experiment. Water and food were available ad lib both in the home cage and in the experimental chamber. The subjects were implanted surgically (under general anesthesia using Metofane®) with a chronic trans-esophageal intragastric cannula that passed subcutaneously to exit the skin at the dorsal surface of the neck or upper back; surgical procedures have been fully described previously [14].

Apparatus

The experimental chambers were clear plastic cylinders of 24 cm height and 25 cm dia. that contained a response-lever, food and water. Each chamber was lighted and enclosed within a ventilated, sound attenuated compartment. When the rat was placed in the chamber, its catheter was attached to a length of flexible needle-tubing that served as both leash and fluid channel, which in turn attached to a leak-proof liquid-swivel at the top center of the chamber. The other end of the swivel was connected by vinyl tubing to

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an infusing pump, which delivered 0.092 ml of solution in 0.25 sec each time it was activated. Bar-presses were registered on electromechanical counters, and programming of contingencies was accomplished by solid-state electronic circuitry.

Procedure of Experiment 1

After allowing five days for recovery from surgery, on Day 0 the subjects were placed in the test chamber with their cannulas attached to the injection system for a 1-hr period of adaptation followed by a 10-hr period during which each bar-press resulted in an intragastric infusion of saline. Bar-pressing during the latter interval was taken as the operant level of responding for a behavioral baseline. Superimposed on the infusion interval was a buzzer presentation, of the same duration as that of the infusion, that was employed to facilitate acquisition. On each of the next five days subjects were allowed a 10-hr period of access on a CRF schedule to infusions of ethanol in a dose of 25 mg/kg/infusion. Responding was extinguished in a 10-hr session on Day 6 by substitution of 0.9% saline for the ethanol solution. In a reacquisition session on Day 7, rats were given a 50 mg/kg intraperitoneal (IP) dose of FLA 57, or an equivalent volume of saline; 4 hr later they were given access to ethanol under the same conditions as for Days 1–5. FLA 57 is the designation of Astra Chemical Co. for 4-methyl-1-homopiperazine-dithiocarboxylic acid; it is used here to designate the sodium salt which we employed. Our dose of 50 mg/kg equals a 44.8 mg/kg dose of the free acid.

Procedure for Experiment 2

Subjects and apparatus were as for Experiment 1. Rats (N=8/group) were given training exactly as in Experiment 1 except that sweetened condensed milk (diluted to 1/2 strength with distilled water) was employed as the reinforcer instead of an ethanol solution.

Procedure for Experiment 3

Subjects were prepared as in Experiment 1. Apparatus was the same except that the response lever was removed during sessions of ethanol-buzzer pairings (Days 2–5).

After recovery from surgery, rats (N=8/group) were given an adaptation and operant level session as during Experiment 1. On Days 2 through 5 they received IP doses of FLA 57 (50 mg/kg) or saline 4 hr before onset of the experimental session, at which time they were placed in the chambers with response-levers removed, attached to the infusion system, and given 50 intragastric doses of ethanol (25 mg/kg) paired with a buzzer presentation without reference to behavior. The infusion volume and duration were as for Experiment 1. Pairings were presented on a variable-time 15-min schedule. On Days 6 through 9 the subjects were allowed to recover from the effects of drug treatment. On Day 10 they were placed in the chambers with the levers in place. During this session each bar-press resulted in a simultaneous presentation of saline infusion and buzzer so as to provide a test for conditioned reinforcement [15]. On Day 11 the rats were allowed to bar-press on a CRF schedule for the 25 mg/kg dose of ethanol used during the pairings. This served to discriminate any rat that would not respond to this dosage of ethanol as a primary reinforcer, since such subjects could not be expected to develop conditioned reinforcement. Failing to respond above its operant level would be cause for removal of a subject from the experiment.

Statistical Analysis

In all three experiments this was performed using two-tailed t-tests for related measures (within group) or independent measures (between control and FLA-57 groups) [7].

RESULTS

The data from Experiment 1, shown in Table 1A, indicate that there was no difference ($p>0.05$) between initial operant levels of control and FLA 57 treated groups or for levels of responding for alcohol in the fifth acquisition session for self-administration. However, a significant difference occurred between the two groups in the session for reacquisition of alcohol self-administration after treatment with saline or FLA 57 ($t=4.172$, $p<0.001$). This difference arose because on Day 7 the saline group reacquired alcohol self-administration, judging by a significant increase ($t=8.066$, $p<0.001$) when compared to their initial operant behavior, whereas the FLA 57 group showed no difference from their operant baseline level of responding ($t=0.570$, $p>0.05$). The latter fact indicates that depression of activity compared to the operant level was not caused by treatment with FLA 57.

The data for Experiment 2 are shown in part B of Table 1. No differences were observed between the two groups in the operant level session or for responding with sweet milk as reinforcer on Day 5 of acquisition. Following extinction there was no difference ($t=1.814$, $p>0.05$) between the two groups on Day 7 in reacquisition of responding for sweet milk. Both groups showed significant increases on Day 7 over their operant level baseline performance ($t=12.064$ and $t=13.968$; each $p<0.001$).

The results for Experiment 3 (Table 2) show no differences between the two groups during the initial operant level test. However, when treatment with saline or FLA 57 was given before buzzer-alcohol pairings, a significant higher ($t=7.199$, $p<0.001$) level of responding was observed for the saline groups during the test for conditioned reinforcement. This difference evidently arises from the buzzer having acquired properties of a conditioned reinforcer in the case of the saline group; i.e., the increase of bar-responses over the operant level for that group was highly significant ($t=6.761$, $p<0.001$). In contrast, the FLA 57 group gave no indication of the buzzer having acquired conditioned reinforcing properties, showing no difference ($t=0.931$, $p>0.05$) between responding in the operant baseline session and in the test for conditioned reinforcement.

DISCUSSION

The results of Experiment 1 show that FLA 57 at a dose of 50 mg/kg did block the reacquisition of self-administration behavior for ethanol. As the response level of the group treated with FLA 57 before the reacquisition session was not depressed below the operant baseline, the hindrance to reacquisition does not seem attributable to a nonspecific central depressant action of FLA 57. Neither do the data give evidence for a negatively reinforcing state resulting from the combination of ethanol and FLA 57, as would be expected for ethanol plus disulfiram. Whether the absence of the aversive effects of high acetaldehyde levels is advantageous to the clinical use of FLA 57 is uncertain and cannot be established from animal data alone.

TABLE 1

EFFECT OF PRETREATMENT WITH SALINE OR FLA 57 (50 MG/KG, IP) BEFORE THE SESSION OF DAY 7 ON INTRAGASTRIC SELF-ADMINISTRATION OF ETHANOL (25 MG/KG/INFUSION) OR SWEET MILK

Pretreatment Agent	Bar-presses per 10-hr Session (Mean ± SEM)‡ Operant Baseline	Acquisition Day 5	Reacquisition Day 7
A. Before ethanol			
Saline	32.8 ± 3.8	84.1 ± 9.1*	98.8 ± 7.0*†
FLA 57	37.6 ± 7.0	88.5 ± 6.9*	35.2 ± 5.5
B. Before sweet milk			
Saline	25.9 ± 3.3	106.6 ± 7.5*	113.6 ± 6.8*
FLA 57	23.5 ± 2.3	96.9 ± 5.1*	102.6 ± 4.3*

*Significantly ($p<0.01$) elevated from operant baseline responding.
†Significantly ($p<0.01$) greater than response level for FLA 57 group.
‡Data for each group represent N's of 8 male Sprague-Dawley rats.

TABLE 2

EFFECT OF SALINE OR FLA 57 (50 MG/KG, IP) 4 HOURS BEFORE FOUR SESSIONS FOR 50 PAIRINGS OF BUZZER AND INTRAGASTRIC DOSES OF ETHANOL (25 MG/KG) ON THE SUBSEQUENT BAR-PRESSING OF RATS UNDER BUZZER CONTIGENCY

Pretreatment Agent	Bar-presses per 10-Hr Session (Mean ± SEM)† Operant Baseline Session (day 0)	Conditioned Reinforcement Test (day 10)
Saline	38.1 ± 7.4	88.9 ± 9.1*
FLA 57	35.4 ± 6.8	30.4 ± 7.1

*Significantly ($p<0.001$) greater than operant baseline or FLA 57 group.
†Data represent N=8 for each group.

The demonstration in Experiment 2 that the same 50 mg/kg dose of FLA 57 did not suppress reacquisition of responding for sweet milk indicates that the results in Experiment 1 may not be attributed to an action of FLA 57 to suppress all appetitive behaviors.

Experiment 3 demonstrated that FLA 57 could prevent the establishment of a buzzer as a conditioned reinforcer through pairings with the primary reinforcing effect of ethanol. The test for this effect of FLA 57 was conducted in the absence of the drug, i.e., five days after the last dose was given. As recovery to control monoamine levels occurred by 24 hr after a single dose of FLA 57 [2], this interval amply assures restoration of normal CA levels in brain. Therefore, the action of FLA 57 exerted on Days 1–5 against ethanol-associated primary positive reinforcement is very likely to be free from any drug effects being exerted yet at the time of testing. Thus, from all of the data it seems that non-specific actions can hardly be invoked to explain these results. Rather, a specific impairment by FLA 57 of the reinforcing action of ethanol is strongly supported by the data.

The FLA 57 treatment used here was on the same time schedule as was used in a prior study for which neurochemical assays were reported [2]. In that case, the brain level of NE at 4 hr after 40 mg/kg of FLA 57 (calculated as the acid) was depleted to 47% of control; levels of DA and serotonin did not differ significantly from the control level at that time. Thus, the inference of Amit and coworkers [2], that depletion of brain NE is responsible for its effects on oral self-administration of ethanol, seems quite applicable to the present data also. This is in accord with our previous finding that intragastric self-administration behavior for ethanol was selectively blocked by another DBH inhibitor, U-14,624 [8], which we also interpreted as arising from the depletion of brain NE and a consequent impairment of the mechanism for positive reinforcement.

The above interpretation is in accord with the findings and conclusions of studies of rats having brain lesions in catecholaminergic pathways induced by 6-hydroxydopamine (6-OHDA) [5, 11, 12]. Drinking of ethanol was markedly suppressed after such treatment of Sprague-Dawley and Wistar rats when both NE and DA were depleted, but not when the NE level was protected and DA alone was depleted. However, one recent study failed to detect a decrease of ethanol preference after selective depletion of brain NE by use of 6-hydroxydopa [13], and another reported an increase in consumption of ethanol after intracerebral 6-OHDA [10].

The pharmacological elimination of ethanol-associated reinforcement by a dose of FLA 57 that did not impair milk-based reinforcement provides a systematic replication of our earlier equivalent finding with U-14,624 [8]. These results indicate that ethanol-based reinforcement can be blocked by DBH inhibitors in a potentially useful fashion. The effectiveness of FLA 57 in preventing the reinforcing action of ethanol, shown in the present study and earlier ones [2,6], suggests that it may be useful for treatment of ethanol abuse. In an animal model, Brown *et al.* [6] provided a demonstration that extinction of ethanol self-administration behavior could be facilitated by treatment with FLA 57 concurrently with oral consumption of ethanol solution. The clinical application of FLA 57 for an analogous therapeutic approach in alcoholics may be deserving of investigation.

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