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# Apomorphine Increases Ethanol Discrimination

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SCHECHTER, M. D. *Apomorphine increases ethanol discrimination.* PHARMACOL BIOCHEM BEHAV 22(2) 179–182, 1985.—Rats were trained to discriminate between the stimulus properties of 600 mg/kg ethanol and saline in a two-lever, food-motivated operant task. Once trained, rats showed a dose-related decrease in discriminative performance with lower ethanol doses and analysis of the dose-response curve indicated an ED50 of 372 mg/kg. Pretreatment with 0.16 mg/kg apomorphine produced increased discriminative performance at each ethanol dose and the combination generated a dose-response curve parallel to ethanol administered alone with an ED50 of 232 mg/kg. This significant shift to the left of the ethanol dose-response curve after apomorphine administration is discussed in relation to dopaminergic neuronal systems and the clinical use of apomorphine in alcoholics.

Drug discrimination Ethanol Apomorphine Dopamine Rats

APOMORPHINE, an emetic drug when ingested in high doses, has been used since the 19th century in smaller non-emetic doses to treat the acute alcohol intoxication phase of chronic alcoholism [15,33]. It has been reported to significantly reduce the craving for alcohol [8, 14, 30]. Apomorphine, at high doses has been reported to be a direct agonist that stimulates dopamine post-synaptic receptors, whereas at lower doses this drug stimulates presynaptic dopamine receptors and, thus, reduces the biosynthesis of dopamine [17,24]. Thus, the effect of apomorphine on alcoholics may not be due solely to a conditioned avoidance reaction induced by vomiting, but also to central dopaminergic effects produced by non-emetic doses [14]. However, apomorphine is a difficult drug to study since it is rapidly metabolized by the liver and the dosage required to produce an emetic effect varies from human subject to subject [8].

Ethanol was shown as early as 1951 by Conger to serve as a discriminative stimulus for differential behavior [11]. Since that time, ethanol has been employed to control discriminative responding in numerous animal [4, 23, 28, 34] and human [13,32] studies. Within this behavioral technique, which is essentially a drug detection procedure, rats are trained to discriminate between a drug, in this case, ethanol, and a non-drug (vehicle or saline) state using operant techniques. Thus, food-deprived rats are trained to press one of two levers for food reinforcement, under the drug state, while on different days, the same animals are trained to press the opposite lever in the non-drug state. Rats learn to associate one lever with drug and the opposite lever with saline such that the drug becomes the stimulus that enables the animal to differentiate between which lever will deliver reinforcement on a given day. The usefulness of this procedure is contingent upon the animal subjects' learning and retaining the acquired discriminative stimulus, i.e., the interoceptive cue produced as a consequence of drug administration. Although somewhat time-consuming as to training duration, this behavioral paradigm has proven to be a sensitive, specific, and stable method to investigate the mechanism of drug action in the central nervous system. Indeed, this behavioral procedure employs drug doses, to serve as stimuli, which are generally below the level at which they affect other pharmacological measures or neurochemical events or cause behavioral disruption or tolerance [16].

The purpose of the present investigation was to train rats to discriminate between ethanol and saline and, once stable discrimination was attained and maintained, to investigate the effect of apomorphine upon the animals' ability to discriminate the behavioral effects of various doses of ethanol. The intent was to establish, in an animal behavioral procedure, the interaction between dopamine receptor stimulation and the effects of ethanol.

## METHOD

### *Subjects*

The subjects were eight male ARS/Sprague-Dawley rats weighing between 380–450 g at the beginning of the study. The animals were housed in individual cages and their weights were maintained at approximately 80–85% of their expected free-feeding weights by partial food deprivation. Water was continuously available in the home cages which were kept in a temperature-controlled (20–22°C) room with a daily cycle of 12 hr (0600–1800) light and 12 hr dark.

### *Behavioral Training*

The behavioral apparatus consisted of four standard two-lever operant chambers (Lafayette Instruments Corp., Lafayette, In) housed within individual sound-attenuating cubicles equipped with an exhaust fan and a 9W house-light. A food pellet receptacle was mounted 2 cm above the grid floor and equidistant between the 2 levers. Solid-state programming equipment (LVB Corp., Lehigh Valley, PA) was used to control and record the sessions and was located in an adjacent room.

TABLE 1

EFFECT OF VARIOUS DOSES OF ETHANOL, WITH AND WITHOUT APOMORPHINE PRETREATMENT, UPON ETHANOL DISCRIMINATION

| Pretreatment | Dose (mg/kg) | Treatment | Dose (No. trials) mg/kg | Quantal | Quantitative (±SD) |
|---|---|---|---|---|---|
| — | | Saline | (12) | 6.8 | 11.5 (7.1) |
| — | | Ethanol | 600 (12) | 97.5 | 82.4 (4.2) |
| — | | | 450 (2) | 62.5 | 54.2 (19.5) |
| — | | | 300 (2) | 25.0 | 33.6 (10.5) |
| Apomorphine | 0.16 | Saline | (2) | 12.5 | 19.7 (9.8) |
| | | Ethanol | 600 (2) | 100.0 | 88.6 (5.4) |
| | | | 450 (2) | 93.8 | 81.3 (16.0) |
| | | | 300 (2) | 87.5 | 75.4 (13.2)* |

*Significant difference from 300 mg/kg ethanol administered alone (Student $t$-test of means; $p<0.04$).

The procedure employed to train the rats to discriminate between ethanol and saline has already been described in detail [29]. In brief, these animals were first trained to lever press for food (45 mg Noyes pellets) reinforcement on a fixed ratio 10 (FR 10) schedule. Throughout this training, the animals received daily intraperitoneal (IP) injections of saline 15 min prior to being placed into the operant chamber. The animals were then trained to discriminate 600 mg/kg of ethanol (10% v/v) administered IP in normal saline. For half the animals, responding on the left lever was reinforced after administration of drug, whereas for the other half, responding on the right lever was reinforced following drug administration. Responses on the opposite levers were reinforced after saline administration. Training criterion was reached when the animal selected the appropriate lever, according to the drug (or non-drug) state imposed, on 8 out of 10 consecutive sessions.

*Dose-Response Studies*

After the rats had attained the discriminative training criterion, testing and training sessions of 15 min duration with alternating administrations of 600 mg/kg ethanol or saline were continued on Mondays, Wednesdays and Fridays. The procedure was meant to insure and maintain discrimination to the training drug conditions. On Tuesdays and Thursdays, challenge compounds consisting of doses of ethanol different from the 600 mg/kg used in training, were administered 15 min before placing the animals into the operant chamber. During these sessions, the animals were allowed to lever press, in extinction, until 10 responses were made on either lever, and were then returned to their home cages.

*Pretreatment With Apomorphine*

Once the dose-response relationship with 300 and 450 mg/kg ethanol doses was completed, challenge days (Tuesdays and Thursdays) were used to test the effects of apomorphine upon discrimination performance with saline and all doses of ethanol. Thus, 0.16 mg/kg of freshly-prepared apomorphine hydrobromide (as base) was administered IP 10 mi prior to a second IP Injection of saline or one of the 3 ethanol doses and, 15 min later, the rat was placed into the operant chamber until it had made 10 responses on either of the two levers. Each co-administration day was preceded by both a saline and a 600 mg/kg ethanol maintenance session to insure discriminative performance to the training conditions. The animal was immediately removed upon making 10 responses upon either lever and the quantal and quantitative measurements were recorded.

*Measurements and Statistical Treatment*

The lever pressed 10 times first was designated as the "selected" lever. The percentage of rats selecting the lever appropriate for the training drug constituted the quantal measurement of discrimination. In addition, the total number of lever presses on both levers, made before 10 presses on either lever were counted, constitutes the quantitative measurement, i.e., the number of responses on the "ethanol-correct" lever, divided by total responses made prior to 10 responses, times 100. The advantages in using both measurements have been discussed by Stolerman and D'Mello [31]. The quantal data for the dose-response experiments were analyzed by the method of Litchfield and Wilcoxon [21] which employs probit vs. log-dose effects and generates ED50's and tests for parallelism. Quantitative measurements were analyzed by application of a two-tailed Student's $t$-test of means.

## RESULTS

The quantal and quantitative measurements for the training conditions, i.e., 600 mg/kg ethanol and saline, and lower ethanol doses, with and without apomorphine pretreatment, appear in Table 1. The 600 mg/kg training dose of ethanol produced 97.5% of first choice responses (selected lever) upon the ethanol-correct lever, whereas saline administration produced 6.8% of quantal responses upon this lever (or 93.2% of selected lever responses upon the saline-correct lever). Administration of decreasing doses of ethanol resulted in decreasing discriminative performance both in terms of quantal and quantitative measurements. Application of the method of Litchfield and Wilcoxon [21] to the quantal data indicates an ED50 of 372 mg/kg (95% confidence limits: 310–446 mg/kg).

Pretreatment with 0.16 mg/kg apomorphine produced an increased quantal discrimination at all ethanol doses. The ED50 for the co-administration dose-response curve was 232 mg/kg (95% confidence limits: 177–304 mg/kg) and analysis of both curves [21] indicate that they are parallel within statistical limits and that the combination produced a significantly ($p<0.05$; PR=1.6>FPR 1.4) more potent effect upon dis-

criminative performance. Lastly, the quantitative measurement of 0.16 mg/kg apomorphine and 300 mg/kg ethanol was significantly ($p<0.04$) greater than that of ethanol administered alone.

## DISCUSSION

Pretreatment of rats trained to discriminate ethanol from saline with apomorphine produced a significant shift to the left of the dose-response curve. Thus, the co-administration of this direct dopaminergic agonist produced a significant increase in the rats' ability to differentially respond to the interoceptive cue produced by various doses of ethanol. A possible link between the behavioral effects of ethanol and its ability to affect brain dopamine levels has been reported. Neurochemical studies indicate that ethanol, or its metabolite acetaldehyde, produces an increase in turnover of brain dopamine [7,18], alters its uptake and release [12] and reduces its endogenous concentration [9]. Based upon indirect evidence, enhanced dopaminergic neuronal activity was reported to enhance ethanol depression in mice [6] and increase intoxication in humans. [1].

Apomorphine, an agent which directly stimulates dopaminergic receptors without affecting noradrenergic receptors [3], has been reported to reduce craving and tension in alcoholics [5,8]. Furthermore, postethanol ingestion of non-emetic doses of apomorphine increased subjective inebriation in moderate drinkers without altering blood ethanol levels or the rate of blood alcohol decline [2]. This increased degree of subjective intoxication after apomorphine treatment may be viewed as responsible for the decrease in craving for the drug in alcoholics.

The parallelism of the ethanol and apomorphine + ethanol dose-response curves seen in the present study would suggest that the drugs are acting via a common site and/or mechanism of action [20]. The 0.16 mg/kg apomorphine dose has been reported to control discriminative responding without producing malaise or behavioral disruption in rats by affecting post-synaptic dopaminergic receptors [27] and this, further, suggests that part of ethanol's discriminative properties may involve central dopaminergic activity [19]. Acute administration of ethanol has repeatedly been reported to activate dopaminergic neurons [7, 9, 18] and this has led to speculation that the dopaminergic neuron systems are involved in expression of the effects of acute doses of ethanol [19]. In addition, recent interest concerning the mechanism of ethanol action in the central nervous system has focused upon the tetrahydroisoquinoline alkaloid salsolinol, formed by the condensation of the ethanol metabolite acetaldehyde with dopamine in the brain. The formation of this simple alkaloid after ethanol catabolism has led to the speculation that salsolinol may play a role in the craving for, and addiction to, ethanol [10]. Indeed, salsolinol has been detected in human urine [22,25] and rat brain [10] and it has been reported to produce ethanol-like discrimination in a paradigm identical to that used in this report [26]. Further studies are necessary to elucidate the importance and role of dopaminergic receptors in mediating apomorphine-ethanol interactions and the involvement of these receptors in mediating or modulating ethanol intoxication.

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