Pharmacology Biochemistry & Behavior, Vol 24, pp 769–771, 1986 © Ankho International Inc Printed in the U S A 0091-3057/86 $3 00 + 00

BRIEF COMMUNICATION

Nicotine-Induced Potentiation of Ethanol Discrimination

STEVEN A SIGNS AND MARTIN D. SCHECHTER[1]

Department of Pharmacology, Northeastern Ohio Universities College of Medicine, Rootstown, OH 44272

Received 22 July 1985

SIGNS, S A AND M D SCHECHTER *Nicotine-induced potentiation of ethanol discrimination* PHARMACOL BIOCHEM BEHAV **24**(3) 769–771, 1986 — Rats were trained in a 2-lever, food-motivated operant task to discriminate intraperitoneal administration of ethanol (600 mg/kg) from vehicle Dose-response curves for the ethanol cue were analyzed before and after pre-treatment of rats with intraperitoneal doses of 0 4 mg/kg or 0 2 mg/kg nicotine Results demonstrate that nicotine potentiates ethanol-appropriate responding in test sessions The results are discussed in light of the recognized correlation between smoking and alcohol intake

Drug discrimination Ethanol Nicotine Smoking behavior Rats

SEVERAL investigations have observed a correlation between ethanol intake and cigarette smoking Alcoholics have been reported to smoke more cigarettes than non-alcoholics [9] and ethanol consumption has been shown to increase tobacco use in man [3] It has been suggested that nicotine derived from cigarette smoke and ethanol may jointly influence poly-synaptic transmission in the central nervous system [8]

Drug discrimination paradigms in animals are behavioral assays that assess "subjectively" experienced effects of drugs Typically, an animal is trained to make a differential response on the basis of a drug-induced interoceptive cue In this paradigm, the drug becomes the discriminative stimulus and the differential behavioral response depends on the perceived drug state The purpose of the present experiment was to train rats to discriminate the interoceptive cue produced by ethanol and to establish a dose-responsive discrimination to that drug In addition, the effect of co-administration of nicotine would be tested upon this dose-response discrimination to ascertain the possible effect of nicotine upon the ethanol-induced drug state

METHOD

The subjects were 6 experimentally-naive female ARS/Sprague-Dawley rats (Zivic-Miller Laboratories, Allison Park, PA) weighing 80±5% of their expected free-feeding weights Water was available ad lib. The experimental space consisted of a 6 standard rodent operant test cages (Lafayette Instrument Corp , Lafayette, IN) equipped with two operant levers and a food receptacle at an equal distance between the two levers. Solid-state equipment (LVB Corp , Lehigh Valley, PA) used to control and record the sessions was located in an adjacent room

The procedure used to train rats to discriminate between ethanol and vehicle has been described in detail elsewhere [13] In brief, daily discrimination training started after initial shaping to lever press on both levers on a food-reinforced fixed-ratio of ten (FR 10) Ten min prior to placement into the test chamber, the rats were injected intraperitoneally (IP) with either 600 mg/kg ethanol (10% v/v in distilled water) or an equal volume (5 ml/kg) of distilled deionized water (vehicle) Depending on whether the rat was administered ethanol or water, it obtained reinforcement by pressing either the ethanol lever (EL) or the vehicle lever (VL), respectively After every tenth press (FR 10) on the appropriate lever, a 45 mg Noyes pellet was delivered through the food receptacle Responses on the incorrect lever were recorded, but produced no programmed consequence

To randomize for possible position preference, lever assignments were ethanol left, vehicle right for half of the rats and ethanol right, vehicle left for the other half These assignments remained constant throughout the experimentation The number of responses made on either lever before 10 responses were made on the correct lever was recorded This number reflects the accuracy of the rats' lever selection

Each rat was run once each weekday for a daily session of

[1]Requests for reprints should be addressed to Martin D Schechter

TABLE 1

DOSE-RESPONSE RELATIONSHIP OF DISCRIMINATION OF ETHANOL WITH AND WITHOUT PRETREATMENT WITH NICOTINE

Ethanol Dose (mg/kg)	Ethanol		0 4 mg/kg Nicotine + Ethanol		0 2 mg/kg Nicotine + Ethanol	
	Quantal	Quantitative	Quantal	Quantitative (±SD)	Quantal	Quantitative (±SD)
900	100 0	94 4 (1 2)	ND*	ND	ND	ND
600	95 1	85 4 (6 6)	100 0	85 5 (7 7)	91 2	80 2 (7 6)
450	66 7	74 1 (13 7)	91 7	79 6 (7 4)	83 3	73 6 (7 4)
300	50 0	52 7 (27 7)	91 7	79 3 (1 0)†	75 0	67 9 (4 2)
150	16 7	26 8 (14 2)	75 0	61 7 (3 8)†	25 0	37 5 (4 3)
75	16 7	26 5 (1 8)	33 3	31 3 (3 6)	25 0	45 2 (2 1)
(vehicle) 00	10 7	16 3 (13 0)	16 7	20 9 (10 4)	16 7	37 4 (0 4)
ED50 (95% conf limit)	223 4 (139 7–357 3)		99 5 (50 8–194 8)		181 8 (107 2–308 5)	
Parallelism (calculated t)		N S ‡ (0 253)		N S ‡ (0 772)		

*N D =not determined

†Significant difference ($p<0$ 05) from quantitative measurement with ethanol administered alone (Student t-test of means)

‡N S =not significant when compared to dose-response curve of ethanol alone, critical t (2 365)>calculated t

15 min duration Ethanol (E) or vehicle (V) injections were given according to a daily two-week pseudo-random sequence E-V-V-E-E and V-E-E-V-V The training criterion was reached when the animals made no more than two incorrect responses prior to 10 correct responses during the course of 8 of 10 consecutive training sessions

Once all rats attained the training criterion, training sessions of 15 min duration, with alternating administrations of 600 mg/kg ethanol and vehicle, were continued on Mondays, Wednesdays and Fridays This procedure endeavored to ensure and maintain behavioral discrimination to the trained drug It was intended that if a rat was observed to make more than two incorrect lever selections in 10 consecutive maintenance sessions, the data on that rat's performance would be deleted from the results This, however, did not occur On Tuesdays and Thursdays, the rats were injected with either 75, 150, 300, 450, or 900 mg/kg ethanol, and 10 minutes later they were placed into the experimental chamber and were allowed to lever press, without reinforcement, until 10 responses were made on either of the two levers When 10 responses were made on either lever, the animal was immediately removed from the experimental chamber to preclude training at an ethanol dose other than that to which the animals were trained, i e , 600 mg/kg. The lever first pressed 10 times was designated as the "selected" lever Each ethanol dose was administered in a random order on 2 occasions with each test session preceded by one vehicle and one 600 mg/kg ethanol maintenance session In this way, the animals' experience on days preceding test days was counterbalanced with respect to any possible after-effects that may have been produced by the training conditions

Nicotine tartrate (ICN Laboratories, Cleveland, OH) was prepared fresh in distilled deionized water for interperitoneal injection The dose of nicotine was calculated as free base and prepared to yield a volume of 1 ml vehicle/kg body weight Nicotine was administered at an intial dose (0 4 mg/kg, IP) previously shown capable of controlling discrimination in a similar operant task [11] In accordance with that study, nicotine was administered 15 minutes prior to task performance, i e , 5 minutes prior to the administration of the various doses of ethanol Dose-response relationships for ethanol after pre-treatment with nicotine were subsequently investigated by halving the nicotine dose to 0 2 mg/kg

The percentage of rats "selecting" the lever appropriate for the training drug was the quantal measurement for discrimination Quantal data are presented as percent correct first choice responses on the ethanol-correct lever The quantal data were subjected to the Litchfield-Wilcoxon procedure [7] that employs probit vs log-dose measurements This computer generated analysis [16] yielded an ED50 for each drug or drug combination and tests for parallelism and potency differences between drugs In addition to the quantal measurement, the total number of lever presses on both levers, made before 10 lever presses on either lever, constituted the quantitative measurement This measurement is derived by dividing the number of responses on the ethanol-appropriate lever by the total responses made on both levers prior to fulfillment of the "selection" criterion The quantitative data has the advantage of allowing the determination of statistically significant differences between drug treatments by application of t-test analysis [15]

RESULTS

The six rats required a mean (±SD) of 13 7 (±9 2) sessions to reach the first of ten consecutive sessions to attain criterion performance [10] with a range of 3 to 25 sessions Thus, by 35 sessions with 600 mg/kg ethanol and vehicle administrations, in a random order, all rats attained the training criterion Maintenance trials with the training dose (600 mg/kg) of ethanol, interspersed between test trials, resulted in 95 1% of all first choices upon the ethanol-correct lever (Table 1), whereas vehicle resulted in 10 7% of "selected" lever responses upon this lever (or 89 3% upon the vehicle-

correct lever) Administration of 900 mg/kg ethanol on two trials resulted in errorless discrimination and decreasing doses of ethanol produced decreased discriminative performance both in terms of quantal and quantitative measurements The ED50 (and 95% confidence limits) of ethanol was calculated [7] to be 223 4 (139 7–357 3) mg/kg

Pre-treatment with 0 4 mg/kg nicotine prior to doses of ethanol from 75–600 mg/kg and vehicle produced increased discrimination at every dose of ethanol The quantitative measurement after 0 4 mg/kg nicotine and 300 mg/kg ethanol was significantly higher ($t=3$ 36, $p<0$ 05) than after 300 mg/kg ethanol administered alone Likewise, 0 4 mg/kg nicotine pre-treatment prior to 150 mg/kg ethanol produced significantly greater discrimination ($t=3$ 80, $p<0$ 05) than 150 mg/kg ethanol administered alone The ED50 of the 0 4 mg/kg nicotine pre-treated ethanol dose-response relationship was calculated to be 99 5 (50 8–194 8) mg/kg and this dose-response line was parallel, within 95% confidence limits [7], to that of the ethanol dose-response line Similarly, pre-treatment with 0 2 mg/kg nicotine prior to all (75–600 mg/kg) ethanol doses produced a linear dose-response curve with an ED50=181 8 (107 2–308 5) mg/kg which was parallel to the ethanol dose-response line (Table 1)

DISCUSSION

The results of the present study demonstrate that nicotine potentiates the interoceptive cue produced by various doses of ethanol The parallel nature of the dose-response curves suggests that the potentiation of ethanol by nicotine is produced by a similar mechanism and/or site of action that elicits the interoceptive cue produced by ethanol [6] However, the exact mechanism responsible for the potentiation of the ethanol cue by nicotine is unclear

Changes in ethanol perception, in human subjects, due to nicotine have been described and have been found not to be due to any significant change in blood alcohol concentration, suggesting that the observed ethanol-tobacco interaction occurs at the level of the CNS [5] In animal studies, the chronic administration of nicotine (5 mg/kg/day, subcutaneously) for three weeks was reported to potentiate the duration of ethanol hypnosis in mice [2] This effect was shown to be independent of any influences on blood-brain barrier or liver microsomal enzyme activity since nicotine did not potentiate barbital hypnosis

The potentiation of an ethanol-induced discriminative cue by a co-administered drug is not a new phenomenon Schechter [14] demonstrated that a post-synaptic activating dose of the dopamine agonist apomorphine potentiated the ethanol cue in rats Curve parallelism indicated that apomorphine accomplished this effect via a mechanism similar to that responsible for the ethanol cue itself, suggesting a role for dopaminergic pathways in this effect Indeed, nicotine has been shown to directly affect dopaminergic neurons [12] and ethanol has been shown to have distinct effects upon dopaminergic neurons [4]

The high degree of correlation between alcoholic beverage consumption and tobacco smoking suggests a commonality of pleasure seeking behavior This study has demonstrated that the primary active ingredient in cigarette smoke, nicotine, acts to potentiate the centrally-mediated interoceptive ethanol cue The mechanism of this effect may be located at a number of sites influenced by nicotine including central dopaminergic, adrenergic, cholinergic, or serotonergic and/or peptidergic systems [1] Further research efforts along these avenues are necessary for the mechanistic elucidation of the nicotine-ethanol interaction

REFERENCES

1 Balfour, D J K The effects of nicotine on brain neurotransmitter systems *Pharmacol Ther* **16:** 269–282, 1982

2 Bhagat, B , T Bayer and C Lind Effects of chronic administration of nicotine of drug induced hypnosis in mice *Psychopharmacologia* **21·** 287–293 1971

3 Griffiths, R R , G E Bigelow and I Liebson Facilitation of human tobacco self-administration by ethanol A behavioral analysis *J Exp Anal Behav* **25·** 279–292, 1976

4 Hunt, W A Neurotransmitter function in the basal ganglia after acute and chronic ethanol treatment *Fed Proc* **40** 2077–2081, 1981

5 Knott, V J and P H Venables EEG alpha correlates of alcohol consumption in smokers and non-smokers *J Stud Alcohol* **40** 247–257, 1979

6 Levine, R R *Pharmacology Drug Actions and Reactions*, second edition Boston Little, Brown, and Co , 1978, pp 169–209

7 Litchfield, J T and F Wilcoxon A simplified method of evaluating dose-effect experiments *J Pharmacol Exp Ther* **96·** 99–113, 1949

8 Lyon, R J , J E Tong, G Leigh and G Clare The influence of alcohol and tobacco on the components of choice reaction time *J Stud Alcohol* **36** 587–596, 1975

9 Maletzky, B M and J Klotter Smoking and alcoholism *Am J Psychiatry* **131·** 445–447, 1974

10 Overton, D A State-department learning produced by depressant and atropine-like drugs *Psychopharmacologia* **10·** 6–31, 1966

11 Rosecrans, J A and W T Chance Cholinergic and non-cholinergic aspects of the discriminative stimulus properties of nicotine In *Discriminative Stimulus Properties of Drugs*, edited by H Lal New York Plenum Press, 1977, pp 155–185

12 Sakurai, Y , Y Takano, Y Kohjimoto, K Honda and H Kamiya Enhancement of (3H)dopamine release and its (3H)metabolites in rat striatum by nicotinic drugs *Brain Res* **242·** 99–106, 1982

13 Schechter, M D Extended schedule transfer of ethanol discrimination *Pharmacol Biochem Behav* **14.** 23–25, 1981

14 Schechter, M D Apomorphine increases ethanol discrimination *Pharmacol Biochem Behav* **22:** 179–182, 1985

15 Stolerman, I P and G D D'Mello Role of training condition in discrimination of central nervous system stimulants by rats *Psychopharmacology (Berlin)* **73·** 295–303, 1981

16 Tallarida, R J and R B Murray *Manual of Pharmacologic Calculations With Computer Programs* New York Springer-Verlag, 1981