Pharmacology Biochemistry & Behavior, Vol. 23, pp. 759–763, 1985. © Ankho International Inc. Printed in the U.S.A. 0091-3057/85 $3.00 + .00

Intravenous Self-Administration of Pentobarbital and Ethanol in Rats

VICTOR J. DENOBLE,[1] PAUL C. MELE AND JOSEPH H. PORTER

Virginia Commonwealth University, Department of Psychology, 810 W. Franklin Street Richmond, VA 23284

Received 6 December 1984

DENOBLE, V. J., P. C. MELE AND J. H. PORTER. *Intravenous self-administration of pentobarbital and ethanol in rats.* PHARMACOL BIOCHEM BEHAV **23**(5) 759–763, 1985.—Rats provided with unlimited access to intravenous doses of ethanol (30, 60, 90, 180, and 360 mg/kg/infusion) failed to initiate and maintain lever pressing that resulted in ethanol delivery. When pentobarbital (0.5 mg/kg/infusion) was substituted for ethanol, lever pressing increased. There were three indications of the positive reinforcing effects of pentobarbital: (1) a greater number of lever presses occurred when pentobarbital was response-contingent than when saline was available; (2) a greater number of responses were made on the pentobarbital lever than on a control "activity" lever; and (3) systematic changes in lever pressing were a function of pentobarbital dose (0.125, 0.25, 0.5, 1.0, and 2.0 mg/kg/infusion). Sequential substitution of ethanol (30, 90, 360 mg/kg/infusion) for pentobarbital failed to maintain lever pressing. However, access to combinations of ethanol (1, 3, 10, 30, 60 mg/kg/infusion) and a nonreinforcing dose of pentobarbital (0.125 or 0.25 mg/kg/infusion) did maintain lever pressing. As the dose of ethanol increased, the daily number of infusions first increased then decreased. Following a history of self-administration of ethanol-pentobarbital combinations, a retest of ethanol alone (10 or 30 mg/kg/infusions) followed by pentobarbital alone (0.125 or 0.25 mg/kg/infusion) failed to maintain lever pressing.

Self-administration Drug history Pentobarbital Ethanol Drug interactions Positive reinforcers Rat

EXCESSIVE ethanol drinking can be produced in animals by the use of schedule-induced polydipsia. In this procedure excessive drinking occurs when food-deprived animals are given small pellets of food on an intermittent schedule [12,13]. Once the animals are trained to accept ethanol they will show a preference for ethanol even when water is available [27]. While oral self-administration of ethanol has been well documented (for reviews see [21,22]), intravenous self-administration of ethanol has been less extensively studied. In addition, intravenous ethanol self-administration has been more reliably demonstrated in nonhuman primates than in rats [4, 9, 10, 20, 37]. For reasons that are unclear, intravenous ethanol self-administration in rats has been demonstrated only at doses below 5.0 mg/kg/infusion [29] or after repeated forced ethanol infusions of 9.0 to 16.0 g/kg/day [23] or under conditions of schedule-induction [24]. In contrast to ethanol, it has been shown that rats will intravenously self-administer barbiturates under conditions of concurrent non-contingent electric shock presentation [7,8], or after being made physiologically dependent (as cited in [25]) or under conditions of non-physiological dependence [6,25].

Drug self-administration is controlled in part by the subject's history of drug exposure. The rate and pattern of behavior maintained by one drug can be influenced by the history of drug exposure [28, 39, 40]. Previous studies have shown that pentobarbital and ethanol can act synergistically in drug discrimination tests [2]. Ethanol has also been shown to modify other effects of barbiturates, such as an enhanced rate of acquisition of both metabolic and functional tolerance, a potentiation of barbiturate induced sleep time, and an increase in the physical dependence liability of barbiturates [30, 34, 35]. Therefore, the purpose of the present study was first to examine intravenous ethanol self-administration in rats before and after a history of pentobarbital self-administration. A second purpose was to determine if low doses of ethanol alter the effectiveness of pentobarbital in maintaining lever pressing that results in its delivery.

METHOD

Subjects

Six experimentally naive male hooded rats (Blue Spruce Farms) weighing 350 to 400 g were used. Rats were anesthetized with ketamine (70 mg/kg/IM) and sodium pentobarbital (18 mg/kg/IP) and implanted with a venous catheter under aseptic conditions. One end of the catheter (inside diameter, 0.30 mm; outside diameter 0.61 mm) was passed by way of the external jugular vein into the superior vena cava at the level of the right atrium. The distal end of the catheter was passed subcutaneously and out through the skin in the middle of the rat's back. This end of the catheter was connected to a stainless steel back plate, and via protective tubing and swivel joints it was connected to a remote infusion pump. Each rat was allowed seven days recovery in their home cage before being placed in the test chamber. In two rats, catheter patency was not confirmed; therefore, results are being reported for four rats. The general catheterization procedure has been described in detail elsewhere [36].

[1]Requests for reprints should be addressed to Victor J. DeNoble, Ph.D., Ayerst Laboratories Research, CN 8000, Princeton, NJ 08540.

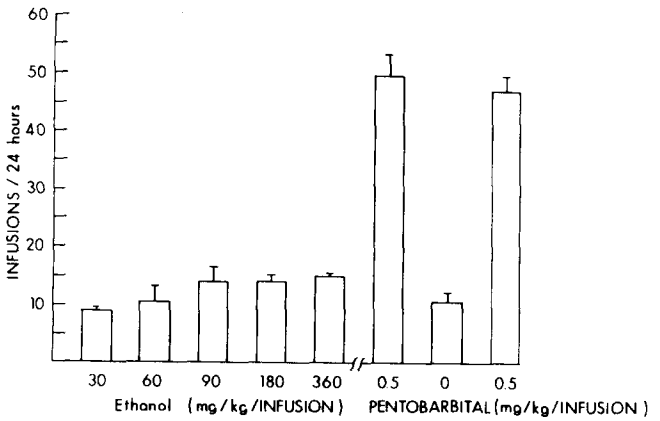

FIG. 1. The number of infusions per 24 hr is shown for ethanol and pentobarbital as a function of dose (mg/kg/infusion). Each bar is a mean of 3–5 consecutive sessions for four rats and the vertical lines show the standard error of the mean.

Apparatus

Four identical operant conditioning chambers (Lehigh Valley Electronics, LVE No. 193-25), each contained in a sound-attenuated cubicle (LVE No. 132-02) were used. Located at one end of the chamber were two levers, a house light and water spout. Presses on the right lever were programmed to activate the infusion pump (Harvard Apparatus Peristaltic pump No. 1201) for 4 sec, delivering an infusion of 200 μl of solution (saline or pentobarbital) into the bloodstream of the rat. To achieve the high ethanol doses (90, 180, 360 mg/kg/infusion) the concentration of ethanol solution was held constant, and adjustments in the volume infused constituted dose variations. The largest volume infused was 500 μl and the longest duration of infusion was 8 sec. Responses on the other lever (control lever) were recorded but had no programmed consequences. The house light (4.5 watts) provided general illumination and blinked at a rate of 10 Hz when the infusion pump was activated. White noise was continuously present and an exhaust fan provided ventilation. Through all experimental sessions, food (20–30 g Purina Rat Chow) and unlimited water were available. Experimental events were scheduled and responses recorded by equipment located in an adjacent room.

Drugs

Physiological saline was used as the vehicle for both ethanol and sodium pentobarbital. The concentration of ethanol was 20% w/v from 95% USP. Doses of pentobarbital are expressed as the salt.

Procedure

Each rat was provided access to the following sequence of ethanol doses (30, 60, 90, 180, and 360 mg/kg/infusion) under a fixed ratio 1 (FR 1) schedule. Each ethanol dose was available 24 hr/day for a minimum of seven days. Next, the rats were given access to the following series of sodium pentobarbital doses under FR 1 schedule: 0.5, saline; 0.5, 2.0, 1.0, 0.5, and 0.125 mg/kg/infusion. Each dose was available for four to seven consecutive sessions until pressing stabilized (3–5 days with no increasing or decreasing trends in the number of infusions per session).

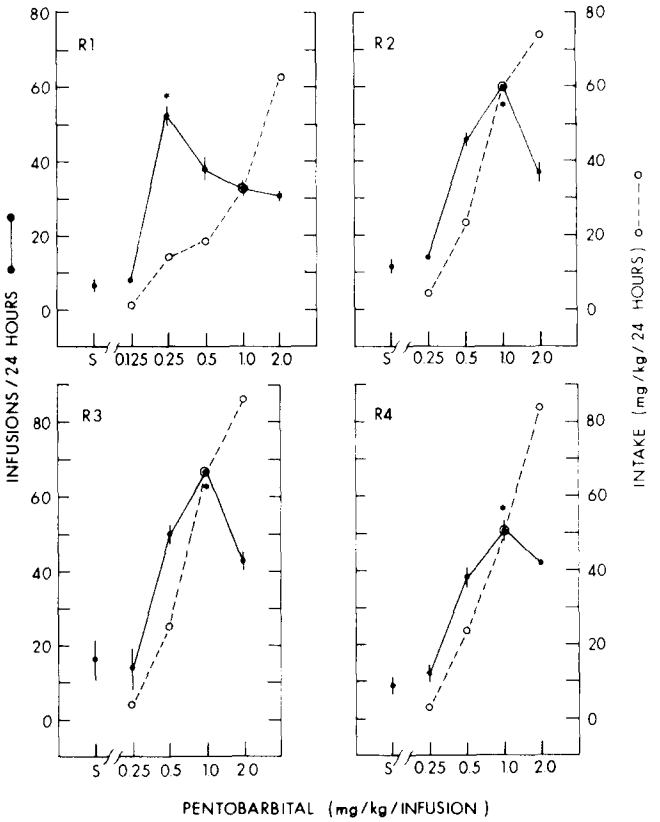

FIG. 2. The number of infusions (solid lines) and the intake in mg/kg/body weight (dashed lines) are shown as a function of pentobarbital dose for individual rats. Points above S indicate lever pressing maintained by saline. Each point is a mean of 3–5 consecutive sessions and the vertical lines show the standard error of the mean. Asterisks indicate a retest of that dose.

After determination of the pentobarbital dose effect function, each rat was provided access to the pentobarbital dose (0.25 or 1.0 mg/kg/infusion) that produced maximal responding, and periods of access to ethanol were alternated with periods of access to pentobarbital as follows: pentobarbital followed by 30 mg/kg/infusion of ethanol, pentobarbital followed by 90 mg//kg/infusion of ethanol, pentobarbital followed by 360 mg/kg/infusion of ethanol, and a pentobarbital retest. Each drug was available for a minimum of five days or until responding stabilized. Following this the rats were provided access to a dose of pentobarbital (0.25 or 0.125 mg/kg/infusion) that did not maintain lever pressing, in combination with ethanol doses of 1.0, 3.0, 10, or 30 mg/kg/infusion. Finally the rats were given access to ethanol alone (10 or 30 mg/kg/infusion) followed by pentobarbital alone (0.125 or 0.25 mg/kg/infusion) for five consecutive sessions each.

Throughout all experimental manipulations, control lever responding was recorded and compared to the rate of responding recorded from the lever resulting in drug infusions.

RESULTS

In all rats unlimited access to intravenous doses of ethanol (30, 60, 90, 180, and 360 mg/kg infusion) failed to ini-

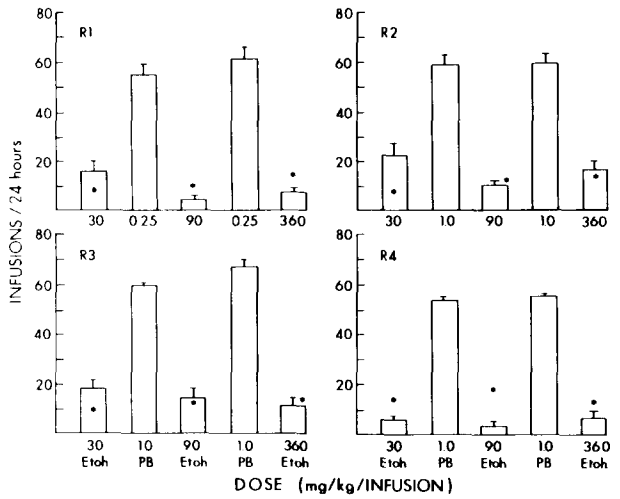

FIG. 3. The number of infusions per 24 hr as a function of ethanol (Etoh) or pentobarbital (PB) availability. Each graph represents an individual rat and each bar represents a mean of 3–5 consecutive sessions. Asterisks indicate the number of infusions for individual rats during the initial ethanol dose effect function. Vertical lines show the standard error of the mean.

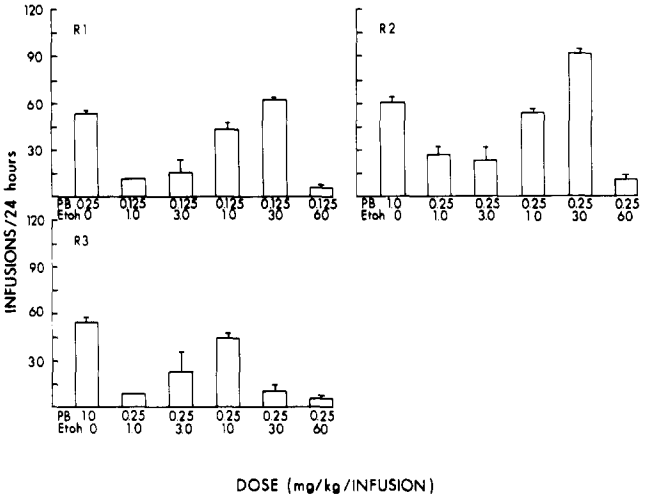

FIG. 4. The number of infusions per 24 hr as function of pentobarbital (PB) ethanol (Etoh) combinations. Each graph represents an individual rat and each bar is a mean of 3–5 consecutive sessions. Vertical lines show the standard error of the mean. Rat R 4 did not complete this part of the experiment.

tiate and maintain lever pressing that resulted in ethanol delivery (Fig. 1). Generally, only 10–15 infusions of ethanol occurred at any dose during a 24 hr session. This is similar to the number of saline infusions which were self-administered during the saline substitution (Fig. 1). In contrast, responding under the FR 1 schedule was initiated and maintained by 0.5 mg/kg/infusion of sodium pentobarbital (Fig. 1) in all rats (49.0 infusions per 24 hr ±7.1 SEM). The number of pentobarbital infusions stabilized in all rats within 13 days. Acquisition patterns were typically 2–4 days of low responding (15–25 infusions/24 hr) followed by gradual increase (2–5 days) to near stable response rates. The within sessions pattern of pentobarbital infusions under the FR 1 scedule was typically a series of closely spaced infusions (4–10 per min), followed by a pause (60–120 min) during which time little or no infusions were taken. Typically, after 5–8 infusions of pentobarbital (0.5 mg/kg) signs of intoxication and ataxia characterized by an inability to stand on the floor grids in the operant chamber were observed.

Substitution of saline for the pentobarbital solution failed to maintain lever pressing (Fig. 1). Saline substitution produced a temporary (12–18 hr) increase in lever pressing, which rapidly declined to 16 infusions (±3.4 SEM) during the following 24 hr session. Observation of the rats during the seven day saline substitution failed to reveal any signs of physiological dependence [38]. When pentobarbital (0.5 mg/kg) was reintroduced, the number of infusions increased to previous levels within 48 hr. When pentobarbital was available, lever pressing occurred almost entirely on the lever, resulting in a pentobarbital infusion. Control lever responses were less than 12% of the total number of responses for all rats.

The effect of varying pentobarbital dose on the number of infusions and session intake (mg/kg of body weight) for individual rats is shown in Fig. 2. As the dose of pentobarbital was increased, the number of infusions first increased then decreased. In contrast, session intake (mg/kg of body weight) increased directly as a function of pentobarbital dose. For three rats (R 2, 3, 4) the pentobarbital dose that maintained maximal responding was 1.0 mg/kg/infusion, whereas for one rat (R 1) 0.25 mg/kg/infusion maintained maximal responding. At the lowest pentobarbital dose tested (0.25 or 0.125 mg/kg/infusion) lever pressing was similar to saline levels. A retest of the dose that produced maximal responding was not different from original values (Fig. 2).

The effect of substituting ethanol for pentobarbital is shown in Fig. 3. Three rats (R 1, 2, 3) showed increases in the number of lever presses at 30 mg/kg/infusions of ethanol compared to the original values, while R 4 showed a decrease. Subsequently, none of the ethanol doses tested maintained lever pressing above either saline levels or above previously determined ethanol levels (asterisks, Fig. 3). When pentobarbital was substituted for ethanol (Fig. 3), lever pressing increased to levels comparable with the original values (Fig. 2).

The effect of adding ethanol to the low, nonreinforcing dose of pentobarbital on the number of infusions is shown in Fig. 4. The first bar in each graph shows that a retest of the dose of pentobarbital that maintained maximal responding did not differ from original values (compare with Fig. 2). The effects of adding successively increasing doses of ethanol to the nonreinforcing dose of pentobarbital (R 1, 0.125; R 2 and R 3, 0.25 mg/kg/infusion) was first an increase in the number of infusions then a decrease. For rats R 1 and R 2 the decrease occurred when 60 mg/kg/infusion of ethanol was presented in combination with pentobarbital, whereas for rat R 3 the decrease occurred at 30 mg/kg/infusion of ethanol. When rats were given access to ethanol alone (R 1 and 2, 30; R 3, 10 mg/kg/infusion) followed by pentobarbital alone (R 1, 0.125; R 2 and R 3, 0.25 mg/kg/infusion) for a minimum of five sessions, lever pressing was not maintained.

DISCUSSION

Access to five doses of ethanol failed to initiate lever pressing that resulted in ethanol delivery. While a number of

studies have reported that monkeys, and to a lesser extent rats, will intravenously self-administer ethanol [9, 10, 20, 32, 37], there have been several reports indicating that one-third to one-half of the animals tested fail to initiate self-administration [1, 31, 37]. The present results with ethanol are in general agreement with other studies, indicating that ethanol does not function reliably as an intravenously delivered reinforcer for rats [6, 17, 18, 23, 29]. In contrast to ethanol, all rats initiated and maintained lever pressing for intravenously delivered pentobarbital. This finding is in agreement with two recent reports of intravenous barbiturate self-administration in nonphysically dependent rats [6,25]. There were three indications of the positive reinforcing effect of pentobarbital: (1) a greater number of lever presses when pentobarbital was response-contingent than when saline was response-contingent; (2) a greater number of responses on the pentobarbital lever than on the control lever; and (3) systematic changes in lever pressing as a function of the pentobarbital dose. In the present study the 1.0 mg/kg dose of pentobarbital maintained the highest number of daily infusions in three of the four rats. This is in agreement with a previous finding showing that a 1.0 mg/kg dose produced the highest abuse liability score in rats using IV self-administration techniques. [6].

Pentobarbital-maintained lever pressing was studied over a range of doses. As the dose of pentobarbital was increased, responding increased then decreased resulting in an inverted V shaped dose-response function (Fig. 2). This general relationship between the magnitude of the reinforcer and frequency of responding found in the present study was similar to that obtained with other reinforcers [11, 15, 26]. In contrast to the inverted V shaped dose-response function, the pentobarbital intake (mg/kg of body weight) increased directly as a function of dose.

Although a history of drug self-administration is not necessary to establish certain drugs as reinforcers, a prior drug self-administration history may increase the probability that certain drugs will maintain behavior. Winger and Woods [37] reported that for some monkeys intravenously delivered ethanol would not initiate responding. However, when intravenous cocaine or methohexital replaced ethanol, responding was initiated and maintained. When ethanol was reintroduced, responding was maintained and did not differ from monkeys that initiated ethanol self-administration without exposure to cocaine or methohexital. Similar results have since been reported [20]. It has also been shown that prior exposure to ethanol by gavage increases the rate of acquisition of ethanol self-administration in rhesus monkeys [10]. In addition, a recent study with rats has shown that both tertiary butanol and ethanol, when substituted for pentobarbital in a discrimination test, will generally result in a pentobarbital choice [3]. Taken together it would suggest that ethanol and pentobarbital share similar interoceptive cues, and exposure to either drug may increase the probability that the other will maintain self-administration behavior. However, in the present study, repeated ethanol substitutions followed by reintroduction of pentobarbital were not sufficient to initiate ethanol maintained lever pressing. The difference in species (rodents vs. nonhuman primates) and barbiturate used (pentobarbital vs. methohexital) may account for the failure of a pentobarbital reinforced history to facilitate ethanol self-administration in the present study.

Interactions between ethanol and barbiturates have been extensively reported in humans and animals [14,35] and have been reported to be both in terms of changes in metabolism [19] and in alterations in sensitivity of the CNS [5]. Using a drug discrimination test, it has been shown that low doses of pentobarbital (5.0 mg/kg) and ethanol (500 mg/kg) administered in combination would result in a drug response, whereas these same doses given alone result in a saline response [2]. The present study extends these findings by showing that the reinforcing properties of pentobarbital are increased in a dose-related fashion by ethanol.

REFERENCES

1. Altschuler, H. L., P. E. Phillips and D. A. Feinhandler. Alteration of ethanol self-administration by Naltrexone. *Life Sci* **26:** 679–688, 1980.
2. Barry, H., III. Classification of drugs according to their discriminable effects in rats. *Fed Proc* **33:** 1814–1824, 1974.
3. Barry, H., III, M. S. McGuire and E. C. Krimmer. Alcohol and meprobamate resemble pentobarbital rather than chlordiazepoxide. In: *Drugs Discrimination: Applications in CNS Pharmacology*, vol VI, edited by F. C. Colparet and J. L. Slangen. Amsterdam: Elsevier, 1982, pp. 219–233.
4. Carney, J. M., M. E. Llewellyn and J. H. Woods. Variable interval responding maintained by intravenous codeine and ethanol injections in the rhesus monkey. *Pharmacol Biochem Behav* **5:** 577–582, 1976.
5. Carney, J. M., J. A. Rosecrans and M. R. Vasko. Barbitone-induced tolerance to the effects of sedative-hypnotics and related compounds on operant behavior in the rat. *Br J Pharmacol* **65:** 185–192, 1979.
6. Collins, R. J., J. R. Weeks, M. M. Cooper, A. I. Good and R. R. Russell. Prediction of abuse liability of drugs using IV self-administration by rats. *Psychopharmacology (Berlin)* **82:** 6–13, 1984.
7. Davis, J. D. and N. E. Miller. Fear and pain: Their effect on self-injection of amobarbital sodium by rats. *Science* **141:** 1286–1287, 1963.
8. Davis, J. D., G. C. Lulenski and N. E. Miller. Comparative studies of barbiturate self-administration. *Int J Addict* **3:** 207–214, 1968.
9. Deneau, G. A., T. Yanagita and M. H. Seevers. Self-administration of psychoactive substances by the monkey. A measure of psychological dependence. *Psychopharmacologia* **16:** 30–48, 1969.
10. DeNoble, V. J. and H. Begleiter. Alcohol self-administration in monkeys (*macaca radiata*): The effect of prior alcohol exposure. *Pharmacol Biochem Behav* **8:** 391–397, 1978.
11. DeNoble, V. J., D. S. Svikis and R. A. Meisch. Orally delivered pentobarbital as a reinforcer for rhesus monkeys with concurrent access to water: Effects of concentration, fixed ratio size, and liquid positions. *Pharmacol Biochem Behav* **16:** 113–117, 1981.
12. Falk, J. L. Production of polydipsia in normal rats by an intermittent food schedule. *Science* **133:** 195–196, 1961.
13. Falk, J. L. Conditions producing psychogenic polydipsia in animals. *Ann NY Acad Sci* **157:** 569–593, 1969.
14. Fraser, H. F., A. Wikler, H. Isbell and N. K. Johnson. Partial equivalence of chronic alcohol and barbiturate intoxication. *Q J Stud Alcohol* **18:** 541–551, 1957.

15. Goldberg, S. R. Comparable behavior maintained under fixed-ratio and second order schedules of food presentation, cocaine injection, or d-amphetamine injection in the squirrel monkey. *J Pharmacol Exp Ther* **186:** 18–30, 1973.
16. Griffiths, R. R., J. V. Brady and L. D. Bradford. Predicting the abuse liability of drugs with animals drug self-administration procedures: Psychomotor stimulants and hallucinogens. In: *Advances in Behavioral Pharmacology*, Vol 2, edited by T. Thompson and P. B. Dews. New York: Academic Press, 1979, pp. 165–208.
17. Grupp, L. A. An investigation of intravenous ethanol self-administration in rats using a fixed ratio schedule of reinforcement. *Physiol Psychol* **9:** 359–363, 1981.
18. Grupp, L. A. and E. Perlanski. Responding under a fixed interval schedule of intravenous ethanol presentation in the rat. *Subst Alcohol Actions Misuse* **6:** 331–336, 1982.
19. Kalant, H., A. E. LeBlanc and R. J. Gibbins. Tolerance to and dependence on some nonopiate psychotropic drugs. *Pharmacol Rev* **23:** 135–191, 1971.
20. Karoly, A. J., G. Winger, F. Ikomi and J. H. Woods. The reinforcing property of ethanol in the rhesus monkey. II. Some variables related to the maintenance of intravenous ethanol-reinforced responding. *Psychopharmacology (Berlin)* **58:** 19–25, 1978.
21. Meisch, R. A. Ethanol self-administration: Infrahuman studies. In: *Advances in Behavioral Pharmacology*, Vol 1, edited by T. Thompson and P. B. Dews. New York: Academic Press, 1977, pp. 35–84.
22. Meisch, R. A. Animals studies of alcohol intake. *Br J Psychiatry* **141:** 113–120, 1982.
23. Numan, R. Multiple exposures to ethanol facilitate intravenous self-administration of ethanol by rats. *Pharmacol Biochem Behav* **15:** 101–108, 1981.
24. Oei, T. P. S. and G. Singer. Effects of a fixed time schedule and body weight on ethanol self-administration. *Pharmacol Biochem Behav* **10:** 767–770, 1979.
25. Pickens, R. D. Muchow and V. J. DeNoble. Methohexital-reinforced responding in rats: Effects of fixed ratio size and injection dose. *J Pharmacol Exp Ther* **216:** 205–209, 1981.
26. Reynolds, R. W. The relationship between stimulation voltage and rate of hypothalamic self-administration in the rat. *J Comp Physiol Psychol* **51:** 193–198, 1958.
27. Samson, H. H. and J. L. Falk. Alteration of fluid preference in ethanol-dependent animals. *J Pharmacol Exp Ther* **190:** 365–370, 1974.
28. Schlichting, U. U., S. R. Goldberg, W. Wuttke and F. Hoffmeister. d-Amphetamine self-administration by rhesus monkeys with different self-administration histories: In: *Proceedings of the European Society for the Study of Drug Toxicity. Excerpa Med Int Congr Ser* **220:** 62–69, 1971.
29. Sinden, J. D. and J. LeMagnen. Parameters of low-dose ethanol intravenous self-administration in the rat. *Pharmacol Biochem Behav* **16:** 181–183, 1982.
30. Smith, C. M. Interaction of drugs of abuse with alcohol. *Ann NY Acad Sci* **281:** 384–392, 1976.
31. Smith, S. G. and W. M. Davis. Intravenous alcohol self-administration in the rat. *Pharmacol Res Commun* **6:** 397–401, 1974.
32. Smith, S. G., T. E. Werner and W. M. Davis. Comparison between intravenous and intragastric alcohol self-administration. *Physiol Psychol* **4:** 91–93, 1976.
33. Smith, S. G., T. E. Werner and W. M. Davis. Intragastric drug self-administration by rats exposed successively to morphine and ethanol. *Drug Alcohol Depend* **7:** 305–310, 1981.
34. Tilstone, W. T. and P. C. Reavy. Interaction of ethanol with amylobarbitone, phenobarbitone, and methaqualone. *Res Commun Chem Pathol Pharmacol* **19:** 233–242, 1978.
35. Wallgren, H. and H. Barry, III. *Actions of Alcohol*. Amsterdam: Elsevier, 1970.
36. Weeks, J. R. Long-term intravenous infusion. In: *Methods in Psychobiology*, Vol 2, edited by R. D. Myers. New York: Academic Press, 1972, pp. 155–167.
37. Winger, G. W. and J. H. Woods. The reinforcing property of ethanol in the rhesus monkey. I. Initiation, maintenance, and termination of intravenous ethanol-reinforced responding. *Ann NY Acad Sci* **215:** 162–174, 1973.
38. Yanagita, T. and S. Takahashi. Dependence liability of several sedative-hypnotic agents evaluated in monkeys. *J Pharmacol Exp Ther* **185:** 307–316, 1970.
39. Young, A. M., S. Herling and J. H. Woods. History of drug exposure as a determinant of drug self-administration. In: *Behavioral Pharmacology of Human Drug Dependence*, edited by T. Thompson and C. E. Johnson. NIDA Research Monograph 37, Rockville, MD, 1981, pp. 75–89.
40. Young, A. M. and J. H. Woods. Maintenance of behavior by ketamine and related compounds in rhesus monkeys with different self-administration histories. *J Pharmacol Exp Ther* **218:** 720–722, 1981.