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Time-Action and Behavioral Effects of Amphetamine, Ethanol, and Acetylmethadol[1]

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DOWNS, D. A. AND M. C. BRAUDE. *Time-action and behavioral effects of amphetamine, ethanol, and acetylmethadol.* PHARMAC. BIOCHEM. BEHAV. 6(6) 671–676, 1977. – As time increased between drug administration and the start of experimental sessions, effects of drugs on food-maintained responding in rhesus monkeys increased to a maximum and then decreased. d-Amphetamine, ethanol, and α-ℓ-acetylmethadol (LAAM) generally decreased high response rates in one component of a chain schedule, while very low response rates in another component were increased reliably only by ethanol. The time of peak LAAM and ethanol concentrations in blood or plasma corresponded with or overlapped the time of maximal behavioral effect, while the time of maximal behavioral effect with d-amphetamine occurred somewhat prior to the time of peak plasma-amphetamine concentration. With d-amphetamine and perhaps with ethanol, effects on operant responding were greater after 30-min pretreatment intervals than after six-hr pretreatment intervals despite higher plasma or blood concentrations at six hours than at 30 min.

d-Amphetamine Ethanol Acetylmethadol Operant behavior Time-action Plasma-amphetamine Acute tolerance Blood-ethanol

QUANTITATIVE and qualitative effects of drugs on operant behavior can be related to a variety of pharmacological and behavioral variables. Baseline response rate, stimulus properties, and experimental history have been shown to interact with drug class and dose to determine the magnitude and/or the direction of behavioral effect (e.g., [10]). Compared to the above variables, time-action relationships have been examined only infrequently in behavioral studies [11,16]. Rather, the relationship between dose and behavioral effect often is evaluated within a fairly narrow time span relative to the total possible course of drug action. In many cases it is not clear whether the overall behavioral effects reported represent the peak effects or some rising or falling levels of drug action. Thus, the purpose of this study was to explore the effects of d-amphetamine, ethanol, and α-ℓ-acetylmethadol (LAAM) on operant responding at various time intervals after drug administration in rhesus monkeys. Blood or plasma concentrations of d-amphetamine, ethanol, LAAM and LAAM metabolites were measured at time intervals which coincided with, or overlapped times of behavioral testing.

METHOD

Animals

Eight experimentally naive male rhesus monkeys *(Macaca mulatta)* weighing from 4.4 to 8.2 kg were used. Each animal was maintained at 80% of its approximate free feeding weight by partial food deprivation. The monkeys were housed in individual home cages with intermittent access to drinking water. Purina® Monkey Biscuits and 1/4 of a fresh orange were fed daily after experimental sessions.

Apparatus

Stainless steel restraint chairs were placed inside plywood or fiberboard cubicles. Each cubicle contained a wall-mounted intelligence panel with two response levers, a food pellet receptacle, and an array of colored lamps. Cubicles were ventilated by exhaust fans, while continuous white noise was delivered through 10 cm diameter speakers to mask extraneous sounds. Experiments were controlled automatically by solid state programming modules; re-

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sponses were recorded on digital counters and on cumulative recorders.

Procedure

Lever pressing was established and maintained under a chain DRO 30 FR 30 schedule of food presentation as in the procedure described by Downs and Woods [3]. Under this schedule, thirty lever presses in the presence of a green light resulted in a single 0.3 g banana-flavored food pellet (FR 30). Upon delivery of each food pellet or after 60 sec of green light illumination, a red light was illuminated. In the presence of the red light, each response delayed the onset of the green light by 30 sec (DRO 30). When 30 sec elapsed without a response in the presence of the red light, the green light was reinstated. A white lamp was illuminated over the food pellet receptacle during each session. Each session was terminated over the fiftieth presentation of the red light. Sessions, which usually lasted from about 40 to 90 min, were conducted daily except weekends. Different pretreatment intervals were accomplished by varying the time between oral dosing and the start of experimental sessions. After dosing, monkeys were returned to their home cages until a few minutes before the scheduled start of a session. Sessions were conducted at approximately the same time each day. All supplemental food was given immediately after each session. Thus, the time between the last supplemental feeding and subsequent oral dosing varied at each different pretreatment interval although all monkeys were fasted for at least 18 hr before dosing.

Venous blood samples were obtained via a polyethylene catheter temporarily inserted into the saphenous vein or by repeated venipuncture of cephalic or saphenous veins. The analyses of blood or plasma concentrations of drugs were conducted on separate occasions from the behavioral testing in the same monkeys. Animals were fasted for 18 hr prior to dosing. Four monkeys were exposed to d-amphetamine and LAAM, while four other monkeys were exposed only to ethanol. Plasma concentrations of d-amphetamine were determined by gas chromatography with electron capture detection according to the method of Anggard *et al.* [1] except that chlorphentermine was used as the internal standard and the extracting solvent was a combination containing 75% hexane and 25% isooctane. Blood-ethanol concentrations were determined by gas chromatography as reported by Jain [8] except that a glass column packed with Porapack A was used, acetone served as the internal standard, and a 100°C oven temperature was maintained throughout. Plasma concentrations of LAAM, norlaam, and dinorlaam were determined by gas chromatography mass spectrometry by Dr. Bryan Finkle, Center for Human Toxicology, University of Utah.

Drugs

Aqueous solutions of d-amphetamine sulfate, ethanol, and α-ℓ-acetylmethadol hydrochloride (LAAM) were administered by gavage at various intervals of time prior to the start of experimental sessions. Deionized water served as the vehicle for all drugs; an isocaloric dextrose solution served as an additional control for ethanol. Doses of d-amphetamine and LAAM are expressed in terms of the salts. Ethanol concentration was never greater than 25% vol./vol. Ethanol and d-amphetamine were given no more frequently than twice weekly. LAAM was given no more often than once weekly.

RESULTS

Amphetamine

d-Amphetamine caused dose- and time-related decreases in response rates in FR components (Fig. 1). Compared to vehicle, d-amphetamine typically caused decreases or no change in response rates in DRO components. Total DRO seconds were essentially unaffected at all doses and pretreatment intervals. With 0.32 mg/kg, the greatest decrease in FR response rate occurred when the drug was administered three hr prior to the start of the session; the 30 min pretreatment interval at that dose produced only slight suppression of FR response rate. When the session was started six hr after dosing with 0.32 mg/kg FR response rate was essentially unaffected.

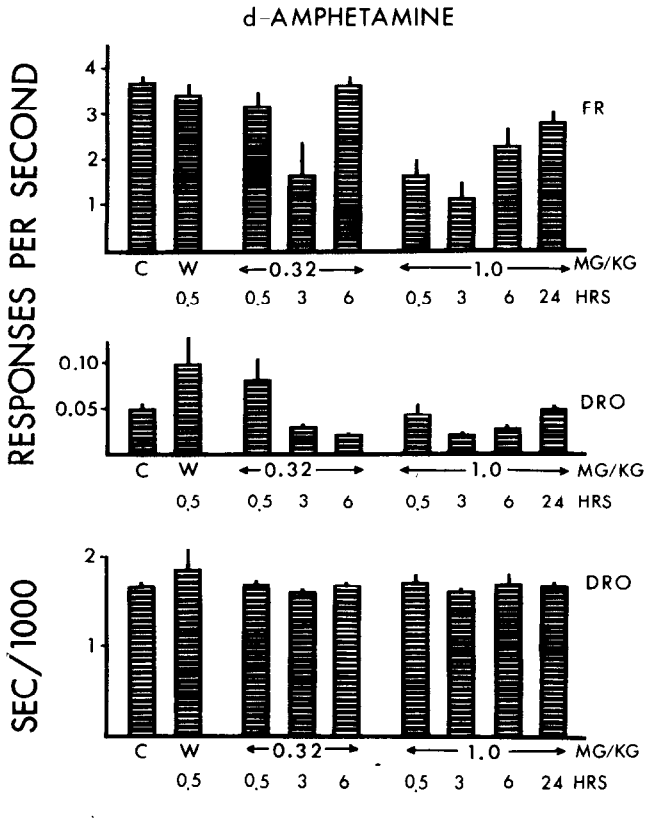

FIG. 1. Dose- and time-effects of orally administered d-amphetamine sulfate (0.32, 1.0 mg/kg) in rhesus monkeys. Each bar represents the mean for four monkeys (6733, 6775, 6751, 6719). Top graph: Response rate in FR 30 components. Center graph: Response rate in DRO 30 components. Bottom graph: Total DRO time per session. The bar at C represents five randomly selected no-treatment control sessions; W represents two tap-water control sessions. All other data are based upon one or two observations per condition in each of the four monkeys. Vertical lines above bars represent one SE.

With 1.0 mg/kg, the greatest FR response rate decrease also was obtained after the three hour pretreatment interval. However, the decrease in FR rate with the 30 min pretreatment interval was almost as great as that obtained

three hr after dosing with 1.0 mg/kg. FR response rate was decreased by about 30% at the six-hr pretreatment interval and a slight decrease in FR rate was evident even 24 hr after dosing with 1.0 mg/kg. In contrast, DRO response rates and total DRO seconds were largely unaffected at any time after dosing with 1.0 mg/kg.

Plasma concentrations of d-amphetamine were determined at various times following 1.0 mg/kg (Fig. 2). Peak plasma d-amphetamine level was obtained at the six hr sample; this was followed by a log-linear decline in plasma d-amphetamine concentration across successive samples through 30 hr. The plasma half-life for d-amphetamine was about ten hr. Comparison of Figs. 1 and 2 reveal that the peak plasma concentration after 1.0 mg/kg did not correspond to the time of the maximal behavioral effect. In fact, greater suppression of FR responding occurred with the thirty-min pretreatment interval than with the six-hr interval (Fig. 1) despite an approximately two-fold lower plasma d-amphetamine concentration at thirty min than at six hr (Fig. 2).

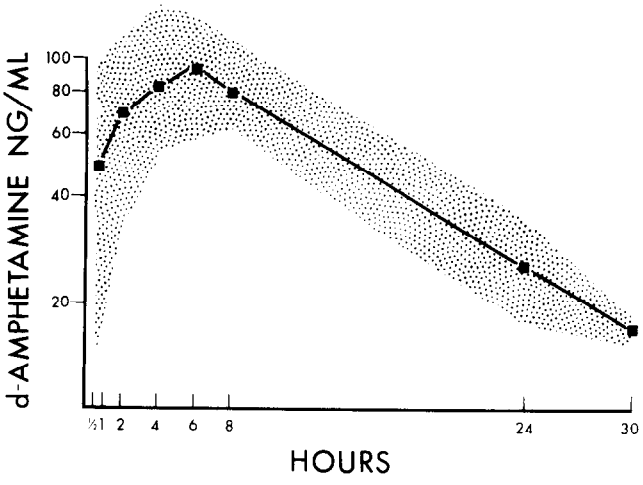

FIG. 2. Plasma d-amphetamine concentration over time (0.5, 2, 4, 6, 8, 24 and 30 hr) following oral administration of 1.0 mg/kg d-amphetamine sulfate. Each point represents the mean of two observations in each of four rhesus monkeys (6733, 6775, 6751, 6719). The shaded area represents the range of all observations.

Ethanol

Like d-amphetamine, ethanol caused dose- and time-related decreases in FR response rates (Fig. 3). The greatest decrease in FR rate was obtained when the session was started two hours after dosing with 3.0 g/kg. Lower doses and other pretreatment intervals resulted in no change or only modest decreases in FR response rates. Dextrose solution, isocaloric to 3.0 g/kg of ethanol, also caused a slight decrease compared to no-treatment control FR rate, but there was no difference between the 30 min and two hr dextrose pretreatment intervals. Unlike d-amphetamine, ethanol caused substantial increases in DRO response rates and total DRO seconds. At the two higher doses, these DRO effects were inversely related to the effects on FR response rates. In contrast, the isocaloric control solution slightly decreased DRO response rate and had no effect on total DRO seconds.

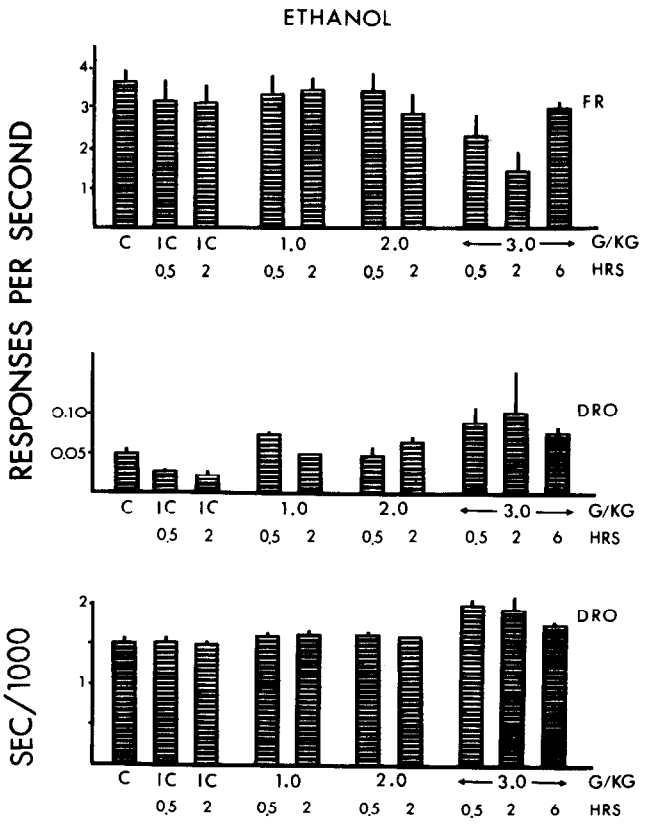

FIG. 3. Dose- and time-effects of orally administered ethanol (1.0, 2.0, 3.0 g/kg) in rhesus monkeys. Each bar represents the mean for four monkeys (466, 436, 6713, 393). Top graph: Response rate in FR 30 components. Center graph: Response rate in DRO 30 components. Bottom graph: Total DRO time per session. The bar at C represents the mean of five randomly selected no-treatment control sessions; IC represents dextrose solution isocaloric to 3.0 g/kg of ethanol. All other data are based upon one or two observations per condition in each of the four monkeys. Vertical lines above bars represent one SE.

Peak blood ethanol concentration (BEC) was obtained at two hr after 3.0 g/kg. This was followed by a roughly linear decline until 13 hr when BEC fell somewhere below 60 mg/100 ml (Fig. 4). This corresponds to an average ethanol disappearance rate of about 20 mg/100 ml/hr. Peak BEC at two hr after 3.0 g/kg corresponded to the time of maximal behavioral effect. However, ethanol was like d-amphetamine in that BEC was substantially lower at 30 min than at six hr, while schedule-controlled responding was more strongly affected at the 30 min pretreatment interval than at six hr.

LAAM

At 2.0 mg/kg, LAAM caused a decrease in FR response rate only at the three-hr pretreatment interval (Fig. 5). A small increase in FR rate occurred with the six-hr pretreatment interval, while there were essentially no effects at thirty min or at twenty-four hr. At the dose tested, LAAM had no effect on DRO performance at any pretreatment interval.

Plasma concentrations of LAAM were detectable only at two hr and at 12 hr (Fig. 6). The highest detectable LAAM

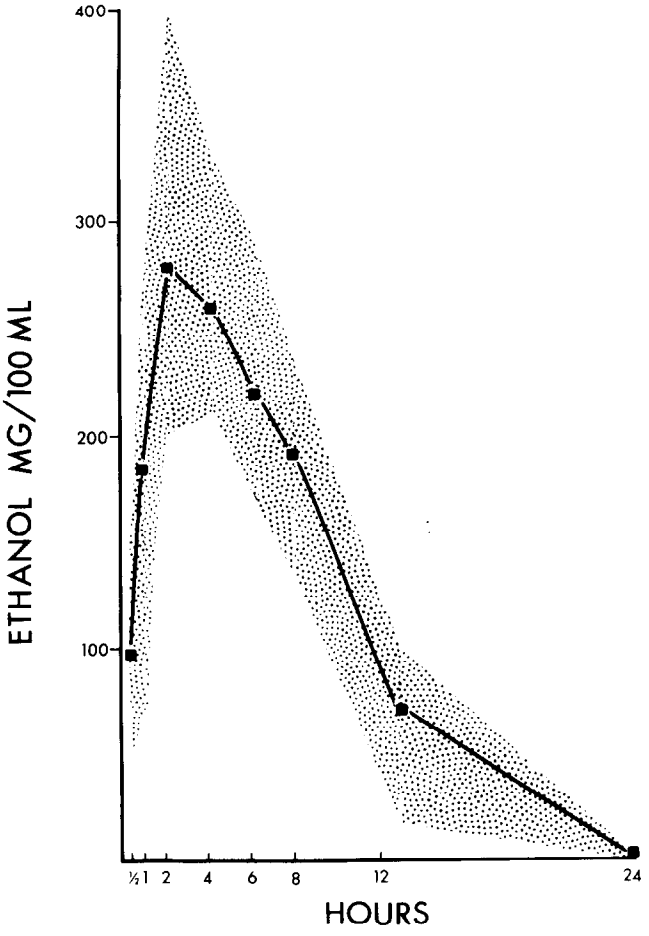

FIG. 4. Blood ethanol concentration over time (0.5, 1, 2, 4, 6, 8, 12 and 24 hr) following oral administration of 3.0 g/kg ethanol (25% vol./vol.). Each point represents the mean of two observations in each of four rhesus monkeys (466, 436, 6713, 393). The shaded area represents the range of all observations.

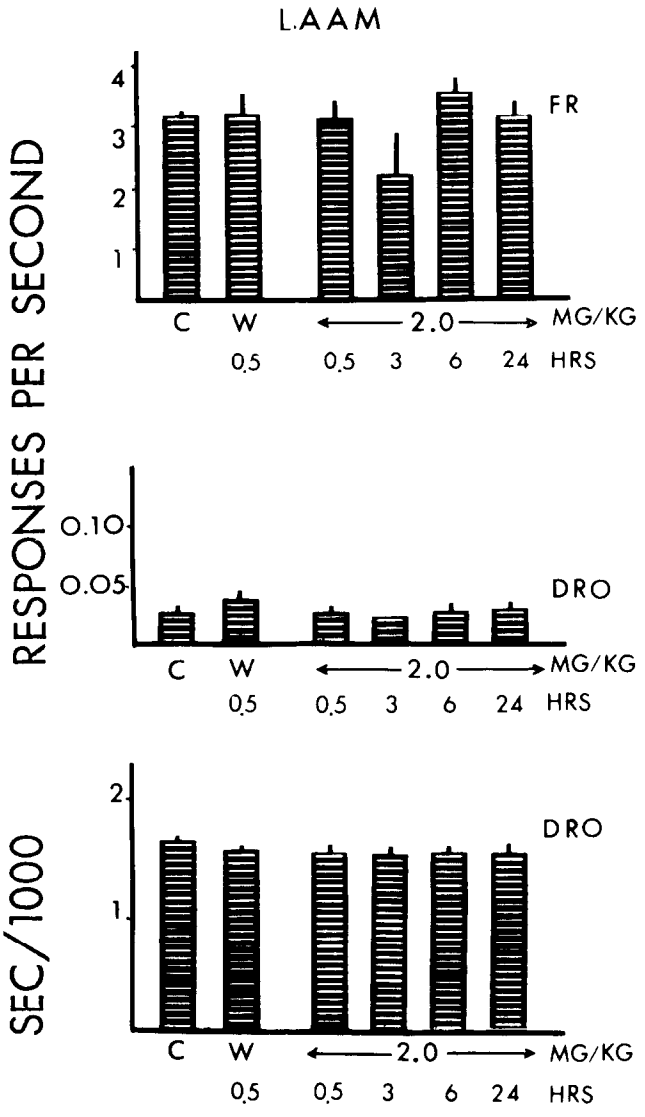

FIG. 5. Time-effects of orally administered α-ℓ-acetylmethadol (LAAM, 2.0 mg/kg) in four rhesus monkeys (6733, 6775, 6751, 6719). Other details as in Fig. 1.

level at two hr occurred near the only behavioral pretreatment interval that produced noteworthy disruption of responding (Fig. 5). Peak plasma levels of norlaam and dinorlaam occurred at one hr after oral administration (Fig. 6). Norlaam declined steadily but was still detectable at 24 hr, while dinorlaam levels remained somewhat more constant from six through 48 hr.

DISCUSSION

Ethanol, d-amphetamine and LAAM each had dose- and/or time-related effects on operant responding in rhesus monkeys. In general, as time increased between dosing and the start of a session, the effect on responding first increased to a maximum and then decreased as time progressed. In some cases, doses of drug which appeared inactive at one pretreatment interval caused pronounced effects on responding when tested sooner or later after dosing. These findings demonstrate that time-action properties of drugs can be evaluated quantitatively using relatively short periods of behavioral observation simply by varying the time between dosing and the start of the observation period. An alternative approach might be to use sessions of schedule-controlled responding which are sufficiently long as to encompass the entire duration of drug effect. However, long session present the potential problems of fatigue, satiation, and relatively fewer animals per experiment.

While all three drugs decreased FR response rate at some point, there was no consistent FR response rate increase at any dose or pretreatment interval. Except with ethanol, there were no consistent increases in DRO response rates or total DRO time. Ethanol did increase the low rates of DRO responding and prolong DRO time substantially at the higher doses. Similar increases in low rates of responding have been reported previously with ethanol in other species and other procedures [2, 6, 14].

The effects of orally administered d-amphetamine in this experiment were similar to those reported for parenterally

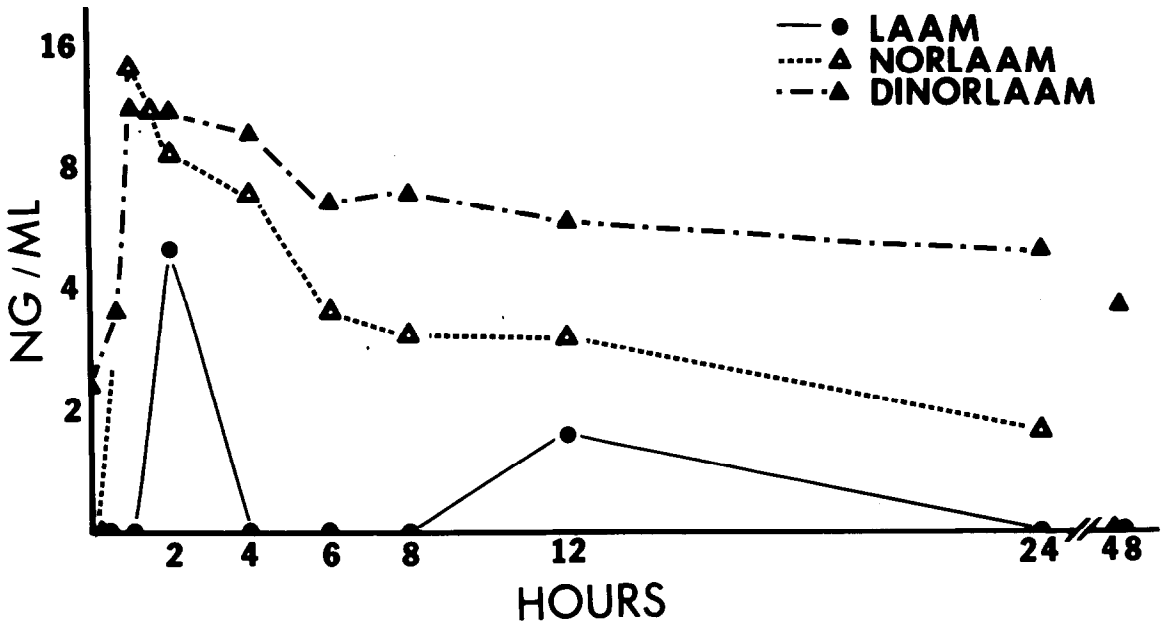

FIG. 6. Plasma concentrations of LAAM, norlaam, and dinorlaam over time (0.5, 1, 1.5, 2, 4, 6, 8, 12, 24 and 48 hr) following oral administration of 2.0 mg/kg of LAAM hydrochloride. Each point represents the mean of a single observation in four rhesus monkeys (6733, 6775, 6751, 6719).

administered amphetamine-like drugs under comparable food presentation schedules in rhesus monkeys [3, 24, 27]. Although amphetamine and related drugs can increase low rates of schedule-controlled responding under some conditions (e.g., [22]), such increases have not been obtained using the chain DRO 30 FR 30 schedule in monkeys (present study, [3, 24, 27]). Similar negative findings concerning increases in low response rates with amphetamine may be attributable to strong stimulus control (e.g., [13]), very low baseline response rates (e.g., [18]), punishment effects (e.g., [5]), or other factors (see [22]).

With LAAM, decreases in FR response rates were observed only at the three-hr pretreatment interval. This was somewhat surprising in view of the long duration of action of this drug in other preparations [9,25]. However, it seems quite likely that higher LAAM doses would cause greater changes in response rates at the longer pretreatment intervals.

In general, the highest plasma levels of LAAM and two active metabolites norlaam and dinorlaam [7, 21, 23] occurred between 30 min and four hr. This overlaps the time of maximal behavioral effect at the three-hr pretreatment interval. Misra *et al.* [20] also have reported that peak plasma levels of LAAM occur between two and six hr after oral dosing with 2.0 mg/kg in rhesus monkeys. It should be noted, however, that Misra *et al.* obtained roughly sevenfold higher peak concentrations of LAAM with detectable levels throughout 48 hr. The reason for the discrepancy between the present results and those of Misra *et al.* is not clear, although it is probably related to the sensitivities of the different analytical methods which were used in the two studies.

Blood ethanol concentrations and disappearance rates obtained in the present study agree with previous reports in rhesus monkeys [4, 19, 28] and in humans [26]. The half-life of d-amphetamine in plasma obtained in monkeys in the present study corresponds to results in humans after oral dosing [12]. Comparison of behavioral effects to these blood or plasma concentrations of d-amphetamine, ethanol, and LAAM revealed interesting relationships. For example, the times of peak blood concentrations of ethanol and LAAM corresponded to or overlapped the times of maximal behavioral effects. With d-amphetamine, however, the time of peak plasma concentration occurred at six hr while maximal behavioral effects were obtained with the three hr pretreatment. Maickel *et al.* [17] reported similar results in rodents in that the times of peak plasma and brain levels of d-amphetamine did not always correspond to the times of maximal effects in a variety of behavioral measures.

With both d-amphetamine and ethanol, effects on operant responding in the present study were greater after 30-min pretreatment intervals than after six-hr pretreatment intervals despite at least two-fold higher plasma or blood concentrations at six hr than at 30 min. Similar findings in rodents have been interpreted as acute tolerance (e.g., [15]). Such an explanation may be appropriate also for the present findings. However, an alternative explanation for the ethanol data may be that blood-ethanol levels were rising rapidly during the session after the 30-min pretreatment; since each session typically lasted about 40 to 45 min and thus actually extended beyond the time of the one-hr sample, it is possible that blood-ethanol levels could have been higher during sessions after 30-min pretreatments than after 6-hr pretreatments. In contrast, such an explanation is clearly inappropriate for the d-amphetamine effect since sessions after 30-min pretreatments always terminated well before peak plasma levels could have been achieved (i.e., between four and eight hr).

In future studies of this type, much shorter behavior sessions may be desirable in order to maximize the

correlation between times of behavioral observation and times of blood samples. Finally, differences between plasma and brain or other tissue concentrations of the drugs were not examined. Thus, it is possible that time-action characteristics of behavioral effects could be predicted more accurately on the basis of drug concentrations in tissues other than plasma.

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