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Interactions Between Depressants (Alcohol-Type) and Stimulants (Amphetamine-Type)[1,2]

R. H. RECH, M. K. VOMACHKA[3] AND D. E. RICKERT[4]

Department of Pharmacology, Michigan State University, East Lansing, MI 48824

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RECH, R. H., M. K. VOMACHKA AND D. E. RICKERT. *Interactions between depressants (alcohol-type) and stimulants (amphetamine-type).* PHARMAC. BIOCHEM. BEHAV. 8(2) 143–151, 1978. – The rotarod disruption in rats by 1.5 g/kg of ethanol was prolonged by combining the depressant with 2 or 8 mg/kg d-amphetamine, but not after combinations with 4 or 6 mg/kg of the stimulant. The combination with 8 mg/kg d-amphetamine also induced a prolonged coma and lethality. Cocaine or methylphenidate in combination form with ethanol also showed prolonged disruption of rotarod performance, but severe depression and lethality were not observed at any dose combination. d-Amphetamine in combination with pentobarbital or diazepam also increased the duration of rotarod impairment. Amphetamine plus methaqualone did not prolong rotarod disruption, but rather showed a trend toward antagonism. These combinations of 8 mg/kg d-amphetamine with depressants other than alcohol did not cause prolonged coma and lethality. Lower doses of ethanol (0.25 and 0.5 g/kg) plus 8 mg/kg d-amphetamine induced a delayed impairment of rotarod performance in rats as well as a comatose state and lethality. Mice showed a similar trend for these interactions between alcohol and d-amphetamine but the influence was much less predictable. Analysis of alcohol levels in rat serum and brain indicated little effect of d-amphetamine on the rate of elimination of ethanol. On the other hand, 1.5 g/kg of ethanol prolonged the d-amphetamine decay from brain and serum. This latter interaction was not observed in rats treated with 8 mg/kg d-amphetamine plus 0.5 g/kg ethanol. Mice treated with 8 mg/kg d-amphetamine plus 2.25 g/kg alcohol showed little trend for changes in rate of elimination of either drug. The behavioral effects of the combination of d-amphetamine and ethanol cannot be explained adequately on the basis of altered pharmacokinetics of either drug.

Drug interactions CNS depressants CSN stimulants Rotarod performance Brain drug levels

FOR many years the interactions between central nervous system stimulants of the amphetamine type and central nervous system depressants of the alcohol or barbiturate type have been considered as antagonistic. The stimulants have been utilized as analeptics in attempts to treat an overdose with one of the depressant drugs [10]. Nevertheless, the utilization of lower dose levels and examination of phenomena other than gross loss of consciousness do not always demonstrate antagonism. The combination of d-amphetamine and amobarbital, in treating obesity, is presumed to allow for the reduction in restlessness and insomnia associated with the stimulant without altering the anorexic influence of d-amphetamine [24]. Rushton and Steinberg [21] showed that this same combination increased Y-maze activity of rats well above the maximum increase with any dose of either drug alone. In an operant procedure with dogs involving food reward, Weiss and Laties [25] demonstrated that amphetamine, ethanol, or pentobarbital increased the number of responses required to achieve each reinforcement. Combining amphetamine with ethanol or pentobarbital caused a substantial increase in the effect over that observed with each drug alone. A more recent article [3] dealt with pigeons in a discriminative operant paradigm using food reward and examined the effects of d-amphetamine and pentobarbital. Both drugs administered alone increased the rate of responding but decreased accuracy; the combination acted synergistically in producing these effects. Rutledge and Kelleher [22] had previously indicated that the interaction between methamphetamine and pentobarbital on key-pecking by pigeons depended on the schedule of food reinforcement. A facilitation of conditioned avoidance acquisition of mice was reported to be greater after combinations of amphetamine and chlordiazepoxide as compared to that after either drug alone [23].

The above brief review does not support the thesis that

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[2] Send reprint requests to: Dr. R. H. Rech, Department of Pharmacology, Michigan State University, East Lansing, MI 48824.

[3] Ms. Vomachka's present address is: University of Kansas Medical School, Department of Physiology, Kansas City, KA 66103.

[4] Dr. Rickert's present address is: C.I.I.T., P.O. Box 12137, Research Triangle Park, NC 27709.

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amphetamine-type stimulants and barbiturate-type depressants generally interact via a physiological antagonism that would tend to cancel out the separate effects of each drug. These antagonisms may operate in reference to gross effects of large doses, i.e., the convulsant activity of amphetamines and the anesthetic properties of central nevous system depressants. The combination of lower doses of these agents may not demonstrate mutually antagonistic properties and, in some cases, appears to enhance an effect that is caused by either agent when administered alone. The purpose of the present study was to explore further the interactions between these classes of drugs, utilizing the rotarod performance of rats. The rotarod test has advantages of simplicity and efficiency in measuring a behavior that demands a reasonable degree of alertness and motor coordination for the animal to complete the task.

METHOD

Female Sprague-Dawley rats weighing from 225 to 3280 g and female Swiss-Webster mice weighing about 25 g were used in this study. The animals were purchased from Spartan Farms, Haslett, MI, and were maintained in animal rooms with controlled temperature (22°C), humidity (45%), and diurnal lighting (light cycle from 7 a.m. to 7 p.m.). Purina Rat Chow and water were available ad lib except on days of testing.

The behavioral test utilized to determine dose-response patterns for behavioral disruption was rotarod performance [19,20]. Training and testing was done between 9 a.m. and 5 p.m. The animals were trained over 3 to 5 trials to walk a rotating cylinder (rotarod) for a period of 180 sec. The cylinder was 5 in. in diameter and 6 in. wide and was rotated at 8 revolutions per min. For the purposes of this investigation the extent of disruption of behavior was quantified by estimating the time required for 50% recovery of rotarod performance. In a few instances this criterion had to be modified to include late decrements in performance during testing when animals had either recovered from or had not demonstrated behavioral impairment at earlier measurements. The general time-course of effects, up to 4 or 5 hr, was determined in groups of 6 to 8 animals which had been treated with various dose combinations of alcohol and/or d-amphetamine. These subjects were observed for gross behavior over the next 24 hr for eventual complete recovery or death.

Other animals were treated in a manner identical to the above and were tested in groups of 6 on the rotarod until various times of sacrifice, when brain and serum samples were collected for drug analysis. Animals were usually sacrificed by decapitation at 5, 15, 30, 90 and 180 min following treatment with ethanol and/or d-amphetamine. For rats, blood was collected in tubes over ice and allowed to clot, and whole brains were removed rapidly and a 33% homogenate was prepared in ice-cold distilled water. For mice, blood was collected by sinus puncture at the inner canthus of the eye in heparin-filled capillary tubes after lightly etherizing the animals. Brains were then removed in the same manner as with rats. Blood and brain tissue from 3 mice were pooled to make up each sample. Ethanol and d-amphetamine levels were quantified by gas chromatographic methods using a Shimadzu 4BMPFE instrument equipped with a flame ionization detector.

Ethanol was measured in serum and supernatants of brain homogenates after centrifugation. Twenty μl aliquots were added to 20 μl of a 0.1% methanol solution. One μl aliquots of the mixture were introduced into the gas chromatograph. The column (2.5 m) was packed with Porapak Q. Injection port and detector temperatures were 300°C and the column temperature was 160°C. Ethanol levels were estimated by the peak height ratio method with methanol as the internal standard. Standard curves were run each day of assay and were linear between 0.05 and 2.5 mg of ethanol per ml of serum or brain supernatant. Recovery of ethanol added to blood or brain homogenates from untreated animals was 100 ± 2%. Acetaldehyde levels were also measured in samples from rats, the assay being accurate to a level of 0.01 mg/ml.

The assay of d-amphetamine was a modification of published methods [13, 16, 18]. Ten μg of methamphetamine was added to 2 ml of serum or brain homogenate as the internal standard. Protein was precipitated by adding 2 ml of 1.2 N perchloric acid, the solution was made alkaline with 5 N NaOH, and the amphetamines extracted into methylene chloride. The resulting organic layer was back-extracted for amphetamines using 1 N HCL and then discarded. The aqueous layer was made basic with NaOH, mixed with 100 μl of methylene chloride (50 μl in assays of mouse tissues), and the layers separated by centrifugation. Three μl aliquots of the methylene chloride layer were introduced into the gas chromatograph. The column (2.5 m) was packed with 10% apiezon-2% KOH. Injection port and flame ionization detector were held at 300°C and the column at 150°C. d-Amphetamine was quantified by the peak height ratio method with methamphetamine as the internal standard. Standard curves for d-amphetamine were linear from 0.5 to 10 μg/ml of serum or brain homogenate extract. Absolute recoveries of the amphetamines varied according to whether they were extracted from water, serum or brain matrices, but the relative recoveries of d-amphetamine and methamphetamine were constant regardless of the source.

Drugs were administered by IP injection with one exception. In one series of rats, ethanol was administered by intragastric intubation as a 30% (w/v) solution. Alcohol for intraperitoneal injection was prepared as a 10% solution for rats and a 40% solution for mice. d-Amphetamine was dissolved in 0.9% saline. For combined injections d-amphetamine was administered just before the alcohol. Rotarod data were analyzed statistically by the Mann-Whitney U test. Data from the chemical analyses were compared by linear regression, computing 95% confidence limits, and Student's t-test [9].

RESULTS

A dose-response pattern for disruption of rotarod performance by various doses of ethanol is indicated in Table 1. One g/kg of ethanol provoked a transient impairment and 1.5 g/kg almost doubled the duration of this effect. On increasing the dose to 2.0 g/kg a much more prolonged impairment was noted, being almost 10 times that of the 1.5 g/kg dose. The 2.5 g/kg dose increased the effect only slightly over the 2.0 g/kg dose, whereas increasing the dose to 3.0 g/kg increased the duration of effect to well over 3 hr.

The effects of d-amphetamine in doses of 2, 4, 6 and 8 mg/kg on rotarod behavior were determined. While there was some decrease in scores from control values, these did not attain a level of 50% at any time, nor were the scores significantly different from controls.

TABLE 1

TIMES TO 50% RECOVERY OF ROTOROD PERFORMANCE AFTER VARIOUS DOSES OF ETHANOL, D-AMPHETAMINE OR THE COMBINATION IN RATS

Treatment	Dose	Duration of Rotarod Impairment (min)
	(EtOH)	
	0.5g/kg	0*
	1.0 g/kg	7.4 ± 2.2†
Ethanol	1.5 g/kg	13.5 ± 6.2
Alone	2.0 g/kg	126.8 ± 17.9
	2.5 g/kg	132.5 ± 21.0
	3.0 g/kg	217.3 ± 46.6
	(d-A)	
d-Amphetamine	2, 4, 6, 8 mg/kg	0*
alone		
	(d-A)	
Ethanol	2 mg/kg	81.6 ± 14.7‡
(1.5 g/kg)	4 mg/kg	6.0 ± 2.6§
plus	6 mg/kg	38.8 ± 16.4
d-Amphetamine	8 mg/kg	−(>300)[c]

*0 denotes no impairment below the 50% level of performance within a 5 hr test period.

†Mean time ± SD for 50% recovery of each group of 6 rats. Time to recovery calculated as described in Methods. Vehicle-treated animals walked the rotarod for 180 sec (cut-off time, expressed as 100%). Only animals that walked for less than 90 sec (less than 50%) were considered significantly impaired, in this and subsequent figures.

‡The time to recovery was significantly longer than that derived from the group treated with ethanol 1.5 g/kg alone ($p<0.05$ by Mann-Whitney U test).

§This measurement was compromised by a later transient decrease in performance below the 50% level at about one hr.

TABLE 2

TIMES TO 50% RECOVERY OF ROTAROD PERFORMANCE AFTER VARIOUS DOSE COMBINATIONS OF ETHANOL WITH COCAINE OR METHYLPHENIDATE IN RATS

Treatment	Dose	Duration of Rotarod Impairment (min)
Ethanol	(EtOH)	
Alone	1.5 g/kg	13.5 ± 6.2
	(coc)	
Cocaine	10, 15, 20, 25	0*
Alone	mg/kg	
	(coc)	
Ethanol	10 mg/kg	41.7 ± 14.9†‡
(1.5 g/kg)	15 mg/kg	50.1 ± 19.2‡
plus	20 mg/kg	59.3 ± 22.6‡§
Cocaine	25 mg/kg	117.5 ± 24.9‡
	(meth)	
Methylphenidate	5, 10, 20, 40	0*
Alone	mg/kg	
	(meth)	
Ethanol	5 mg/kg	15.6 ± 5.2
(1.5 g/kg)	10 mg/kg	77.8 ± 25.6‡
plus	20 mg/kg	45.1 ± 17.3‡
Methylphenidate	40 mg/kg	48.2 ± 16.1‡

*0 denotes no significant impairment below the 50% level of performance within a 5 hr test period.

†Mean time ± SD for 50 % recovery of each group of 6 rats.

‡The time to recovery was significantly longer that that derived from the group treated with ethanol alone ($p<0.05$ by Mann-Whitney U test).

§This measurement was compromised by a later transient decrease in performance at about 2 hr.

Since 1.5 g/kg of ethanol was about an intermediate dose for bringing about rotarod impairment, that dose was chosen for combination with the dose range of d-amphetamine. Table 1 displays the effects of the combination on rotarod performance. Ethanol combined with 2 mg/kg d-amphetamine resulted in a duration of rotarod disruption of 81.6 min, significantly longer than the 13.5 min measured after ethanol alone. After combining the depressant with 4 or 6 mg/kg of d-amphetamine, there was no significant prolongation of the impairment. With the combination of ethanol and 8 mg/kg of the stimulant, the rotarod performance was disrupted for the entire period of 300 min of testing. As these latter rats were observed over the next 24 hr, they were noted to become severely depressed, comatose, and hypothermic, and approximately two-thirds died within this time.

To explore further the interactions of stimulants with ethanol, cocaine and methylphenidate were combined with the depressant (Table 2). Both of the stimulant drugs were found to prolong the disruption from 1.5 g/kg of the depressant. All 4 doses of cocaine tested showed an interaction. Doses of cocaine larger than 25 mg/kg were not tested since pilot studies showed them to elicit marked tremors and preconvulsive twitches. Methylphenidate showed the interaction in all but the lowest dose used (5 mg/kg). Nevertheless, all animals recovered to control levels of rotarod performance within 150 min of drug administration. Furthermore, on following these animals over the next 24 hr, none showed any lasting influences of the drug treatments in terms of central nervous depression, hypothermia, or death.

d-Amphetamine was also combined with pentobarbital or diazepam (Table 3). The doses of the depressant drugs to induce an intermediate duration of rotarod impairment were determined from previous pilot studies. The pattern of interaction of the stimulant with pentobarbital resembled somewhat the interaction with ethanol. That is, 2 or 8 mg/kg of d-amphetamine combined with pentobarbital increased the duration of impairment over pentobarbital alone, but 4 or 6 mg/kg of the stimulant in combination with the depressant provoked the same effect as the depressant drug alone. On the other hand, rotarod performance after the combination with 8 mg/kg of d-amphetamine recovered almost completely by 5 hr. None of these animals exhibited prolonged or severe central nervous depression, hypothermia, or lethality. In the case of interactions of diazepam with the stimulant, the 2 or 4 mg/kg combination showed a prolonged rotarod disruption while the 6 or 8 mg/kg combination did not. The combinations with diazepam in any dosage did not demonstrate the prolonged and severe coma and lethality found after ethanol combined with 8 mg/kg of d-amphetamine. Methaqualone (15 mg/kg) was also combined with the 4 doses of the stimulant and no clear-cut prolongation of the

rotarod disruption was noted (not shown in the tables). In fact, some dose combinations yielded evidence that d-amphetamine can antagonize the rotarod impairment from methaqualone. The impairment was less ($p<0.05$, Mann-Whitney U test) at 15 and 30 min after methaqualone plus 6 mg/kg d-amphetamine and at 30 and 90 min after methaqualone plus 8 mg/kg d-amphetamine as compared to the rotarod performance of rats treated with methaqualone only.

Since the ethanol plus d-amphetamine interactions described above may relate to an altered metabolism of one or both drugs, we decided to measure the blood and brain levels of these agents. Both drugs were quantified after single or combined administration at 5, 15, 30, 90, and 180 min (and in one dose combination at 360 min) after IP injection. Figure 1 illustrates the serum (left-hand panels) and brain (right-hand panels) levels of ethanol (mg/ml; mg/g) at various times after administering 1.5 g/kg of ethanol alone or in combination with 2 and 8 mg/kg d-amphetamine to rats. Since the results of interacting 4 and 6 mg/kg of d-amphetamine with this dose of ethanol are essentially identical to those depicted for 8 mg/kg of the stimulant with ethanol, they have not been shown. Only minor changes in the decay curve for ethanol were observed when ethanol was administered with amphetamine. Regression analyses indicated no significant differences, although Student's t-test at individual points indicated a few minor differences. If anything, the combination results in a slightly faster rate of decay. Analysis

TABLE 3

TIMES TO 50% RECOVERY OF ROTAROD PERFORMANCE AFTER VARIOUS DOSE COMBINATIONS OF d-AMPHETAMINE WITH PENTOBARBITAL OR DIAZEPAM IN RATS

Treatment	Dose	Duration of Rotarod Impairment (min)
	(Pb)	
Pentobarbital Alone	12 mg/kg	137.8 ± 17.9*
	(d-A)	
Pentobarbital	2 mg/kg	194.1 ± 24.3†
(12 mg/kg)	4 mg/kg	119.2 ± 22.7
plus	6 mg/kg	128.4 ± 26.6
d-Amphetamine	8 mg/kg	200.9 ± 27.5†
	(Diaz)	
Diazepam Alone	5 mg/kg	103.0 ± 20.6
	(d-A)	
Diazepam	2 mg/kg	196.6 ± 31.2‡
(5 mg/kg)	4 mg/kg	163.5 ± 25.8‡
plus	6 mg/kg	99.6 ± 22.5
d-Amphetamine	8 mg/kg	114.9 ± 24.7

*Mean time ± SD for 50% recovery of each group of 6 rats.

†Time to recovery after the combination was significantly longer than that derived from the group treated with pentobarbital alone ($p<0.05$ by Mann-Whitney U test).

§Time to recovery after the combination was significantly longer than that derived from the group treated with diazepam alone.

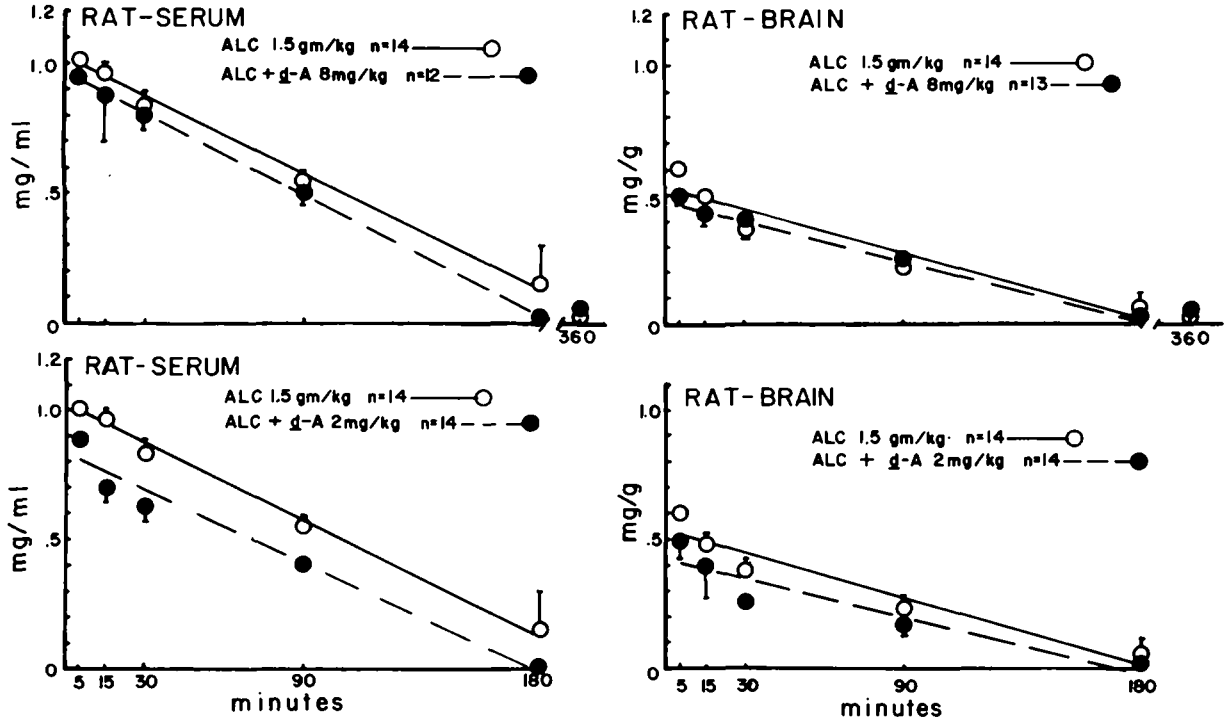

FIG. 1. Time-course study of decay of alcohol levels in serum and brain in rats treated with alcohol 1.5 g/kg alone (0–0) or d-amphetamine 8 or 2 mg/kg plus alcohol 1.5 g/kg (●– – – –●). The drugs were injected IP. The vertical lines through the symbols indicate the standard error of the mean value for each group; where standard errors are not shown, they are smaller than the radius of the symbol, in this and subsequent figures. The slopes represent regression analyses for 5 to 180 min determinations from both brain and serum samples. Values were also determined over 180 min for the combinations of d-amphetamine, 6 mg/kg, plus alcohol, 1.5 g/kg, and d-amphetamine, 4 mg/kg, plus alcohol, 1.5 g/kg, but these are not shown since the plots were essentially identical to those for d-amphetamine, 2 mg/kg, plus alcohol, 1.5 g/kg. Regression analysis indicated no significant differences in alcohol decay as a consequence of being combined with d-amphetamine.

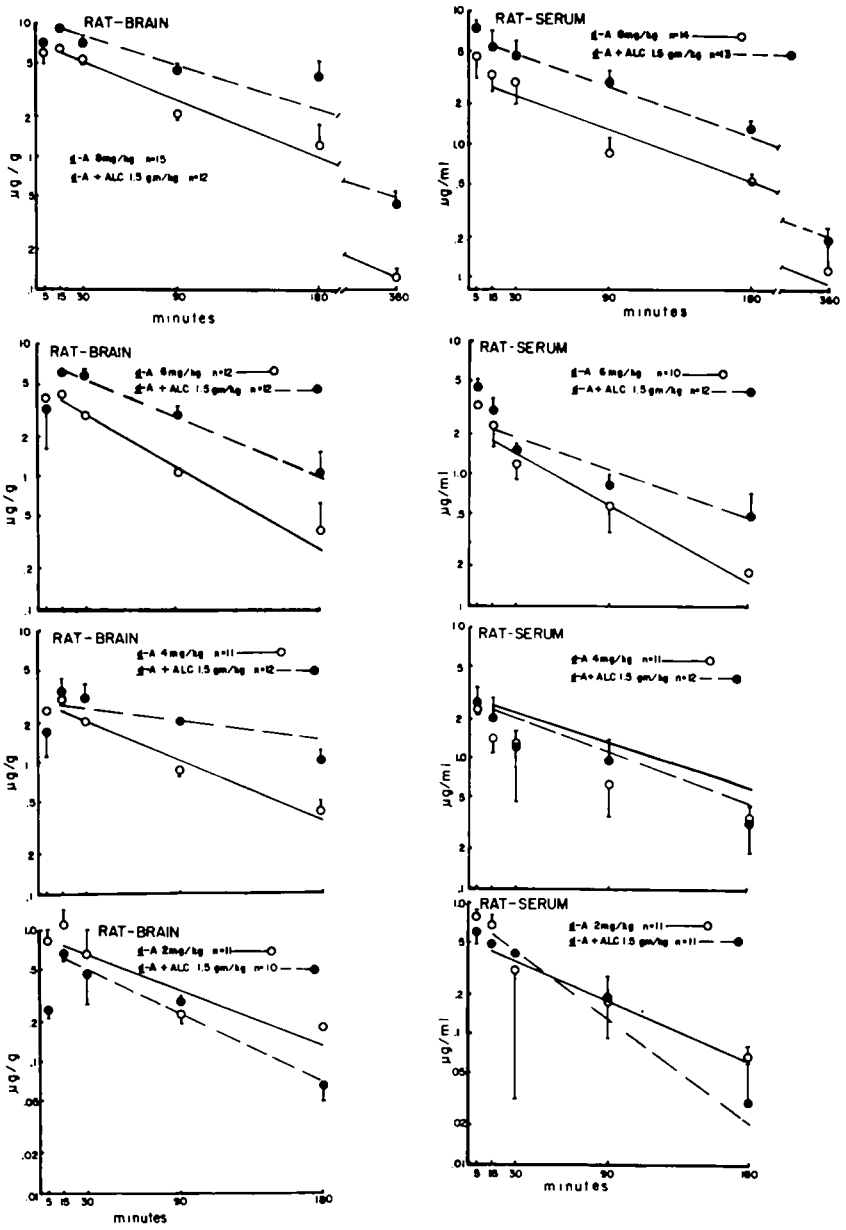

FIG. 2. Time-course of decay of d-amphetamine levels in serum and brain in rats treated with 2, 4, 6, or 8 mg/kg of d-amphetamine alone (0–0) and in animals treated with these doses of d-amphetamine plus alcohol, 1.5 g/kg (●– – – –●). The drugs were injected IP. The slopes represent regression analyses for all values excluding the 5 min determinations. Both serum and brain levels of d-amphetamine were significantly retarded, by regression analysis, after combining 8 mg/kg with alcohol. Brain levels of d-amphetamine were significantly prolonged after combining 6 or 4 mg/kg d-amphetamine with alcohol.

for serum and brain levels of acetaldehyde showed no significant levels at the limit of the assay sensitivity (0.01 mg/ml). This was true for all experiments involving ethanol administration.

Figure 2 depicts the serum (right-hand panels) and brain (left-hand panels) levels of d-amphetamine (μg/g) at various times after administering 2, 4, 6, or 8 mg/kg d-amphetamine alone or in combination with 1.5 g/kg of ethanol to rats. Regression analyses indicated significant differences between d-amphetamine levels in animals administered the stimulant alone and those treated with the combination in the following instances: serum levels of d-amphetamine are greater after the combination involving 8 mg/kg of the stimulant; brain levels are greater after the combination involving 8, 6 and 4 mg/kg d-amphetamine. These differences are based on 95% confidence limits of each slope. The slopes themselves did not differ at any dose level, comparing d-amphetamine alone with the combined administration. Student's *t*-test at individual points also indicated these significant differences. It should be em-

TABLE 4

ROTAROD PERFORMANCE OF AND LETHALITY IN RATS TREATED WITH 8 MG/KG d-AMPHETAMINE IN COMBINATION WITH LOWER DOSES OF ALCOHOL

Drugs, Dose	Scores at Various Times (min) After Ethanol Injection									
	5′	30′	60′	90′	120′	150′	180′	210′	240′	Percent Deaths‡
d-A + Ethanol 0.5 g/kg	126	101	93	58*	50	54	46	52	54	65%
d-A + Ethanol 0.25 g/kg	136	123	89	90	38*	32	30	46	39	50%
d-A + Ethanol 0.1 g/kg	150	134	102	67*	88	76	53	65	62	0%
d-A + Ethanol 0.05 g/kg	171	159	125	97†	92†	93†	98†	96	98	0%

*All scores following this one in this series were significantly lower than control, $p<0.05$. Scores are the mean number of seconds that each group walked the rotarod.

†Scores thus labelled in this series were signifcantly lower than control ($p<0.05$ by Mann-Whitney U test).

‡Percent of animals treated that suffered lethality within 24 hr of completing the rotarod tests.

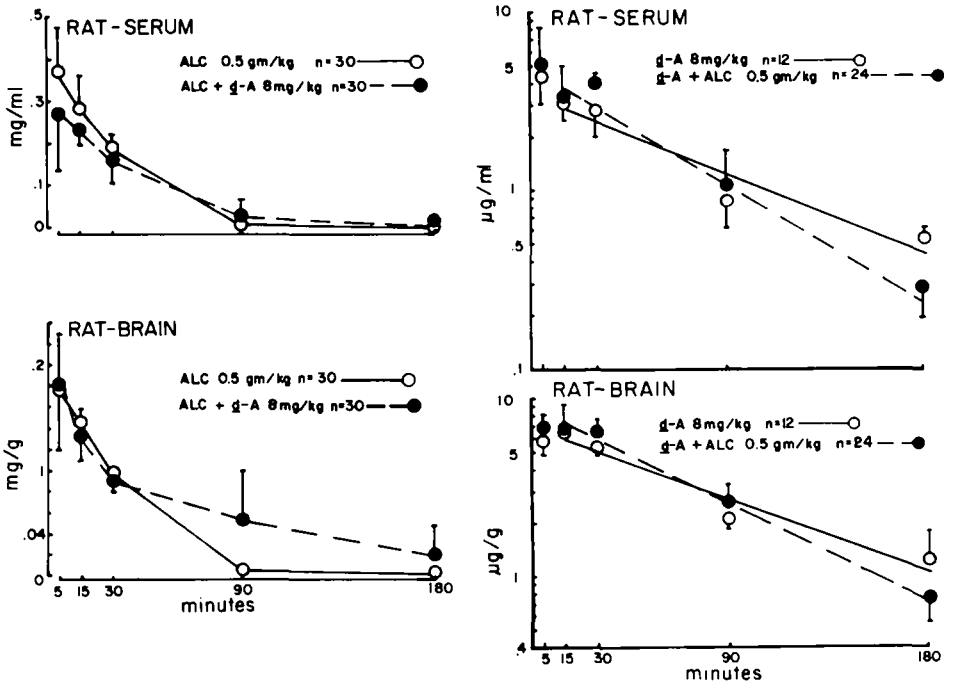

FIG. 3. Time-course of decay of alcohol (left-hand panels) and d-amphetamine (right-hand panels) in serum (upper panels) or brain (lower panels) of rats. Subjects were administered 0.5 g/kg alcohol or 8 mg/kg d-amphetamine alone (0–0), or were injected with the combination (•– – – –•). The drugs were injected IP. The alcohol levels after 0.5 g/kg did not appear to decay linearly, so that curves connecting the points for alcohol levels were fit by eye in the graphs. The decay in alcohol was not altered significantly as a consequence of combining it with d-amphetamine. Regression lines are plotted for all d-amphetamine values, excluding the 5 min determinations. No significant differences were noted between d-amphetamine values derived from rats treated with d-amphetamine alone and those receiving the combination.

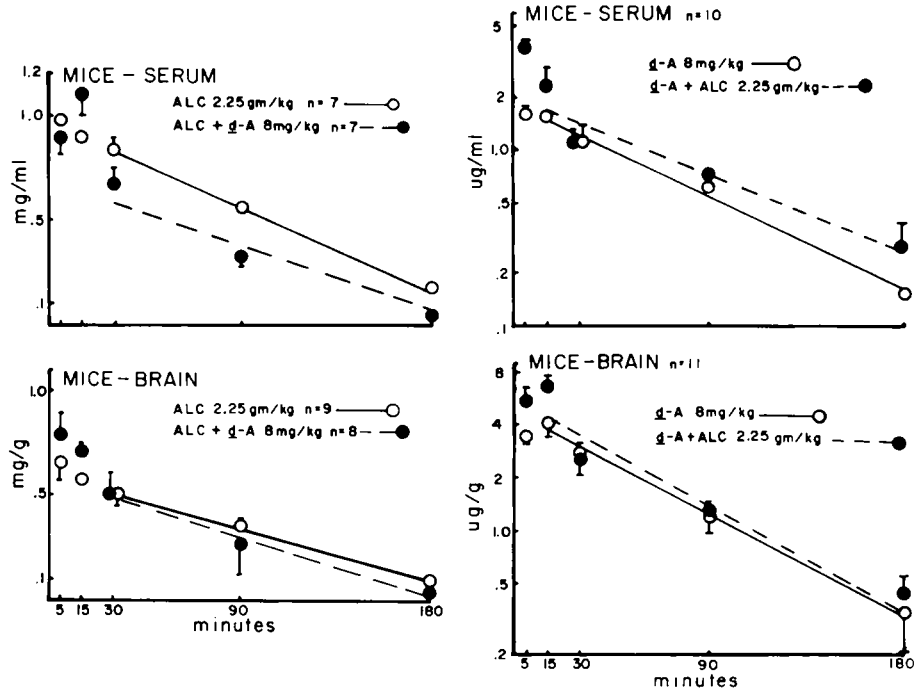

FIG. 4. Time-course of decay of alcohol (left-hand panels) and d-amphetamine (right-hand panels) in serum (upper panels) and brain (lower panels) in mice. Animals were administered 2.25 g/kg of alcohol or 8 mg/kg d-amphetamine alone (0–0) or were treated with the combination (●– – – –●). The drugs were injected IP. Regression slopes were determined for alcohol decay excluding the 5 and 15 min values. Regression slopes were calculated for d-amphetamine decay excluding the 5 min values. No significant differences were found between levels of either drug, comparing values derived from mice treated with alcohol or d-amphetamine alone with those from animals treated with the combination.

phasized that these rats sacrificed for serum and brain levels of drugs were tested on the rotarod up to the time of sacrifice. This was done, since the stress of repeated testing on the rotarod was involved in the ethanol plus d-amphetamine interaction. For example, animals treated with 8 mg/kg d-amphetamine plus 1.5 g/kg of ethanol and tested only once on the rotarod 3 hr after ethanol administration showed much less impairment and incidence of lethality (20%) than those tested repeatedly. Rats tested only once at 3 hr walked the rotarod for 95 ± 18 sec (mean ± SE), while those tested repeatedly walked the rotarod for 31 ± 7 sec.

Since the ethanol-amphetamine interaction varied as a function of the dose of d-amphetamine, the influence of varying the dose of ethanol was explored. Table 4 lists rotarod scores over time for rats treated with 0.5, 0.25, 0.1 and 0.05 g/kg of ethanol in combination with 8 mg/kg d-amphetamine. None of these doses of alcohol when administered alone caused a significant impairment of rotarod or any lethality. The combination with 0.5 g/kg ethanol resulted in a late decrease in rotarod scores and lethality similar to those observed after the combination of 8 mg/kg d-amphetamine and 1.5 g/kg alcohol. The combination with 0.25 g/kg of ethanol demonstrated a similar rotarod impairment although lethality was reduced. The ethanol doses of 0.1 and 0.05 g/kg combined with the stimulant still caused significant disruption of rotarod performance, although no lethality was seen. Because the 0.5 g/kg dose of alcohol combined with the stimulant yielded almost as great an interaction as the 1.5 g/kg dose, the time course of drug elimination from the body after 0.5 g/kg ethanol plus 8 mg/kg d-amphetamine was determined. The results are shown in Fig. 3. Changes in the decline of brain and serum levels of ethanol over time when combined with the stimulant were not significant by regression analysis. The *t*-tests generally supported this finding. The levels of d-amphetamine when combined with ethanol do not differ from control values by regression analysis. By the *t*-test there are some minor differences but no systematic changes. Therefore, with interactions of d-amphetamine and alcohol at these dose levels, there is no clear-cut delay in the rate of elimination of the stimulant from the body.

To ascertain to what extent the d-amphetamine-ethanol interactions occur in other species, mice were tested with similar treatments. Doses of 2 or 2.25 g/kg, IP, of ethanol were combined with 1, 2, 4 or 8 mg/kg d-amphetamine, IP, and the mice tested at various times over the next 4 hr for impairment of rotarod performance. While the results showed trends in the same direction as the comparable rat data, the interaction was far from clearcut. Some groups of mice demonstrated the greater disruption of rotarod behavior after ethanol plus low or high doses of the stimulant, but others did not show it. Serum and brain levels of ethanol and d-amphetamine were determined in mice treated with 2.25 g/kg ethanol and 2, 4 or 8 mg/kg d-amphetamine. Drug levels for the combination with the highest dose of the stimulant are shown in Fig. 4. Lower doses of d-amphetamine combined with ethanol presented comparable patterns of decay slopes for ethanol and

d-amphetamine, relative to respective controls. Regression analysis of alcohol decay curves was done excluding the 5 and 15 min determinations, and no significant differences were apparent, comparing ethanol levels after this drug alone with those after administering the combination. d-Amphetamine decay slopes for these mice were comparable, by regression analysis, whether the stimulant was administered alone or in combination with alcohol. The related *t*-tests for ethanol or d-amphetamine levels at the various times of analysis, comparing groups administered ethanol or d-amphetamine alone with those receiving the combined treatment, showed some statistical differences. However, these analyses generally indicated that both alcohol and d-amphetamine brain levels were lower after the combination. Thus, mice differ from rats in that d-amphetamine levels in the brain do not tend to persist after administering higher doses of the stimulant in combination with ethanol.

DISCUSSION

This study has demonstrated that central stimulants of the amphetamine type and depressants of the barbiturate-ethanol class interact to influence rotarod performance of rats in ways not predictable on the basis of the separate effects of each agent. The test employed is rather simple, although it does involve a learned behavior requiring the integrity of systems for skeletal muscle coordination. Previous experience with the rotarod indicated that drugs affecting attention, alertness, or motor coordination can impair performance in this test [15, 17, 20].

There was greater disruption of rotarod behavior by ethanol, 1.5 g/kg, combined with 2 mg/kg d-amphetamine, as compared to the effects of this dose of ethanol alone (Table 1). This interaction may be the result of mixed depressant and stimulant drug influences which are not counterbalanced during the combined effects and are more disruptive to performance than either drug effect in isolation. This suggestion gains support from the fact that ethanol in combination with other stimulants (Table 2) or d-amphetamine combined with other general central nervous system depressants (Table 3) also prolonged rotarod impairment as related to the effect of the depressant drug alone. Of the depressants studied only methaqualone showed a pattern of antagonism when combined with d-amphetamine. This agent may differ in its central actions in some fundamental way from the other 3 depressant drugs. Analysis of serum and brain levels of ethanol showed that less drug was present at various time after coadministering it with 2 mg/kg d-amphetamine than after ethanol alone. Therefore, an increase in half-life of ethanol in the rat as a consequence of combining it with d-amphetamine is not the basis of the prolonged rotarod disruption.

The more prominent interaction between ethanol 1.5 g/kg and 8 mg/kg d-amphetamine, compromising the rotarod performance for more than 5 hr and including late onset of unconsciousness and death, appears to have a different basis than that seen with ethanol and the lower dose of d-amphetamine. In the interaction involving the higher dose of d-amphetamine, substituting a similar type stimulant for d-amphetamine or replacing ethanol in the combination with another depressant did not result in a long-term rotarod impairment, coma, and lethality. Furthermore, much lower doses of ethanol in combination with 8 mg/kg d-amphetamine (Table 4) provoked only a delayed type of interaction. The interaction involving 0.5 g/kg ethanol plus 8 mg/kg of the stimulant was almost as intense as observed after 1.5 g/kg ethanol plus this dose of d-amphetamine, for the late decrease in rotarod scores, comatose state, and percent dying. It is also clear that this latter interaction involves the stress of repeated rotarod testing, since the same drug combination in untested subjects provoked less intense effects. In addition, these results indicate that the interaction was not simply the additive effects of the separate impairments of ethanol and d-amphetamine, since the 0.5 g/kg dose of ethanol alone caused no impairment of rotarod performance.

In analyzing serum and brain levels of drugs after ethanol 1.5 g/kg plus 8 mg/kg d-amphetamine, some prominent changes were noted in reference to levels attained after each drug administered alone. Ethanol levels after the combination were very nearly identical to those found in rats treated only with ethanol (Fig. 1). However, the decay in d-amphetamine levels with time was greatly retarded as a result of combining the two drugs (Fig. 2). This was actually a dose-related phenomenon, gradually increasing in prominence from 4 to 8 mg/kg d-amphetamine treatments, and being most noticeable in the brain levels. Jonsson and Lewander [14] have previously demonstrated that ethanol can inhibit the metabolism of d-amphetamine in the rat. These altered patterns of d-amphetamine disposition were not observed in mice treated with the combination, at least as related to brain values (Fig. 4). Mice also showed an equivocal interaction in terms of rotarod performance and lethality after the combination. Thus, it may appear that the persistent d-amphetamine levels in rats and the disrupted motor performance, coma, and late deaths in this species are related. However, a consideration of the data derived from rats treated with the combination of 0.5 g/kg ethanol and 8 mg/kg d-amphetamine does not support this contention (Table 4, Fig. 3). While the rotarod impairment, late depression and lethality of this combination were similar to that seen with the higher dose of ethanol, the effect on d-amphetamine disposition was not. That is, d-amphetamine in these rats decayed at an identical rate in groups treated with 8 mg/kg alone or with 8 mg/kg of the stimulant in combination with 0.5 g/kg ethanol. Thus, the behavioral influences and altered d-amphetamine brain levels as a consequence of the interaction do not seem to be causally related.

Other possibilities may be entertained to explain the interaction between ethanol and 8 mg/kg d-amphetamine. A considerable amount of evidence has become available over the last several years to indicate that ethanol may activate brain catecholamine mechanisms and thereby stimulate certain types of behavior [5, 6, 7]. This appears to be true also in the human [1]. Combining ethanol with sympathomimetic agents, however, has variable effects [2, 4, 8, 11, 12, 25]. It is possible that d-amphetamine and ethanol may synergize in causing increased catecholamine release in certain brain pathways, particularly in the presence of stress. This may produce an imbalance in various brain functions so as to impair behavior even more than ethanol alone. A late onset of coma and lethality may have signalled the exhaustion of these overstimulated systems. A greater intensity of overt stimulation during the early phase may be blunted by the presence of the direct depressant effects of ethanol that are not related to an increased dopamine synthesis. In any case, this hypothesis should be tested in the future with more definitive experiments.

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