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EFFECT OF INHIBITORS AND INDUCERS OF DRUG METABOLISM ON ETHANOL METABOLISM *IN VIVO*

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Abstract—SKF 525-A (β-diethylaminoethyl diphenylpropyl acetate), in a dose of 50 mg/kg (i.p.), had no effect on ethanol sleeping time or on the rate of disappearance of ethanol from the whole body in mice, or on the slope of linear decrease of blood ethanol concentration in rats. It appeared to delay the absorption or distribution of ethanol, as shown by a cross-over in the blood curves. Chronic pretreatment with chlorcyclizine or phenobarbital, in doses which significantly shortened the pentobarbital sleeping time, had no effect on ethanol sleeping time or ethanol metabolism in rats, although onset of sleep was delayed in rats pretreated with phenobarbital. It is concluded that the hepatic microsomal drug-metabolizing systems are probably not involved in ethanol metabolism *in vivo*, or in cross-tolerance between ethanol and other drugs.

CROSS-TOLERANCE among various drugs which are metabolized by the hepatic microsomal drug-metabolizing systems (MDMS) has been demonstrated in various species.[1] The cross-tolerance is, in part, the result of induction of this nonspecific hepatic microsomal system by one agent, so that another agent, metabolized by the same system, disappears more rapidly.[2–4] Fraser *et al.*[5] reported that barbiturate addicts showed only moderate signs of intoxication after very large doses of ethyl alcohol when it was substituted for barbiturates. Recently, Lind and Parkes[6] reported a significant decrease in ethanol sleeping time in mice after pretreatment with chlorpromazine, pentobarbital, amytriptyline or imipramine, and a 4-fold prolongation of ethanol sleeping time in mice injected with SKF 525-A (β-diethylaminoethyl diphenylpropyl acetate). On the basis of these findings, the authors suggested that ethanol might be oxidized by the liver microsomes, but they did not study the rate of disappearance of ethanol.

Pretreatment with chlorcyclizine, an inducer of the hepatic MDMS, has been reported to produce 62 per cent reduction in blood alcohol during the 4 hr following a 3 g/kg dose of ethanol by intubation.[7] Daily administration of phenobarbital and hexobarbital for 4–5 days has also been reported to increase the rate of ethanol disappearance from the blood by 15–40 per cent in rabbits[8] and by about 20 per cent in mice.[9] Surprisingly, hexobarbital was more effective than phenobarbital, even though the latter is a much more effective inducer of the MDMS.[4]

These experiments leave a number of unanswered questions. Cross-tolerance, as indicated by measurements of sleeping time, could depend upon changes in the sensitivity of the nervous system, and not only upon modification of drug metabolism. Also, the increases in ethanol disappearance rate reported by these investigators vary considerably in size. The greater effect of hexobarbital than of phenobarbital in raising the rate of ethanol disappearance appears at odds with the suggestion that

their effect is exerted by induction of the hepatic MDMS. For these reasons, we have investigated the effects of pretreatment with SKF 525-A, chlorcyclizine and phenobarbital upon both the sleeping time and the metabolism of ethanol under comparable conditions.

MATERIALS AND METHODS

Animals

Male Wistar rats and male and female Swiss albino mice obtained from Canadian Breeding Laboratories were used in this experiment. The animals were allowed Purina chow and tap water *ad lib.*

Materials

Nicotinamide adenine dinucleotide (NAD) and yeast alcohol dehydrogenase were obtained from the Sigma Chemical Company. Phenobarbital sodium was purchased from British Drug Houses. SKF 525-A was kindly donated by Smith, Kline & French Laboratories and chlorcyclizine hydrochloride by Burroughs Wellcome & Company (Canada).

Acute drug treatments

SKF 525-A, 50 mg/kg (i.p.), was administered 45 min before a challenge dose of ethanol (2 g/kg, i.p.). This dose of SKF 525-A was double that used by Lind and Parkes.[6] The control groups received the equivalent amount of saline in place of SKF 525-A. Previous work in this laboratory had confirmed that a dose of 40 mg/kg drastically reduced the rate of hexobarbital metabolism.[10]

Chronic drug treatments

Chlorcyclizine hydrochloride (50 mg/kg) and phenobarbital sodium (100 mg/kg) dissolved in distilled water were administered daily by intubation. The duration of treatment differed in the various experiments, as described below. The control rats received an equal volume of water to balance the effect of stress due to intubation. The last dose of drug was given either 24 or 48 hr before administration of ethanol, as detailed below for individual experiments. Food was withheld for 24 hr before the alcohol tests.

Ethanol metabolism in rats

Ethanol, in a dose of 2 g/kg, was injected intraperitoneally as a 20% (v/v) solution in 0·9% saline. Blood samples of 0·1 ml were taken from the cut tails of the rats directly into heparinized blood pipettes. Samples were obtained from each animal at 60, 120, 180 and 240 min after administration of the test dose of ethanol. The disappearance rates of blood ethanol (β) were calculated from the slope of the linear descending portion of each curve. Values for C_0 (theoretical alcohol concentration in blood immediately after administration, assuming complete absorption and uniform distribution) were found by extrapolating this portion of the blood alcohol curve to zero time. The values of r (fraction of body mass in which ethanol is equilibrated with the blood) were determined by dividing the dose of ethanol administered, in milligrams per 100 g, by C_0. The rate of ethanol disappearance in milligrams per kilogram per hour was calculated as the product of β, r and the weight of the animal.[11] Blood ethanol concentration was measured enzymatically as described previously.[12]

Ethanol metabolism in mice

The dose of ethanol was 2 g/kg, administered intraperitoneally as a 20% (v/v) solution in saline. At intervals of 30, 60, 90, 120 and 160 min, the mice were decapitated with scissors, immediately dropped into a Waring blendor, and homogenized with 70 ml of cold water in a room at 4°. The homogenate was centrifuged at 7800 *g* for 30 min and the clear supernatant fraction removed. The pellet was suspended in distilled water and centrifuged again at 7800 *g* for 30 min. The two supernatant fractions were combined and the total volume of the two was made to 250 ml;1 ml of this pooled supernatant fraction was used for analysis of ethanol as described previously.[12] A control mouse, analyzed in the same way, served as the blank for each assay.

Measurement of sleeping time

For measurement of sleeping time, ethanol was injected intraperitoneally as a 20% (v/v) solution in saline. The dose was 4·5 g/kg in mice and 3·0 g/kg in rats. The time between the injection and the loss of righting reflex was measured with a stopwatch, and recorded as the induction time. Sleeping time was the interval between loss and return of the righting reflex. Recovery was verified by again placing the animal on its back, and the initial measurement was accepted only if the animal again righted itself at once. Drug pretreatments are described in relation to the results of individual experiments.

RESULTS

Effect of SKF 525-A treatment on ethanol sleeping time and metabolism

Table 1 shows the onset and duration of sleep produced by ethanol in mice pretreated

TABLE 1. EFFECT OF SKF 525-A ON ONSET AND DURATION OF SLEEP

Expt.	No. of mice	Body wt. (g)	Onset of sleep (min)	Duration of sleep (min)
I: Males				
Controls	18	35 ± 1·4*	3·0 ± 0·4	122 ± 6·8
SKF 525-A treated	17	38 ± 1·1	4·4 ± 0·7	111 ± 11·5
II: Females				
Controls	20	31 ± 0·7	3·9 ± 0·5	141 ± 7·5
SKF 525-A treated	20	32 ± 0·7	4·4 ± 0·4	160 ± 11·6

* Results are expressed as mean ± standard error.

45 min earlier with SKF 525-A or saline. Two separate experiments were performed, one with males and the other with females. There was no difference in onset or duration of sleep between the two treatment groups in either experiment.

Figure 1 shows the curves for mean body ethanol concentration in mice after a test dose of 2 g/kg of ethanol. It is evident that administration of SKF 525-A, 45 min before the dose of ethanol, did not affect the shape of the ethanol disappearance curve. There was no significant difference between the two groups at any of the times of sampling. The mean rates of ethanol disappearance were obtained by subtracting the residual ethanol concentration at any given time from the initial dose, and dividing

by the elapsed time. Calculated rates for the 150-min period were 594 ± 35mg/kg/hr for the controls and 566 ± 42 for the mice pretreated with SKF 525-A. The difference was not significant.

The mean blood ethanol curves in two separate experiments with rats are shown in Fig. 2. Pretreatment with SKF 525-A appeared to retard the absorption of ethanol. The blood ethanol concentration at 1 hr, which is shown in Fig. 2a, appeared lower in the pretreated animals than in the controls, but the difference was not significant. However, in Fig. 2b the difference was in the same direction and was significant ($P < 0.02$). In contrast, the concentrations were higher in the pretreated animals than in the controls at 2, 3 and 4 hr in Fig. 2a ($P < 0.01$, 0.01 and 0.05 respectively) and at 3 and 4 hr in Fig. 2b ($P < 0.05$ in both cases). In the control curves, moreover, the 1-hr value is the highest, while in the pretreated group in Fig. 2b the 2-hr value is higher than the 1-hr values. This crossover of the blood alcohol curves is consistent with the suggestion that pretreatment with SKF 525-A delayed the absorption or distribution of ethanol, though the present work does not indicate the mechanism of such an effect. For this reason, only the 2- to 4-hr values were used in calculation of the

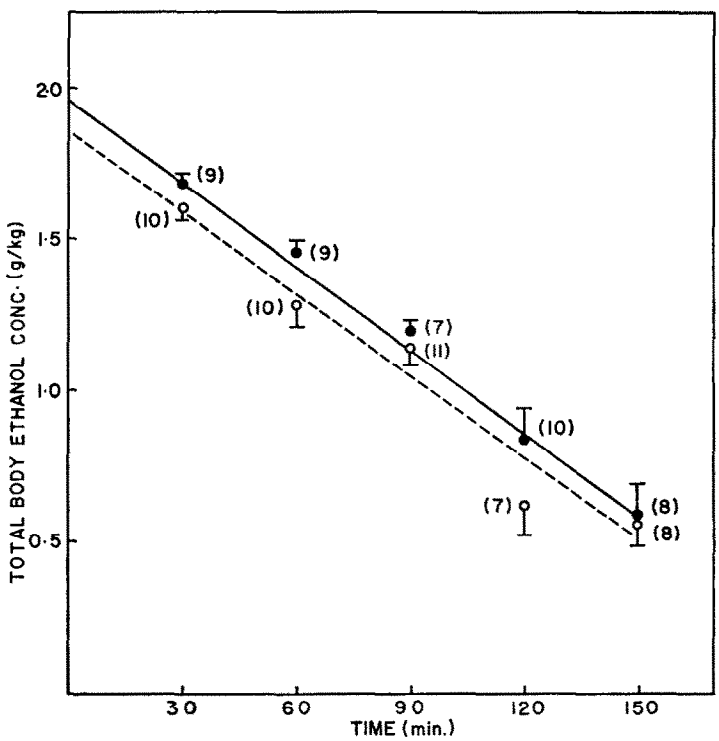

FIG. 1. Concentration of ethanol in whole bodies of mice at various times after intraperitoneal injection of a 2 g/kg dose 45 min after intraperitoneal injection of either SKF 525-A, 50 mg/kg (●), or saline (○). Number of animals for each point is shown in parentheses. Vertical lines indicate positive or negative half of standard error. Regression lines were calculated by the method of least mean squares: ———, SKF 525-A animals; – – – – –, controls.

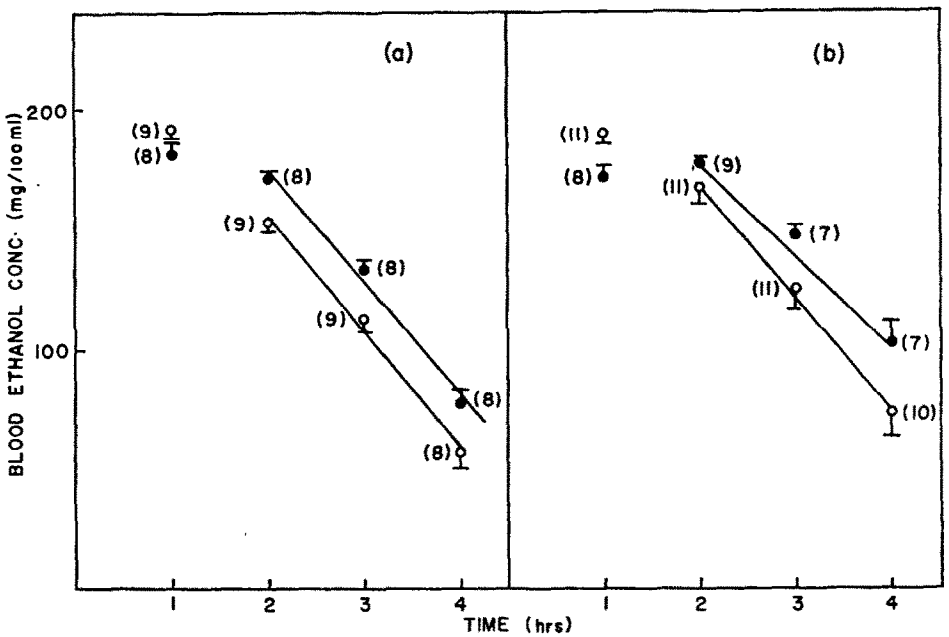

FIG. 2. Concentration of ethanol in blood of rats at various times after intraperitoneal injection of a 2 g/kg dose 45 min after intraperitoneal injection of either SKF 525-A 50 mg/kg (●), or saline (○). (a) Experiment I; (b) experiment II. Number of animals per point is shown in parentheses. Vertical bars indicate standard errors. Lines showing descending slope of blood alcohol curves were calculated for best fit of 2- to 4-hr values.

value of β for each animal. Even the 2-hr value in Fig. 2b probably does not represent the post-absorptive state, thus biasing the results in favor of a difference between the two groups with respect to the slope of the descending portion of the curves. Despite this, the mean β values were not significantly different in either experiment.

Effect of chronic chlorcyclizine treatment on ethanol sleeping time and ethanol metabolism

Chronic treatment with chlorcyclizine has been reported to shorten barbiturate sleeping time and increase the rate of barbiturate metabolism.[13] To be sure that the dose of chlorcyclizine used in the present work was effective, a preliminary experiment with pentobarbital was done. Six rats were pretreated for 7 days with chlorcyclizine, while six control animals received water. After a 24-hr fast following the last dose of chlorcyclizine, both groups received a 30 mg/kg dose of pentobarbital, i.p., and sleeping time was measured. The mean values were 48 $\pm$ 5·4 min for the controls and 7 $\pm$ 2·3 min for the pretreated animals; two of the latter did not go to sleep at all. The highly significant difference ($P < 0{\cdot}001$) confirmed the effectiveness of the pretreatment schedule.

Table 2 shows that chlorcyclizine pretreatment for 2 or 5 weeks had no significant effect on the onset and duration of sleep produced by a test dose of ethanol given 24 hr after the last dose of chlorcyclizine. The chlorcyclizine-treated animals did not grow as much as the controls, so that their total dose of ethanol per animal was not as large. It seems likely that any bias which this might have introduced into the results would be

TABLE 2. EFFECT OF CHRONIC CHLORCYCLIZINE TREATMENT ON ETHANOL SLEEPING TIME AND BLOOD ALCOHOL LEVELS AT AWAKENING

Treatment	Duration (weeks)	No. of rats	Body wt. (g)	Onset of sleep (min)	Duration of sleep (min)	Blood alcohol levels at awakening (mg/100 ml)
Controls	2	11	204 ± 6*	3·5 ± 0·3	50 ± 4·5	
Chlorcyclizine-treated	2	11	174 ± 5	4·4 ± 1·0	41 ± 6·1	
Controls	5	10	265 ± 6	3·7 ± 0·8	52 ± 11	338 ± 12
Chlorcyclizine-treated	5	10	229 ± 9	2·7 ± 0·3	37 ± 5·2	343 ± 1·8

* Results are expressed as mean ± standard error.

TABLE 3. EFFECT OF CHRONIC CHLORCYCLIZINE TREATMENT ON ETHANOL METABOLISM IN RATS

Expt.	Duration (weeks)	No. of animals	Body wt. (g)	Disappearance of ethanol from blood (mg/100 ml/hr)	Ethanol metabolized (mg/kg/hr)
I					
Controls	1	9	131 ± 3·6*	45·2 ± 3·7	419 ± 17
Chlorcyclizine-treated	1	7	137 ± 4·2	49·2 ± 3·7	458 ± 20
Controls	3	10	185 ± 8·7	38·3 ± 2·2	330 ± 16
Chlorcyclizine-treated	3	10	172 ± 6·2	32·3 ± 2·4	298 ± 17
II					
Controls	1	8	195 ± 3·7	41·9 ± 1·5	372 ± 13
Chlorcyclizine-treated	1	9	184 ± 4·3	46·6 ± 2·1	381 ± 11

* Results are expressed as mean ± standard error.

in the direction of creating, rather than obscuring, a difference in sleeping time. The blood alcohol levels on awakening were practically identical in the two groups.

In two separate experiments, the effect of 1 or 3 weeks' pretreatment with chlorcyclizine on ethanol metabolism *in vivo* was investigated (Table 3). In the first experiment, the same animals were examined after 1 week and then again after 3 weeks of pretreatment. In the second experiment, a different group of animals was used after 1 week of pretreatment. In each case a 24-hr interval was left between the last preceding dose of chlorcyclizine and the test dose of ethanol. There was no significant effect of chlorcyclizine in either group, at either 1 or 3 weeks, on the rate of fall of blood ethanol concentration or on the calculated rate of ethanol metabolism.

Effect of chronic phenobarbital treatment on ethanol sleeping time and ethanol metabolism

As with chlorcyclizine, a preliminary experiment was carried out to confirm that the dose of phenobarbital used was sufficient to decrease the pentobarbital sleeping time. After 1 week of phenobarbital treatment, the sleeping time following pentobarbital (30 mg/kg, i.p.) was reduced from a control value of 36·6 ± 0·87 min to 4·6 ± 2·8 min, with 5 animals per group ($P < 0.001$). Three of the phenobarbital-pretreated group did not go to sleep at all after pentobarbital injection.

In the main experiment (Table 4), phenobarbital or water was administered daily for 5 weeks. Blood ethanol curves, after a test dose of 2 g/kg, were obtained after 1 week

TABLE 4. EFFECT OF CHRONIC BARBITURATE TREATMENT ON ETHANOL SLEEPING TIME AND ON DISAPPEARANCE OF ETHANOL

Treatment	Duration (weeks)	No. of rats	Body wt. (g)	Onset of sleep (min)	Duration of sleep (min)	Disappearance of ethanol from blood (mg/100 ml/hr)	Ethanol metabolized (mg/kg/hr)
Controls	1	6	227 ± 6*			37·8 ± 7·0	306 ± 60
Barbiturate-treated	1	7	220 ± 8			36·4 ± 5·2	318 ± 40
Controls	3	10	249 ± 4	3·9 ± 0·6	100·8 ± 26·2		
Barbiturate-treated	3	10	251 ± 7	6·1 ± 0·7	81·4 ± 18·4		
Controls	4	7	278 ± 7			39·0 ± 2·4	305 ± 14
Barbiturate-treated	4	9	258 ± 9			33·9 ± 4·1	272 ± 22

* Results are expressed as mean ± standard error.

and 4 weeks. Ethanol sleeping time was measured in the same animals after 3 weeks. Blood alcohol level on awakening was determined after the fifth week, on blood samples obtained by decapitation. In the test after 3 weeks' pretreatment, a 48-hr interval was left between the last dose of phenobarbital and the test dose of ethanol, because preliminary experiments showed that a 24-hr interval was insufficient to permit clearing of residual phenobarbital, so that synergism with ethanol occurred. The onset of sleep was significantly delayed in the phenobarbital group, but the duration of sleep was not significantly affected. The rate of decrease of blood ethanol concentration and the calculated rate of ethanol metabolism did not differ significantly in the two groups at either 1 or 4 weeks, and at 5 weeks the blood alcohol levels on awakening were practically identical (controls, 334·0 ± 12·1 mg/100 ml; phenobarbital pretreated, 333·0 ± 10·0; eight animals per group).

DISCUSSION

The above results show that an inhibitor of the hepatic MDMS, and two inducers of it, in doses shown to be effective in modifying barbiturate metabolism, had no effect on ethanol sleeping time or the rate of ethanol metabolism. These results therefore seem inconsistent with the suggestion that ethanol is metabolized to a significant extent by the MDMS *in vivo*.

The reports by Fischer and Oelssner[8, 9] of increased rate of ethanol disappearance after barbiturate pre-treatment do not provide sufficient information to permit statistical evaluation of the significance of the reported differences. Indeed, Fischer himself does not regard the effects as evidence of participation of the MDMS in ethanol metabolism,[14] a view which is supported by the greater efficacy of hexobarbital than of phenobarbital. Lind and Parkes[6] reported a 4-fold increase of ethanol sleeping time by SKF 525-A in mice, based on experiments with 10 animals per group. Using 17–20 animals per group and a larger dose of SKF 525-A, we have been unable to confirm their finding. The reason for the discrepancy is not certain, but it may lie in the variation about the dose–response curve for ethanol. Figure 1 of Lind and Parkes[6] shows the expected large variation which is usually found with ethanol sleeping times, and the points plotted for the 5 g/kg dose indicate a value of about 16 ± 3 (S. E. M.).

In contrast, their Table I reports a value of 7·0 ± 1·1 min for the same dose. In view of this variability, it is important to use large numbers of subjects for intergroup comparisons at each dose.

Wooles[7] reported a 62 per cent reduction in blood ethanol concentration 4 hr after a test dose in rats which had been pretreated with chlorcyclizine for 3 days. This also differs from our findings. However, he administered the ethanol by stomach tube as a 50% solution, which in itself may cause marked retardation of gastric emptying[15] and ethanol absorption.[16] Since chlorcyclizine has some anticholinergic effect, it may well retard ethanol absorption still further. The blood ethanol concentrations shown in Wooles' Table 4 indicate erratic absorption during the first 4 hr, and effects of chlorcyclizine on blood levels during this period cannot be accepted as evidence of an increase in the rate of ethanol metabolism. The importance of drug effects on ethanol absorption and distribution is indicated in the blood ethanol curves in Fig. 2, in which SKF 525-A altered the time course of the ethanol concentration even when the ethanol was given intraperitoneally. Unfortunately, Wooles[7] did not study the blood ethanol curve during the post-absorptive period, but the 16-hr concentrations in treated and control animals were virtually identical.

No attempt was made in the present work to study directly the ethanol tolerance of the central nervous system in barbiturate-tolerant animals. Two minor points of evidence suggest conclusions which appear mutually contradictory. Blood ethanol concentrations on awakening were not significantly different in controls and in animals pretreated with chlorcyclizine or phenobarbital for 5 weeks. This would suggest that tolerance of the central nervous system to ethanol had not been increased by these pretreatments. In contrast, the longer induction time of sleep after ethanol in phenobarbital-treated rats (Table 4) suggests either a higher threshold of the nervous system or a delayed distribution of ethanol into the brain. Resolution of this apparent contradiction will require direct measurement of ethanol concentrations in the brain.

The present findings do not support the concept that any significant role in ethanol metabolism is played by the hepatic MDMS *in vivo*. Hepatic microsomal preparations have been shown to oxidize ethanol to acetaldehyde *in vitro*.[17, 18] However, this action may depend upon artifactual conditions introduced by disruption of cellular organization.[19, 20] Further evidence in support of the latter suggestion will be published separately.[21]

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