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Toxicity of ethanol-barbiturate mixtures

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The simultaneous administration of ethanol at doses of either 2, 3, or 4 g/kg intraperitoneally produced a dose-related decrease in the intraperitoneal LD50 for thiopentone, pentobarbitone, amylobarbitone, phenobarbitone and barbitone in rats. The most marked ethanol-barbiturate interaction was with the long-acting, poorly metabolized, less potent barbiturates phenobarbitone and barbitone. Similarly, a non-hypnotic dose of ethanol (3 g/kg, i.p.) produced a much greater prolongation of the sleeping time with non-hypnotic doses of phenobarbitone and barbitone, than with threshold doses of the shorter acting barbiturates. Various postulates are advanced to explain the underlying mechanism of the barbiturate-ethanol interaction.

Many deaths—both accidental and deliberate—occur each year from the coincidental ingestion of alcoholic beverages and barbiturates. Bogan & Smith (1967) in a survey of 85 fatal barbiturate poisonings noted that ethanol was present in 58% of the cases, and that in these the mean blood barbiturate was only 50% of the level found where poisoning was by barbiturate alone. The interaction of these compounds in the living organism has interested many investigators (cf. Gores, 1964) yet the underlying mechanism whereby the potentiation effects are elaborated is still obscure. Since ethanol and barbiturates are both central nervous system depressants, classical pharmacologists have invoked the traditional concepts of drug synergism—potentiation and addition—to explain their observations (Veldstra, 1956).

However their interaction could have a biochemical basis. Ethanol, for example, could suppress barbiturate metabolism, or conversely barbiturates could inhibit the oxidation of ethanol (Whittlesey, 1954; Melville, Joron & Douglas, 1966; Seidel, 1967). It is known that certain barbiturates can inhibit the reoxidation of NADH to NAD (Pumphrey & Redfearn, 1963; Erwin & Heim, 1963). This inhibition might curtail the oxidation of ethanol since both alcohol dehydrogenase and aldehyde dehydrogenase require NAD (Gores, 1964; Lieber, 1967).

Unfortunately there has been little systematic investigation of the ethanol-barbiturate interaction. A review of the pertinent literature reveals a heterogeneous collection of unrelated experiments which employ several species of animals, differences in times and routes of dosing and a variety of response parameters (death, sleeping times, blood decay curves, tissue levels), which makes it difficult to compare the results in different reports. Furthermore, many barbiturates have been used with differing potencies, pharmacologic effects and metabolic fate. Many workers have chosen hexobarbitone for study even though it is rarely employed in current medical practice. For the above reasons a comprehensive systematic investigation of the ethanol-barbiturate interaction is underway in our laboratory.

Since deaths caused by ethanol alone are infrequent, in the first phase of the study, we have investigated the effect of ethanol on the LD50 and threshold hypnotic doses of several commonly used and prescribed barbiturates. Thiopentone, pentobarbitone,

amylobarbitone, phenobarbitone and barbitone were selected because they differ in pharmacological activity and metabolic fate. The results of these investigations are reported in this paper.

EXPERIMENTAL

Male albino rats of the Wistar strain, weighing 180–220 g were housed 10 animals per cage and acclimatized to the laboratory environment for at least one week. They were fasted overnight and then administered simultaneously an aqueous solution of ethanol (15% w/v) and barbiturates by the intraperitoneal route at a volume of 20 ml/kg. After dosing, they were housed in individual cages and observed for 24 h. The number of deaths and time to death were recorded. The LD50 values of the barbiturates were determined in the presence of ethanol at 0, 2, 3, and 4 g/kg and calculated by the moving average method of Thompson (1947) using the tables provided by Weil (1952) for 4 doses of 5 animals each and a dose-interval of 1·5.

The effect of ethanol on barbiturate sleeping time was investigated by treating rats simultaneously with 3 g/kg ethanol and a threshold or subthreshold hypnotic dose of the barbiturate. The drugs were given by the intraperitoneal route. The sleeping time represented the elapsed time in minutes between the loss and the reappearance of the righting reflex. Control studies were made in which ethanol or barbiturates were given separately.

RESULTS

Acute toxicity

The results of the LD50 determinations are shown in Fig. 1. The LD50 (mg/kg) for the five barbiturates were: thiopentone, 53·0 (45·0 to 62·3); pentobarbitone, 69·0

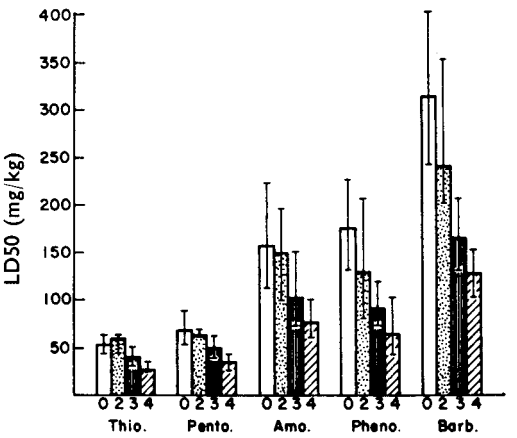

FIG. 1. The effects of ethanol on the LD50 of barbiturates in rats. Figures on the abscissa are doses of ethanol (mg/kg).

(53·4 to 98·1); amylobarbitone, 155 (108 to 221); phenobarbitone, 171 (131 to 224); and barbitone, 312 (241 to 387). These values are in good agreement with published data (Barnes & Eltherington, 1964; Wiberg & Grice, 1965; Coldwell & Peters, 1968).

The presence of ethanol produced a dose-dependent decrease in the LD50 values for all five barbiturates. The decreases were statistically significant ($P < 0·05$) for

phenobarbitone and barbitone at a dose of 3 g/kg ethanol and for thiopentone, pentobarbitone and amylobarbitone at 4 g/kg ethanol. The ratios of the LD50 values without to those with ethanol (4 g/kg) were approximately 2 for thiopentone, pentobarbitone and amylobarbitone, and 3 for phenobarbitone and barbitone.

In our strain of rat the intraperitoneal LD50 of ethanol was 6·5 (6·1–6·9) g/kg. The estimated LD 0 dose was 5·0 g/kg hence it was unlikely that ethanol *per se* produced any fatalities. The cause of death was respiratory failure. Observations on mortality were terminated after 24 h since intraperitoneal injection of ethanol can produce a fatal chemical peritonitis. However, deaths from this cause do not occur within the first 48 h after injection of ethanol (15 to 20% w/v).

The addition of ethanol to the barbiturate reduced the induction time for the appearance of the loss of righting reflex, prolonged the sleeping time of the survivors and shortened the survival time for the fatal doses. The extent of these differences became more marked as the dose of ethanol was increased.

Prolongation of sleeping time

Ethanol (3 g/kg) greatly prolonged the barbiturate sleeping time (Table 1). Where possible, threshold hypnotic doses of barbiturate were employed; this was not feasible

Table 1. *Prolongation of barbiturate sleeping times by ethanol*

Drug(s)	Dose/kg	No. of animals	Median induction time (min)	Median sleeping time + range (min)	Remarks
Ethanol	3 g	20	0	0	Non-hypnotic dose
Thiopentone	30 mg	20	4	24 (5–118)	11/20 did not sleep
Thiopentone + ethanol	3 g	20	2	217 (150–268)	3 fatalities
Pentobarbitone	30 mg	15	4·5	36 (21–42)	3/15 did not sleep
Pentobarbitone + ethanol	3 g	15	2	194 (132–243)	
Amylobarbitone	60 mg	20	5	39 (21–55)	
Amylobarbitone + ethanol	3 g	20	2·5	171 (121–245)	
Phenobarbitone	50 mg	15	0	0	Non-hypnotic dose
Phenobarbitone + ethanol	3 g	15	5	245 (173–359)	
Barbitone	125 mg	20	0	0	Non-hypnotic dose
Barbitone + ethanol	3 g	17	5	426 (264–623)	

with phenobarbitone and barbitone, since the threshold hypnotic dose of these drugs combined with 3 g/kg ethanol was lethal. This effect of ethanol was more marked with phenobarbitone and barbitone than with the shorter-acting barbiturates.

DISCUSSION

Considerable controversy exists as to whether the ethanol-barbiturate interaction is a true potentiation or merely an additive effect. Veldstra (1956) has reviewed the earlier literature and agrees with Gruber (1955) that the interaction is additive and not synergistic. These authors suggest that so-called evidence for a synergism has arisen through failure to consider differences in the characteristics of the barbiturates (long acting, short acting) and the effects of different routes of administration. Nevertheless, considerable experimental evidence accumulating in our laboratory indicates that this interaction involves more than a single additive response of two CNS depressants.

Although ethanol increased the toxicity of all five barbiturates, the interaction was most pronounced with barbitone, the barbiturate least subject to biotransformation. This would suggest that the increase in toxicity did not result from the inhibition of barbiturate metabolism by ethanol but might have been caused by other factors such as: (i) concerted depressant action of ethanol and barbiturate on the CNS, (ii) inhibition of ethanol metabolism by the barbiturates, (iii) altered tissue distribution of the two drugs, (iv) changes in lipid-water partition coefficient of the barbiturates, and (v) changes in protein binding capacity. There is no reason to assume that only one of these proposed mechanisms is involved, it is quite likely that several could operate in concert.

In the present work, since the drugs were given intraperitoneally, the circulating levels of barbiturate and ethanol probably reached their respective maxima rapidly and at approximately the same time. This might not occur after oral administration because of differences in the rates of absorption from the gastrointestinal tract. However, Ramsey & Haag (1946) reported that ethanol ingestion decreased the oral toxicity of quinalbarbitone in mice.

The sleeping time experiments offered some insight to the nature of the interaction. It has been shown that thiopentone is first taken up by the brain and then released for storage in the fat depots, and that the duration of the thiopentone sleep is related to its level in the brain, (Price, Kovnat & others, 1960). Since ethanol prolonged the thiopentone sleeping time it might delay the migration of thiopentone from the brain to the fat depots. Non-hypnotic doses of phenobarbitone or barbitone in the presence of a non-hypnotic dose of ethanol produced very prolonged sleeping times. This did not appear to be an additive effect. Barbitone, which is only slightly metabolized, produced the longest sleeping time which would imply that impairment of barbiturate metabolism is not a major factor in the interaction mechanism.

Ethanol reduced the induction times for the appearance of the loss of righting reflex for all five barbiturates (data from LD50 experiments). The induction times (2–5 min) were too short to be associated with altered rates of ethanol or barbiturate degradation but most likely reflected levels in the brain. There is some evidence that ethanol facilitates the passage of barbiturate into the brain. Seidel (1967) found higher levels of pentobarbitone but not of thiopentone or barbitone in the brains of mice treated with ethanol. We are determining currently the "t-1/2" and rate constants of decay curves for ethanol and several barbiturates alone and in combination in several tissues of rodents and pigs. Although these studies are not completed we have found higher levels of phenobarbitone and barbitone in brains of rats dosed with barbiturate and ethanol than in those given barbiturate only. Ramsey & Haag (1946) noted that in mice barbitone did not change the blood ethanol curves and that the blood barbitone curves were practically identical with and without ethanol. Whittlesey (1954) observed that pentobarbitone decreased the rate of fall of blood ethanol in dogs. Melville & others (1966) reported significant decreases in the rate of disappearance of blood levels of quinalbarbitone and ethanol in dogs given oral doses of these drugs alone and in combination. Seidel (1967) found that the concentration of pentobarbitone in the blood decreased more slowly in mice pretreated with ethanol, but that the rates of disappearance of barbitone and thiopentone were not affected.

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