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LETTERS TO THE EDITOR

Interaction and acute cross tolerance between ethanol and hexobarbitone in the rat

Sleeping times (the times without righting reflex) have been extensively used to evaluate the interaction between ethanol and barbiturates (see for instance Forney & Hughes, 1968; Gruber, 1955; and Wiberg, Coldwell & others, 1968). Some controversy exists about the mode of interaction (Forney & Hughes, 1968). From theoretical considerations Gruber (1955) concluded that the data so far suggested an additive effect.

After single administrations of ethanol (Mirsky, Piker & others, 1941) or barbiturate (Brodie, Mark & others, 1951) a phenomenon occurs which has been called acute tolerance. Acute tolerance may be measured as a difference between the concentrations of the drug at the appearance and subsequent disappearance of symptoms of CNS depression. It has also been measured as a positive correlation of the blood concentration of the drug at the disappearance of certain neurological signs of intoxication, and the magnitude of the administered dose (Brodie & others, 1951; Maynert & Klingman, 1960). The study of Maynert & Klingman (1960) included two barbiturates, ethanol, trichlorethanol and paraldehyde. Acute tolerance thus seems to be a common property of depressant drugs and it is possible that there is a cross-tolerance between different drugs. Such an acute cross-tolerance has been recorded in the present investigation between hexobarbitone and ethanol.

The threshold dose of hexobarbitone needed to obtain suppression for 1 s or more of the bursts in activity seen in the EEG after barbiturate was determined (Wahlström, 1966a). Briefly, hexobarbitone as the racemate was infused at a constant rate of 0·25 mg/kg s^{-1} into the tail vein of male rats (350 g). When the first EEG burst suppression with a duration of 1 s or more appeared in the record, the infusion was stopped and the ensuing sleeping times recorded (Wahlström, 1966b). Animals were kept in darkness between 8 a.m. and 8 p.m. and were well adapted to this rhythm before the experiments. All experiments were done between 9 a.m. and 3 p.m.

Ethanol was given intraperitoneally as a 20% (w/v) solution in isotonic saline. All blood samples were taken from a tail vein; in the threshold experiments the sample was obtained with a new needle and syringe after the infusion. Ethanol was measured by gas-chromatography (F and M model 402) (Curry, Walker & Simpson, 1966), with 1-pentanol as internal standard. Injection port and column temperature 85°; detector temperature, 105°; carrier gas, N_2, flow rate, 50 ml/min. The relation between peak heights were used to determine the ethanol concentration. Planimetry gave no higher precision.

The approximate times at which the threshold determinations needed to be made were estimated by measuring blood ethanol after intraperitoneal injection of 2 g/kg in a separate group of 5 animals (Fig. 1A).

In the rats in the main experiment (n = 18) four barbiturate threshold analyses were done before ethanol was given. The first one was discarded (Wahlström, 1966a) and a pre-ethanol mean of threshold dose and ensuing sleeping time was calculated on the remaining three. The mean and standard error for all participating rats were 59·7 $\pm$ 1·1 mg/kg and 18·9 $\pm$ 1·3 min respectively. The threshold measurements were then done for groups of 6 rats 1·25, 2·75 and 5·75 h after the injection of 2·0 g/kg ethanol. Each group was tested at one time only. Average blood concentrations of ethanol when the threshold measurements were made are in Fig. 1A.

The relation between ethanol concentration and hexobarbitone threshold dose is

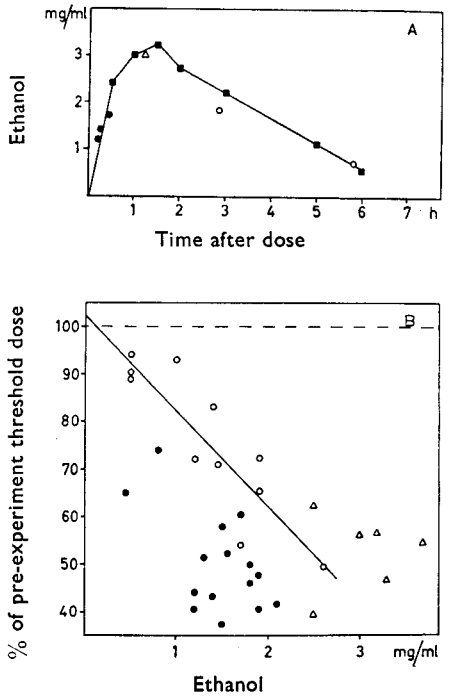

Fig. 1. A. Average ethanol concentration in blood after intraperitoneal injection of 2 g/kg. ■ Preliminary experiment in a separate group of rats (n = 5). The averages were obtained by graphical interpolation of the curves obtained from the individual rats. Samples were obtained approximately at the times used. △○● Average ethanol concentrations obtained after two threshold determinations in 3 groups of rats as described in text.

B. Relation between ethanol blood concentrations and per cent of pre-ethanol threshold dose of hexobarbitone needed to obtain a burst suppression of 1 s or more. The unbroken line indicates the regression line calculated on the data obtained on the falling ethanol concentration curve (○). The same animals were used on two occasions. A few threshold determinations could not be evaluated because of subcutaneous infusions. ● Ethanol increasing. ○ Ethanol decreasing. △ Ethanol around maximum.

given in Fig. 1B. It is evident that there was a linear relation (linear regression coefficient = $-20{\cdot}0 \pm 3{\cdot}5$ d.f. = 9) between blood ethanol concentration and decrease in barbiturate threshold dose when the threshold measurements were made on the decreasing part of the blood ethanol concentration curve (Fig. 1B). This line does not deviate significantly from the point 0 mg/ml; 100%. This is a strong indication that Gruber's (1955) conclusion of an additive effect is correct for hexobarbitone and ethanol on the decreasing blood ethanol curve, 1 mg/ml of ethanol corresponding roughly to a decrease of 20% (approx. 12 mg/kg) of hexobarbitone. The sleeping times before ethanol were not different from those after ethanol, the difference ±s.e. being $-1{\cdot}3 \pm 3{\cdot}5$ min (n = 10). The decrease in threshold dose thus compensated for the additional effect of ethanol. A small decrease would be expected as the ethanol concentration decreased.

The threshold measurements made around the peak ethanol blood concentration (Fig. 1B) deviated slightly to the right compared with the regression line for the decreasing ethanol blood concentrations. More observations are needed to decide whether this deviation is significant. The sleeping time in this group of animals was $14{\cdot}5 \pm 2{\cdot}7$ min longer than the pre-ethanol time (n = 6) which might indicate that the threshold was measured before the peak ethanol concentration in the brain.

Before the subsequent experiments a new threshold determination was made without ethanol. 0·2–0·4 h before barbiturate infusion, saline was given intraperitoneally: barbiturate dose and the sleeping time were unchanged compared with the pre-experimental ones.

Threshold measurements on the rising part of the blood ethanol curve were then made on the same animals (3 groups of 6 rats in each) 0·20, 0·25 and 0·40 h after ethanol injection. The interval between the two ethanol experiments in the same animal was 2–3 weeks. The average ethanol concentrations are shown in Fig. 1A, and the threshold measurements in Fig. 1B. Larger decreases in the barbiturate threshold doses were encountered on this part of the ethanol curve than on the decreasing part. Because of the accumulation of data between 1 and 2 mg/ml of ethanol, no definite statement about linearity can be made. The sleeping times were increased by $18{\cdot}0 \pm 3{\cdot}6$ min ($n = 13$) over the pre-ethanol times. As the experiments were done on a rapidly rising part of the ethanol curve such an increase is to be expected.

Two kinds of systematic error will affect the information obtained from the ethanol determinations more markedly on the rapidly rising part of the blood ethanol curve than on the more slowly decreasing part. Since the ethanol concentration is rising, blood sample measurements taken after the threshold determinations (the time lag approximately 0·1 h) will give an over-estimate of the ethanol concentration in the blood at the time of the threshold determination. Also, blood concentration will be greater than CNS concentration.

Since both errors overestimate the critical concentration of ethanol on the increasing part of the ethanol concentration curve, and to a much smaller extent underestimate them on the decreasing part, the relevant values are probably even smaller than those recorded (Fig. 1B). The difference in hexobarbitone needed to obtain a suppression of EEG bursts of 1 s or more at similar ethanol concentrations on the increasing and decreasing part of the concentration curve is thus probably even larger than the one recorded (Fig. 1B). An acute cross tolerance thus seems to exist between hexobarbitone and ethanol.

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