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Tolerance experiments with barbitone-dependent rats

SIR,—Recently Ungar & Cohen (1966) found that morphine tolerance could be transferred to mice by the injection of a brain extract prepared from morphine-tolerant rats or dogs. It has also been reported that the respiration of brain slices removed from morphine-tolerant rats is refractory to the depressant effect of morphine (Takemori, 1961; 1962). Adopting experimental procedures similar to those of the above authors we have looked at barbiturate tolerance in experiments with barbitone-dependent and barbitone-withdrawn rats.

Female Wistar rats, about 50 g at the beginning of the experiment, were made dependent on barbitone by the administration of up to 400 mg/kg/day barbitone sodium in the drinking water over a five week period. Withdrawal was effected by replacing barbitone solution by drinking water. Control animals received drinking water throughout. Animals were killed after five weeks of barbitone treatment or 48 hr after withdrawal.

Groups of mice were injected intraperitoneally with a whole brain homogenate (0·5 ml/25 g) prepared from barbitone-dependent, withdrawn or control rats. Each ml of homogenate contained 400 mg of brain in normal saline. Either 3 or 24 hr after administration of the homogenate, the sleeping time after intraperitoneal injection of 100 mg/kg hexobarbitone sodium was measured. At the same time hexobarbitone metabolism *in vitro* was assayed on liver microsomal preparations from other groups of similarly treated mice. The method used involved the determination of the metabolite formed from ^{3}H-labelled hexobarbitone.

Respiratory rate was measured by conventional manometric techniques. Two cortical slices, one from each hemisphere, were placed in each flask and after an initial equilibration period of 15 min at 37° respiration was measured over 60 min. The effect of barbitone (5×10^{-3}M) was measured on the respiration of both unstimulated and stimulated (0·1M KCl) cortical slices taken from barbitone-dependent, withdrawn and control rats.

Although Ungar & Cohen (1966) found that pentobarbitone tolerance was not transferred, their method of producing tolerance was such that it is unlikely that the tolerance observed was due to a central mechanism but rather that the drug stimulated the activity of enzymes concerned with its metabolism. Our method to produce physical dependence on barbiturates was that first described by Crossland & Leonard (1963) who showed that a characteristic withdrawal syndrome developed on cessation of barbitone administration.

TABLE 1. HEXOBARBITONE SLEEPING TIME AND RATE OF HEXOBARBITONE OXIDATION *in vitro* BY LIVER MICROSOMES OF MICE TREATED WITH RAT BRAIN HOMOGENATE

	Pretreatment (hr)	Type of brain homogenate		
		Control	Barbitone-dependent	Withdrawn
Sleeping time (min)	3	61 ± 20(8)	56 ± 14(8)	49 ± 13(8)
Sleeping time (min)	24	66 ± 20(8)	46 ± 15(8)*	55 ± 16(8)
Hexobarbitone metabolism[1]	24	2·8 ± 0·9(4)	3·8 ± 0·9(4)	—

* $P < 0.05$.
[1] μmoles hexobarbitone oxidized/9,000 g supernate from 1 g liver/30 min.

TABLE 2. EFFECT OF BARBITONE (5×10^{-3}M) ON THE RESPIRATION OF BRAIN SLICES OF BARBITONE-DEPENDENT AND WITHDRAWN RATS

Incubation conditions	Oxygen uptake (μM/g/hr)		
	Control	Barbitone-dependent	Withdrawn
Unstimulated	49·0 ± 2	50·4 ± 9	50·0 ± 3
Unstimulated + barbitone	40·3 ± 7	41·3 ± 6	39·8 ± 5
Stimulated[1]	85·3 ± 4·5	90·2 ± 9	83·9 ± 6
Stimulated + barbitone	48·4 ± 7·5	51·6 ± 12	54·3 ± 12

[1] Incubations carried out in the presence of 0·1M KCl.
Figures denote mean ± standard deviation of six observations.

We find hexobarbitone sleeping time to be reduced in mice which 24 hr previously had been injected with brain homogenate prepared from barbitone dependent rats (Table 1). Since the injected brain extract might have affected the activity of hepatic drug-metabolizing enzymes the ability of microsomal preparations to oxidize hexobarbitone *in vitro* was measured in this group of mice. The fact that this activity was somewhat elevated suggests the possibility that the reduction in sleeping time was due to a stimulation of hexobarbitone oxidation and not to a reduced sensitivity of the brain to barbiturate. Support for this view comes from the observation that the barbitone sleeping time (250 mg/kg i.p.) was the same in this group of mice (210 ± 23(8) min) as in the control group (205 ± 18(8) min). It is possible that this enzyme induction may have been brought about by the barbitone present in the brain homogenate from barbitone-treated rats.

The respiratory rate of cortical slices removed from barbitone-dependent or withdrawn rats was the same, and was stimulated to the same extent by 0·1M KCl, as those taken from controls (Table 2). Barbitone depressed unstimulated respiration by about 20% and KCl stimulated respiration by about 40%, again there being no difference between the results obtained with slices from the 3 kinds of experimental animal. The greater sensitivity of stimulated respiration to barbiturates is well known (McIlwain, 1953, Ghosh & Quastel, 1954).

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