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Effect of analeptics on brain pentobarbital levels and sleeping time in mice

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IN THE treatment of barbiturate poisoning the therapeutic emphasis has shifted from the use of analeptics and efforts at arousal to a regimen of physiologic supportive therapy.[1] For some time, though, analeptics were drugs of choice, the results being transferred from experimental evidence[2, 3] despite some reports[4, 5] about the ineffectiveness of these drugs in animals given large doses of barbiturates. Theoretically, stimulant therapy with drugs like nikethamide, pentylenetetrazol and picrotoxin might seem appropriate. Nilsson,[6] and subsequently others,[7, 8] have given up their use because of severe untoward side effects and higher mortality. According to Nilsson,[6] when the nervous system is doubly challenged by the depressant action of one compound and the stimulant action of another, the system tends towards instability and the resultant patterns of nervous activity are abnormal. In a previous experiment[9] we observed that mice given hexobarbital and nikethamide simultaneously showed a tremendous increase in the sleeping time (loss of righting reflex), even though there were convulsive movements and increased respiration during sleep. In the present report we have extended this observation to an examination of the brain–barbiturate levels, using pentobarbital as the depressant and nikethamide, pentylenetetrazol and pictrotoxin as analeptics.

Female white mice (Haffkine Institute strain) weighing between 25–28 g were used throughout the experiments. For barbiturate estimation brains of three mice were pooled after decapitation and dissection. The samples were duplicated after extraction in petroleum ether and estimated spectrophotometrically[10] with a modification[11] where the final extraction was in 0·5 N NaOH. This method is specific for unchanged barbiturate. The specificity of this method of estimating pentobarbital was confirmed by chromatographing the petroleum ether extract of the pooled brains on thin layer (silica gel G + alumina, 1:1) using cyclohexane–isopropanol and ammonia solution as a solvent system.[12] This method gave only one u.v. spot with an R_f value of 0·85. The anleptics used did not show any change in this spot and did not give a u.v. spot themselves when used concurrently as standards. The sleeping time was studied in groups of mice and was considered as the interval between the loss of righting reflex after injection and the regaining of the righting reflex (thrice within a minute), measured by a stop watch. The time has been expressed only in minutes, the seconds being rounded off to the nearest minute. Table 1 shows all the results.

It can be seen that treatment with analeptics increases the brain barbiturate level to many times the control. Even where the sleeping time is decreased, as with picrotoxin, the barbiturate level in brain is high. There does not seem to be any particular waking level of brain barbiturate after treatment with analeptics. The stimulation of the central nervous system with analeptics might alter the cellular permeabilities to favor an excess entry of the barbiturate. This would cause a delay in the excretion of barbiturate and the higher mortality with the use of analeptics observed in other literature.[13–15]

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TABLE 1. BRAIN LEVELS OF PENTOBARBITAL AND SLEEPING TIME IN MICE AFTER TREATMENT WITH ANALEPTICS*

	Brain levels in μg/g of tissue				Sleeping time
	30 min	60 min	90 min	120 min	(min)
Pentobarbital in mg/kg					
30	35 ± 7	16 ± 4			4 ± 5† (10)‡
40	39 ± 3	20 ± 1			24 ± 5 (11)
50		30 ± 4			64 ± 6 (12)
Pentobarbital and niketamide 150 mg/kg simultaneously					
30	131 ± 5	119 ± 3	105 ± 4	40 ± 4	20 ± 2 (8)
40	162 ± 12	140 ± 1	125 ± 5	44 ± 4	54 ± 8 (8)
50		149 ± 7			164 ± 10 (14)
Pentobarbital and pentylenetetrazol 10 mg/kg simultaneously					
40	128 ± 7				20 ± 4† (11)
50		112 ± 11			124 ± 9 (15)
Pentobarbital and picrotoxin 6 mg/kg simultaneously					
40	144 ± 13				nil (10)
50		105 ± 15			24 ± 5 (10)
Pentobarbital and niketamide 150 mg/kg after 30 min					
50		89 ± 7			126 ± 9 (10)
Pentobarbital and pentylenetetrazol 10 mg/kg after 30 min					
50		75 ± 9			80 ± 9 (9)
Pentobarbital and picrotoxin 6 mg/kg after 30 min					
50		51 ± 8			37 ± 4 (10)

* All drugs were given intraperitoneally in a concentration so adjusted to give the dose of each drug in a volume of 1 ml/100 g of mouse weight, mean ± S.D. For brain levels figures are mean ± S.D. of six groups each.

† Figures in parenthesis are number of animals for sleeping time.

‡ Some of the animals do not sleep; their sleeping time has been taken as zero.

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Drug-induced porphyrin biosynthesis—I. The effect of porphyria-inducing drugs on *N*-demethylase activity of chick embryo liver

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THE ADMINISTRATION of phenobarbital and a variety of other drugs to experimental animals leads to an increase in activity of liver microsomal drug-oxidizing enzymes which is paralleled by an increase in the amount of microsomal cytochrome P-450.[1–3] This cytochrome plays an important role in drug oxidation, and it has been suggested that the increased activity of drug-oxidizing enzymes depends mainly on increased synthesis of this cytochrome.[1] Since phenobarbital and a variety of other drugs induce the formation of δ-aminolevulinic acid (δ-ALA) synthetase in liver mitochondria with a concomitant increase in porphyrin synthesis, Granick[4] suggested the following sequence of events in response to a porphyria-inducing drug. Derepression of δ-ALA synthetase in mitochondria leads to increased porphyrin and heme formation. The heme is utilized for the prosthetic group of microsomal cytochrome P-450 so that an increased amount of cytochrome is available and hence an increased level of drug-oxidizing activity. The objective of this study was to test the validity of this hypothesis. In view of the fact that Granick's studies were carried out in chick-embryo liver cells, which were responsive to porphyria-inducing drugs from the time the embryo was 9 days old, our first experiments were directed to demonstrating the presence and inducibility of drug-oxidizing enzymes in these livers. In our next series of experiments, a positive correlation was sought between the ability of several analogues of 3,5-diethoxycarbonyl-1,4-dihydro-2,4,6-trimethylpyridine (DDC; see Fig. 2b) to induce an increased level of drug-oxidizing enzymes in liver and their porphyria-inducing activity. As an approximate index of the level of drug-oxidizing activity in the liver, the oxidative demethylation of aminopyrine was measured.

MATERIALS AND METHODS

Fertilized eggs used were of a White Leghorn strain obtained from the University of Alberta Farm and stored at 10° for no longer than 7 days prior to incubation at 38°. The age of the embryo was taken as the number of days from the beginning of incubation.

Measurement of N-*demethylase activity.* The embryos were killed by decapitation and the livers immediately removed and homogenized in a Potter-Elvehjem apparatus with 5 vol. of 1·15% potassium chloride solution in the cold. The homogenate was centrifuged at 9000 *g* for 20 min at 4°. The demethylation of aminopyrine was estimated by measuring the amount of formaldehyde liberated according to the method of Nash,[5] as modified by Cochin and Axelrod.[6] The incubation mixture used was as follows: microsomes plus soluble fractions obtained from 0·4 g liver were incubated for 1 hr at 37° in air with 5·0 μmoles aminopyrine, 0·6 μmole NADP, 6·0 μmoles glucose 6-phosphate, 50 μmoles nicotinamide, 45 μmoles semicarbazide hydrochloride, 25 μmoles $MgCl_2$, and 3 ml of 0·1 M potassium phosphate buffer (pH 7·4) in a total volume of 6·0 ml.