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PRELIMINARY COMMUNICATION

Nitroglycerin inhibition of pentobarbital metabolism by liver microsomes

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IT WAS reported earlier[1, 2] that anti-anginal organic nitrates prolonged the sleeping time induced in mice by barbiturates. As a possible mechanism for this effect, it was suggested[2] that organic nitrates may inhibit barbiturate metabolism to nonanesthetic products. Since barbiturates are known to be oxidized by hepatic microsomal enzymes,[3, 4] the postulated mechanism was evaluated experimentally by studying the effect of nitroglycerin upon the metabolism of ^{14}C-pentobarbital by mouse liver microsomes *in vitro*.

Liver microsomes. The preparation of mouse liver microsomes was carried out in a manner analogous to that recommended by Leadbeater and Davies[5] for rat liver microsomes. The livers were removed from 30 female mice (Millerton) immediately after sacrifice and washed with ice-cold 0·1 M Tris-HCl buffer (pH 7·5). The washed livers (39·4 g) were suspended in buffer (about 120 ml) to a total volume of 150 ml and homogenized in a 250-ml capacity Waring Blendor at top speed for 20 sec. The resulting homogenate was then centrifuged for 20 min at 11,000 *g* in a Spinco model R ultracentrifuge (No. 30 rotor). The temperature was kept at 0–5° throughout the procedure. The 11,000 *g* supernatant fraction contained 26·6 mg protein/ml, as determined by the method of Lowry *et al.*[6]

^{14}C-pentobarbital metabolism. The pentobarbital incubations with the 11,000 *g* supernatant fraction were conducted in two 25-ml flasks shaken in a water bath at 37°. In addition to 1·0 ml of the mouse liver 11,000 *g* supernatant fraction, each flask contained 0·3 ml water, 1·5 ml 0·1 M Tris-HCl (pH 7·5) buffer, and 1·0 ml of a solution containing 50 μmole nicotinamide, 0·2 μmole NADP, 13 μmole glucose 6-phosphate, 1·0 μg glucose 6-phosphate dehydrogenase (Calbiochem, sp. act. 140), and 25 μmole magnesium chloride. The control flask contained 4·0 mg CP lactose and the other flask contained 4·0 mg of a mixture of 1 part nitroglycerin and 19 parts lactose. (Nitroglycerin is mixed with lactose to prevent explosion.) After incubating the mixtures for 5 min, the substrate was added as 0·2 ml of an aqueous solution containing 197 μg/ml of sodium pentobarbital-2-^{14}C (Tracerlab, Waltham, Mass., sp. act. 15·65 μc/mg). The total volume of each mixture was 4·0 ml. Immediately after the addition of the substrate, a 0·5-ml aliquot was removed for assay. Aliquots were also removed for assay after 20, 40, and 60 min.

TABLE 1. NITROGLYCERIN INHIBITION OF PENTOBARBITAL METABOLISM BY MOUSE LIVER MICROSOMES

Expt.	Nitroglycerin (mg)	Time (min)	% Conversion*		% Inhibition
			Control†	Nitroglycerin	
I	0·2	20	6·3	4·2	33
	0·2	40	14·7	9·0	39
	0·2	60	28·8	12·3	57
II	2·0	30	14·7	0	100

* Incubation mixture (4 ml) contained 0·16 μmole ^{14}C-pentobarbital, 50 μmole nicotinamide, 0·2 μmole NADP, 13 μmole glucose 6-phosphate, 1·0 μg of glucose 6-phosphate dehydrogenase (Calbiochem, sp. act. 140), 25 μmole $MgCl_2.6\ H_2O$, 1·0 ml mouse liver 11,000 *g* supernatant containing 27 mg protein and was 0·06 M in Tris-HCl buffer at pH 7·50.

† The control incubation mixture contained lactose in the same quantity as its companion incubation mixture contained nitroglycerin.

A second experiment was performed similarly with 40 mg of nitroglycerin–lactose (1:19) and with 40 mg of CP lactose in the control flask. In this experiment the aliquots were removed for assay only at the initial time and after 30 min.

Assay. Each 0·5-ml aliquot withdrawn from the incubation mixtures was added immediately to 1·0 ml of ice-cold 5 N HCl and extracted with three 3-ml portions of ether. The ether extracts were combined; scintillation counting showed that the extraction of radioactive compounds was quantitative. The ether solution was concentrated to a small volume for chromatography on Whatman No. 1 paper strips in 1-butanol saturated with 1 % ammonium hydroxide.[7] The developed chromatograms were scanned on a Packard model 7200 radiochromatogram scanner to determine the radioactivity of the unchanged pentobarbital (R_f 0·87) and of its metabolite (R_f 0·4–0·7).

The tabulated results show that there was sufficient NADP to sustain a linear reaction rate for the 1-hr study period and that nitroglycerin inhibits pentobarbital metabolism by the 11,000 *g* supernatant fraction of mouse liver. Consequently, it seems feasible to consider that mice treated with organic nitrates slept longer because the administered pentobarbital was metabolized at a slower rate.

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