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Pentobarbital-induced reduction of pyridine nucleotide measured by surface fluorometry in perfused rat heart

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Pentobarbital [5-ethyl-5(2-pentyl) barbituric acid] has been used to limit the deleterious effects of cerebral ischemia, such as neurological deficit and infarct size [1–4]. Plasma concentrations resulting from this use of pentobarbital are about 10-fold those obtained in general anesthesia (0.3–0.5 mM vs 0.03–0.04 mM respectively [3, 5]). Such use of high-dose barbiturates gives rise to the need for investigation of their dose-related effects on various organs. This is especially true for the heart, since barbiturates are well-known myocardial depressants [6, 7]. These experiments use NADH surface fluorometry [8–10] in perfused rat heart to demonstrate that pentobarbital significantly inhibits heart mitochondrial NADH oxidation at concentrations which have reduced post-ischemic neurological deficits in baboons [3]. This effect of pentobarbital on mitochondrial NADH is found to be similar to that of Amytal [amobarbital, 5-ethyl-5(3-methyl butyl) barbituric acid], an established inhibitor of NADH dehydrogenase [11, 12]. Barbiturate-induced reduction of pyridine nucleotide is compared with titrated effects on cardiac performance, and the mechanism of cardiac depression by these drugs is discussed with respect to effects on ATP production vs consumption.

Male Sprague–Dawley rats were sacrificed by cervical dislocation. After rapid heart excision, the aorta was cannulated such that the perfusate (35–37°) was delivered by a peristaltic pump into the coronary circulation and exited, unrecirculated, via the pulmonary artery [13]. The perfusion fluid was Krebs–Ringer bicarbonate buffer containing 5 mM dextrose and 2.4 mM Ca^{2+}, equilibrated with 95% O_2+5% CO_2. Drugs were delivered into the perfusate by means of a variable speed syringe driver calibrated to allow constant drug infusion into the perfused heart. The left ventricular peak-developed systolic pressure and heart rate were both measured with a 25-gauge needle placed in the left ventricle and connected to a Millar pressure transducer.

The reduced component of the NADH–NAD complex has an excitation-emission interval of 366–450 nm. Thus, the redox state of pyridine nucleotide can be measured fluorometrically. This was done as follows. Left ventricular surface fluorescence was monitored by a branched light guide contacting the left ventricle, supplying 366 nm excitation light through some fibers and transmitted 450 nm fluorescent light through others [10]. The latter led to a photomultiplier tube, thus allowing 450 nm NADH fluorescence, left ventricular pressure and heart rate to be simultaneously recorded on a Bell & Howell oscillograph.

Bovine heart mitochondria were prepared by the method of Green *et al.* [14] and used at a protein concentration of 0.75 mg/ml. Mitochondria were continuously stirred in an air-saturated medium of 20 mM *N*-2-hydroxytheyl piperazine-*N'*-2-ethanesulfonic acid, 250 mM sucrose, 5 mM glutamate and 5 mM malate at pH 7.5. Drugs were added with a Hamilton syringe and oxygen concentration was detected with a Clark oxygen electrode.

Pentobarbital sodium (Nembutal sodium) was from Abbott. Amobarbital sodium (Amytal sodium) was obtained from Lilly.

Inhibition of mitochondrial respiration by equilibration with Amytal (at the level of NADH dehydrogenase) is non-transient and can be titrated by increasing Amytal concentrations in the perfused heart without drug washout between concentration increments. The increase in 450 nm NADH fluorescence with increasing Amytal concentrations is shown in Fig. 1. Amytal caused a maximal 42.5 per cent increase in NADH fluorescence (increased NADH/NAD ratio) at 5 mM. Heart rate declined steadily during the titration from 240 to 0 beats/min, the latter at 3.4 mM Amytal. The course of left ventricular pressure paralleled the decline in heart rate, from 75 to 0 torr, the latter at 2.3 mM Amytal. The Amytal concentration producing half-maximal NADH fluorescence increase was 0.9 mM.

Figure 2 illustrates the qualitative similarity between pentobarbital and Amytal. Left ventricular pressure and heart rate both declined with increasing pentobarbital concentrations and fell to 0 at 3.6 mM pentobarbital. Although both pentobarbital and Amytal caused maximal increases in NADH fluorescence at 5 mM, the extent of this change was greater for pentobarbital (75.0 per cent) than for Amytal (42.5 per cent, Fig. 1). The pentobarbital concentration producing the half-maximal NADH fluorescence increase was 0.76 mM. Thus, pentobarbital is a somewhat more efficacious inhibitor of NADH oxidation than Amytal.

To confirm that the NADH pool affected by pentobarbital is in the mitochondria (as is the case for Amytal [15]), an experiment with isolated mitochondria was performed. Addition of 2.02 mM pentobarbital to bovine heart mitochondria caused the respiratory rate to go from 100 ng-atoms 0/min to 4 ng-atoms 0/min, a 96 per cent inhibition of respiration (data not illustrated). This correlated well with the fact that 2.02 mM pentobarbital accounted for 67.5/75 = 90 per cent of the maximum increase in NADH fluorescence attainable with this drug (cf. Fig. 2).

Daniel *et al.* [16] found a 50 per cent decline in amplitude

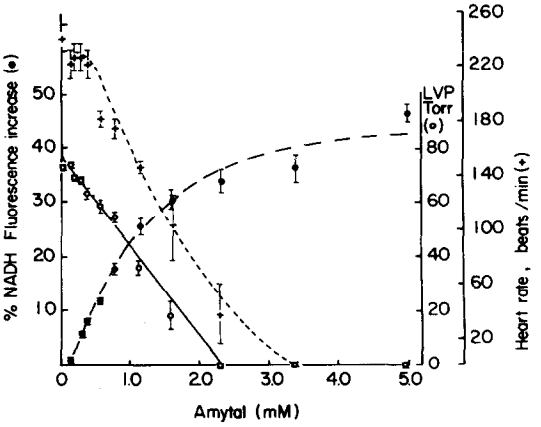

Fig. 1. Effects of Amytal on NADH fluorescence, heart rate and left ventricular pressure (LVP). Points represent mean values and bars represent the standard error for five different experiments. Per cent changes in NADH fluorescence are calculated from recorded arbitrary fluorescence units.

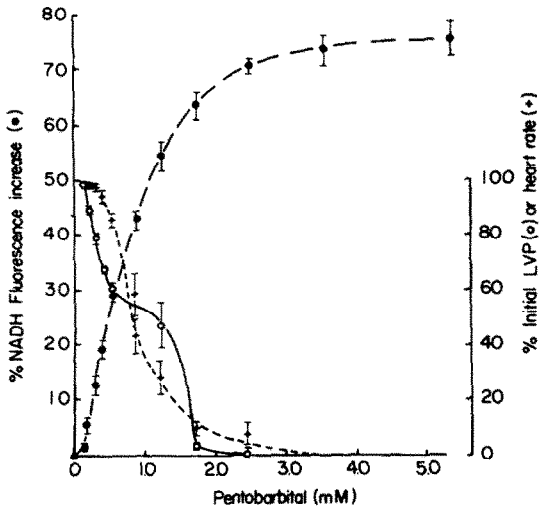

Fig. 2. Effects of pentobarbital on NADH fluorescence, heart rate and left ventricular pressure (LVP). Points represent mean values and bars represent the standard error for five different experiments. Per cent changes in NADH fluorescence are calculated from recorded arbitrary fluorescence units.

of contraction of ventricular strips at 0.6 to 0.8 mM pentobarbital, similar to our finding of a 40 per cent decline (Fig. 2) in left ventricular pressure at the same concentrations. However, those authors found (analogous to previous studies [17, 18]) that the same concentrations of pentobarbital left ATP and creatine phosphate concentrations unchanged. They attributed this to decreased ATP utilization related to pentobarbital-induced inhibition of calcium release and secondarily decreased muscle contraction. More recently, Nayler and Szeto [19] similarly determined that 0.4 mM pentobarbital, while causing a 15 per cent decline in tension of isometrically contracting trabecular muscle (compared here with a 33 per cent decrease in left ventricular pressure at the same concentration, Fig. 2), simultaneously diminished the amount of calcium displaced by La^{3+} from that preparation. In the present experiments, 0.4 mM pentobarbital caused 25 per cent of the total pentobarbital-induced increase in NADH fluorescence in addition to decreases in left ventricular pressure and heart rate (Fig. 2). These results point out the magnitude of high-dose barbiturate-induced inhibition of NADH oxidation in the heart. The relative contributions of depressed cardiac function (with secondarily decreased ATP utilization) and direct inhibition of mitochondrial respiration to inhibition of NADH oxidation by barbiturates are at present uncertain.

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University of Pittsburgh School of Medicine, Pittsburgh, PA 15261, U.S.A. JEFFREY P. WEISS

Johnson Research Foundation, University of Pennsylvania School of Medicine, Philadelphia, PA 19174, U.S.A. CLYDE H. BARLOW, BRITTON CHANCE

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