Diurnal variation of hepatic amphetamine concentrations in mice fed freely and fed single daily meals

Scheving, Vedral & Pauly (1968) demonstrated a circadian rhythm in studies of the mortality of rats maintained in light-dark, continuous light and continuous dark environments to (+)-amphetamine. This may represent a rhythm in the rate at which the drug is metabolized. The activities of several hepatic oxidative drug-metabolizing enzymes in mouse and rat liver have been shown to vary with the time of day (Radzialowski & Bousqet, 1969). The possibility that the cause of the rhythmic response to the drug is related to the rate at which amphetamine is metabolized has been examined.

Feeding schedules have been shown to influence metabolism (Fuller & Snoddy, 1968; Fuller & Diller, 1970; Fuller, 1970). We now report that hepatic amphetamine concentrations in mice fed freely vary significantly over a 24 h period, and that the concentrations in mice fed a single daily meal appear to be shifted 12 h out of phase.

Retired female breeder albino mice (Charles River Mouse Farm, Wilmington), 30–60 g, were housed three per cage in large ventilated room. Fluorescent lights were mechanically switched on at 7.00 a.m. and off at 9.00 p.m. daily. Thirty mice were fed freely, and 30 were given food from 8.00 a.m. to noon daily for 4 weeks before being killed.

Three mice from each feeding schedule were injected intraperitoneally with aqueous solutions of crystalline (+)-amphetamine sulphate (K & K Laboratories, Inc.) at a dose of 10 mg/kg every 3 h within the same 24-h period. 1 h after injection, the mice were decapitated and their livers were quickly removed and frozen on dry ice. Amphetamine concentration were measured (Dubnick, Leeson & others, 1963).

Hepatic amphetamine concentrations were highest during the night and lowest during the day in mice fed freely (Fig. 1). A second rhythm was found in the meal-fed

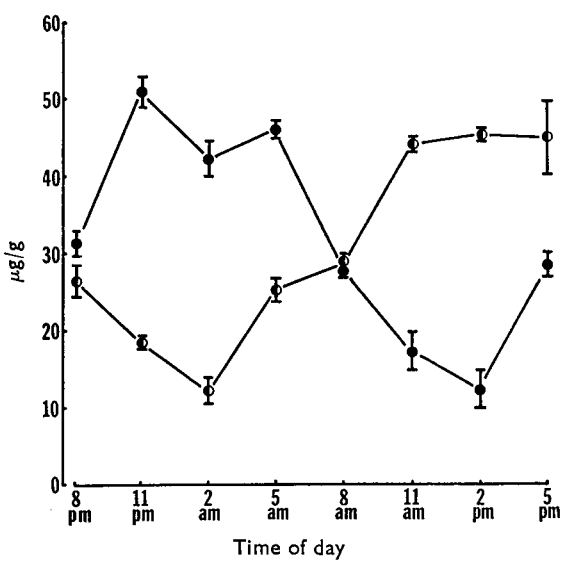

FIG. 1. Amphetamine concentration in mouse liver. The amount of drug in the 9000 *g* supernatant fraction of liver homogenates, expressed on the basis of wet tissue weight, is shown. Mean values and standard errors are shown for 3 mice per group (2 mice in the 2 a.m. and 5 a.m. groups allowed free access to food. ● Free access to food. ◐ Fed 8 a.m.–noon.

mice, indicating that a phase shift induced by meal-feeding had occurred. These results are consistent with those of Scheving & others (1968) in that high concentrations of amphetamine are correlated in time with high susceptibility to the drug. Since Radzialowski & Bousquet (1969) reported the activities of several oxidative drug-metabolizing enzymes in mice to be highest at night, the rate at which amphetamine is metabolized may not cause the rhythm. It remains to be determined that the rhythm represents a rhythm of metabolism rather than of uptake by the liver.

The Department of Biology,
The College of Wooster,
Wooster, Ohio, U.S.A.

W. STEPHEN BAUER
J. E. PERLEY

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Preliminary evidence that syrosingopine produces a selective depletion of central stores of sympathomimetic amines

The convulsive effects of leptazol are markedly facilitated by pretreating laboratory animals with reserpine (Jenney, 1954; Chen & Bohner, 1956; Kobinger, 1958; Lessin & Parkes, 1959; Pfeifer & Galambos, 1967). Reserpine is reported to produce non-selective depletion of both central and peripheral amine stores (Carlsson, 1964). These results have been confirmed in our laboratories.

Syrosingopine is a synthetic analogue of reserpine reported to produce a selective depletion of peripheral stores of sympathomimetic amines (Plummer, Barrett & others, 1959; Brodie, 1960; Orlans, Finger & Brodie, 1960).

Sixty male Porton Wistar albino rats, 200–250 g, were divided into groups of five. After pretreatment with syrosingopine at 0·4, 0·8 and 2·0 mg/kg (administered in 1 ml/kg dissolved in a mixture of 4% w/v propylene glycol, 4% ethanol and 2% lactic acid in distilled water into the penile vein of rats lightly anaesthetized with halothane), leptazol (65 mg/kg) was administered subcutaneously to the animals 4 h later and the number of clonic phases in the following 30 min period recorded, and expressed as a percentage maximum clonic convulsions (Spencer & Turner, 1969). Control animals received the vehicle intravenously under the same conditions. The mean and standard error of not less than three determinations was calculated. The results are shown in Fig. 1 (a). At 0·4 and 0·8 mg/kg there was no significant decrease of the leptazol threshold but at 2 mg/kg the number of clonic convulsive phases increased 100%. It seemed likely that at 0·8 mg/kg only peripheral amines were depleted and at 2 mg/kg there was a depletion of central amines which produced the marked decrease in leptazol threshold. Therefore, the effect of these two doses of syrosingopine on brain and cardiac amine concentrations was determined.

Thirty male Porton Wistar albino rats, 200–250 g, were divided into three groups of ten. Two groups received the syrosingopine at 0·8 or 2 mg/kg administered as before and the third group received the vehicle under identical conditions. Four h later the animals were killed and the brains and hearts removed for determination of