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DAILY RHYTHM OF METYRAPONE METABOLISM IN RATS

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Abstract—Plasma levels of metyrapone were measured in rats standardized in an alternating 12-hr-light and 12-hr-dark regimen, every 4 hr over a 24-hr period.

The results indicate a circadian periodicity with a half-life at 22·00 hr (10 p.m.) which was approximately 2·5-fold longer than that observed at 10·00 hr (10 a.m.).

METYRAPONE (SU-4885 or 2-methyl-1,2-*bis*-(3-pyridyl)-1-propanone, Metopirone®), a relatively specific 11-β-hydroxylase inhibitor, is currently used for testing the pituitary ACTH reserve.[1]

Administration of this drug to normal subjects results in a fall of cortisol secretion and a consequent release of ACTH which induces an increase of total 17-hydroxycorticosteroids in blood[1–3] due to 11-deoxycortisol and its metabolites. In normal subjects the response to metyrapone has always been significantly greater when the compound was infused in the morning than in the afternoon or the evening.[4,5] Ertel *et al.*[6,7] have reported alterations in the LD_{50} of metyrapone in mice depending on the time of day and that the peak LD_{50} values occurred at about the beginning of the dark period. It was therefore of interest to establish if such differences could have been ascribed to a different metabolism of metyrapone at different times of the day. This hypothesis was strengthened by the findings that metyrapone is reduced by the liver[8] and that some liver enzyme activities follow a circadian rhythm.[9–11]

Male Sprague–Dawley rats (120–140 g) were kept in plastic cages with food and water *ad lib.* for 2 weeks, before the experiment, in a sound-proof room with controlled temperature (24 $\pm$ 1°) and illumination (9.00 a.m. to 9.00 p.m.). Metyrapone hydrochloride (kindly obtained from CIBA, Milan), 66 mg/kg dissolved in saline, were injected i.p. in various groups of rats at different times of the day. The rats were sacrificed at 5, 15 and 30 min after the drug administration. Previous studies indicated that the disappearance of metyrapone from plasma showed the same slope when metyrapone hydrochloride was injected either i.p. or i.v.

The plasma concentration of metyrapone was determined by a newly developed method.[12,13] Plasma samples were extracted with methylene chloride (1·5:6 v/v), metyrapone was separated from its reduced metabolite on Kieselgel G thin layer plates by methylene chloride: ethanol (100:4 v/v) chromatographic system. Spots were localized by iodine vapor and then the one pertaining by the pyridyl ring specific

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color reaction of König[14] and measured spectrophotometrically at 470 mμ. The concentrations of metyrapone were plotted against time; regression lines for calculating the plasma half-life ($t_{\frac{1}{2}}$) were determined according to the method of the least squares. The experiment was repeated three times.

Extrapolation of the regression line to $t = 0$ have a volume of distribution. In our experimental conditions the distribution volume at different hours of the day remained

TABLE 1. LEVELS OF PLASMA METYRAPONE IN RATS TREATED WITH METYRAPONE (66 mg/kg i.p.) AT DIFFERENT TIMES OF THE DAY

Time of the day (hr)	Plasma level of metyrapone (μg/ml ± S.E.) min after injection		
	5	15	30
06	34·71 ± 2·28 (7)	14·87 ± 1·21 (8)	7·48 ± 0·68 (9)*
10	34·25 ± 3·30 (8)	14·66 ± 1·24 (9)	5·11 ± 0·81 (9)
14	32·55 ± 2·45 (9)	17·25 ± 0·83 (8)	8·59 ± 0·49 (9)*
18	37·55 ± 2·24 (9)	22·85 ± 1·36 (7)	13·11 ± 0·41 (9)†
22	35·00 ± 2·21 (9)	23·55 ± 1·40 (9)	15·38 ± 0·97 (8)†
02	36·75 ± 2·86 (8)	23·00 ± 1·34 (6)	13·63 ± 0·97 (9)†

In brackets the number of rats.
* $P < 0.01$ in respect to levels obtained at 10.00 a.m.
† $P < 0.001$ in respect to levels obtained at 10.00 a.m.

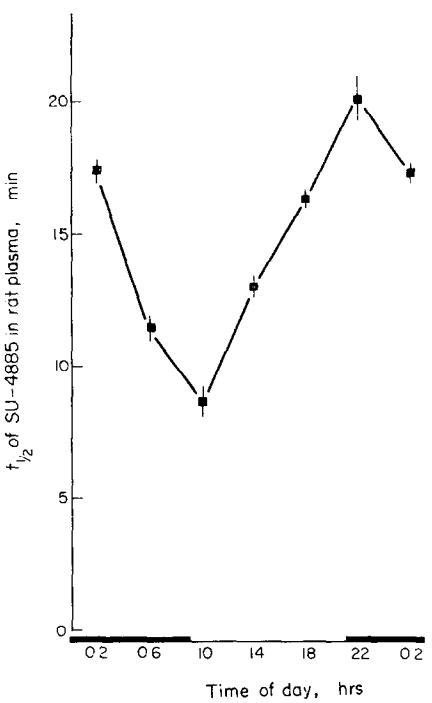

FIG. 1. Half-life ($t_{\frac{1}{2}}$ ± S.E.) of metyrapone (SU 4885) in plasma at different times of the day. Metyrapone (hydrochloride) was given i.p. at the dose of 66 mg/kg.

constant. The plasma concentration of metyrapone 30 min after the injection differs at various times with a minimum at 10.00 a.m. and a maximum at 10.00 p.m. (see Table 1).

From these data it was possible to construct the graph of Fig. 1, showing that the half-life of metyrapone was 8·6 ± 0·6 min at 10 a.m. and 20.3 ± 0·9 min at 10 p.m. These data may offer an explanation to the findings that metyrapone is more toxic to mice in the evening than in the morning.[6,7] It is also tempting to extrapolate these findings to humans suggesting that a different rate of removal of metyrapone from plasma at different times of the day may be responsible for the observed period-of-the-day-dependent different effect.[4,5]

It should be recalled that the reduced metabolite of metyrapone, 2-methyl-1, 2-*bis*-(3-pyridyl)-1-propanol or SU 5236, is considerably less active than metyrapone.[15]

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