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A daily rhythm in the rate of depletion of brain norepinephrine by reserpine

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IN THE course of investigations using reserpine in the rat, variation in mortality rate with time of day was observed. Several regions of the rat brain are subject to a circadian* cycle in endogenous norepinephrine content,[1,2] and it has been observed that metabolism[3] and efficacy[4] of some drugs oscillate daily. This communication describes a circadian rhythm in the rate of depletion of brain norepinephrine by reserpine and reports an environmental factor capable of entraining the cycle.

Female, Sprague–Dawley (160-200 g) rats were housed in clear, plastic cages and provided with Purina chow and water *ad libitum*. The animals were subjected to 50–75 ft-c. of cool white fluorescent light from 5 a.m. to 7 p.m., except where otherwise noted, for 7 days prior to each experiment.

All drugs were administered in aqueous solution by the intraperitoneal route in a volume of 1 ml. Reserpine was obtained from Ciba. Alpha-methyl-paratyrosine (α-m-t) was kindly donated by Dr. Clement Stone (Merck, Sharpe & Dohme). Rats were killed at various times over a 24 hr period by cervical dislocation and tissues were immediately removed and frozen on dry ice. Catecholamines were isolated and determined by methods previously described.[5,6] No significant differences in either whole brain or heart endogenous norepinephrine was observed among control groups killed throughout the day. Consequently, the degree of depletion by reserpine in each experiment was computed on the basis of pooled data from controls killed along with treated groups over the experimental period. DL-7-^{3}H-norepinephrine was employed in the study of turnover in brain norepinephrine. The isotope (specific activity, 9·7 c/m-mole), obtained from New England Nuclear Corp., was purified before use by elution from alumina columns. After appropriate dilution with Elliot's "B" solution (Baxter), the tracer was administered by the intracisternal route[7] to rats anesthetized with ether. All rats received 0·5 μc ^{3}H-norepinephrine (9 ng) in a volume of 25 μl.

Groups of rats were treated with 1 or 2 mg/kg of reserpine throughout the day and killed, together with controls, 4 hr later. Reduction of whole brain norepinephrine varied with time of day. Maximal depletion was observed during the dark period (Fig. 1). Reduction of endogenous norepinephrine varied significantly at a dose of 1 or 2 mg/kg from a trough at 10 a.m. to 2 p.m., to a peak at 2 a.m. With a dose of 0·5 mg/kg, no significant norepinephrine rhythm was apparent (Fig. 1).

To determine whether the observed rhythm was restricted to the central nervous system or involved organs throughout the body, heart and salivary gland were examined. With doses of 0·5, 1·0 or 2·0 mg/kg of reserpine, no statistically significant rhythm in depletion of cardiac norepinephrine was observed. Depletion of salivary gland norepinephrine with reserpine showed no consistent rhythm.

To examine whether the observed rhythm reflected differences in the rates of norepinephrine

* The term circadian is here used to denote a rhythm with a period of 24 hr without implying either an endogenous or exogenous nature.

depletion, rats were killed at nadir and peak times 8 hr (instead of 4 hr) after reserpine (2·5 mg/kg) administration. (A longer time interval was not examined, since this would involve crossing large time segments during the day.) No significant rhythm was observed. After 8 hr, norepinephrine was almost 90 per cent depleted. These results indicate that there is a diurnal rhythm in the rate, but not in the maximum depletion.

Since the catecholamine-depleting action of reserpine may be dependent upon norepinephrine turnover,[8] the half-life of whole brain norepinephrine was estimated at times of day corresponding to

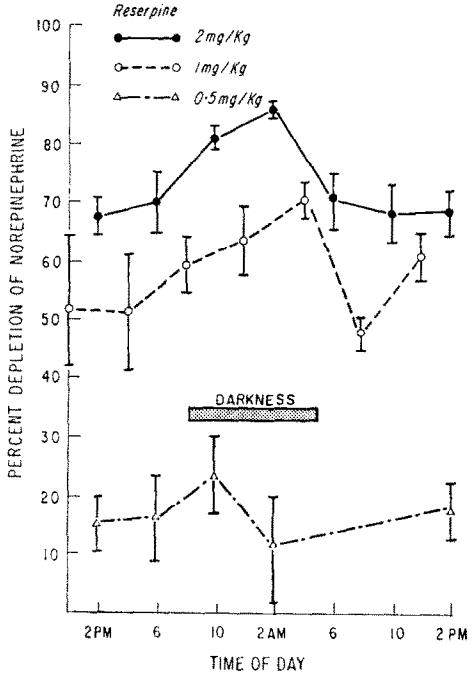

FIG. 1. Release of brain norepinephrine by reserpine at different times of the day. Rats were killed 4 hr after the intraperitoneal administration of reserpine. Results are expressed as mean depletion ± standard error. Rhythms of norepinephrine depletion are statistically significant in the cases of 2 mg/kg and 1 mg/kg at $P < 0{\cdot}01$.

nadir and peak depletion. Brain norepinephrine turnover[9] was estimated by employing the intracisternal injection of ^{3}H-norepinephrine. Rats were treated at 2 p.m. and at 8 p.m. and groups were killed 1, 3 and 5 hr later. Although the half-life was decreased at night, differences were not significant (Table 1). Similar results were obtained using α-m-t for estimation of brain norepinephrine turnover.

TABLE 1. HALF-LIFE OF BRAIN NOREPINEPHRINE AT DIFFERENT TIMES OF THE DAY*

		2 p.m.	8 p.m.
^{3}H-NE	Slope	−0·2369 ± 0·0363	−0·2913 ± 0·0242
	Half-life (hr)	2·92	2·38

* Experiments with ^{3}H-norepinephrine are described in the text. Data were plotted semilogarithmically by the method of least squares, weighted for animal number and standard error. Slopes are expressed ± S.E. Half-life was calculated from the equation $T\frac{1}{2} = \log_{10} 2/K$, where $T\frac{1}{2}$ is the half-life and K is the slope.

The effect of environmental lighting on the norepinephrine depletion rhythm was investigated by subjecting rats to a reversed schedule for 1 week, with lights on from 7 p.m. to 5 a.m. Under these conditions, the rhythm was reversed in time, with maximal depletion during the period of darkness (5 a.m. to 7 p.m.; Fig. 2).

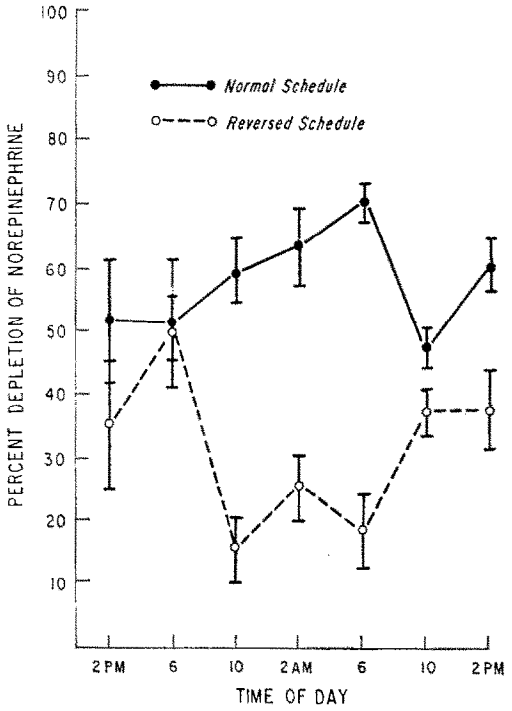

FIG. 2. Release of brain norepinephrine on a reverse lighting schedule. Rats were treated with reserpine (1 mg/kg) intraperitoneally as in Fig. 1. The normal lighting schedule consisted of lights on from 5 a.m. to 7 p.m. In the reversed schedule, lights were on from 7 p.m. to 5 a.m. Both rhythms are significant at $P < 0{\cdot}01$.

It is apparent that environmental lighting is capable of entraining the rhythm, since a reversed lighting schedule shifts the phase of the rhythm by approximately 180°. However, the data presented do not establish an endogenous nature of this rhythm.

Although our studies indicated a more rapid turnover of brain norepinephrine at night, differences were not statistically significant. It is possible that differences in turnover in discrete brain areas might not be discernible by our methods. Circadian rhythms in drug metabolism or drug absorption would be expected to produce a generalized rhythm of depletion involving the heart and salivary glands as well as the brain. Restriction of the rhythm in reserpine depletion of norepinephrine to the central nervous system tends to implicate an inherent characteristic of the brain rather than a generalized oscillation in the organism. A diurnal variation in the sensitivity of the noradrenergic storage vesicle to norepinephrine release by reserpine may reflect either a rhythm residing in the vesicle or an oscillation of factors affecting vesicles, such as nerve impulse frequency.

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