Circadian Rhythm in Adrenal Adenyl Cyclase and Corticosterone Abolished by Medial Forebrain Bundle Transection in the Rat

The rat adrenal gland exhibits a circadian rhythm in corticosterone content which is under the control of environmental light[1]. The visual control of the rhythm is mediated by the hypothalamus and anterior pituitary[2,3], and recent evidence indicates that the adrenocorticotrophic hormone (ACTH) affects corticosterone synthesis by increasing cyclic AMP (adenosine 3′,5′-monophosphate) in adrenal cortical cells[4]. The synthesis of cyclic AMP is carried out by the enzyme, adenyl cyclase[5]. And, although there is in vitro evidence that ACTH stimulates adrenal adenyl cyclase activity[4,6], this has not been demonstrated clearly in the intact animal. In the present study we obtained further evidence for this by demonstrating a diurnal rhythm in adenyl cyclase with timing very similar to that for the release of ACTH from the pituitary. In addition we have found that the integrity of a major hypothalamic projection system, the medial forebrain bundle (MFB), is necessary to maintain these rhythms in the rat adrenal.

70-day-old female albino rats (Holtzman Co., Madison, Wisconsin) were placed in clear plastic cages in racks illuminated on a diurnal schedule (lights on 07.00 h to 19.00 h) by Vita-Lite fluorescent bulbs (Duro-Test Corp., Chicago, Illinois). With the lights on, the rats were exposed to approximately 50 foot-candles of light. 3 groups of rats were used, normal, sham operated, and MFB lesions. Bilateral transection of the MFB was performed in the later group using a modified Halasz knife[7]. Histological study of the brains from the MFB group confirmed that the MFB lesions transected that tract in the lateral hypothalamus at the level of the ventromedial hypothalamic nucleus. None of the operated animals developed any abnormality of eating or drinking behavior and their body weights at the time of sacrifice did not differ significantly from the control groups. The animals were killed by decapitation 3 weeks after operation at 3 h intervals (4 animals from each group at each interval) beginning at 04.00 h and ending at 22.00 h. Their adrenals were removed rapidly, weighed and frozen on dry ice. Subsequently they were homogenized in *Tris* buffer (pH 7.3, $4 \times 10^{-2} M$) and one aliquot was taken for corticosterone analysis by a modification of the method of SILBER et al.[8]. A second aliquot was used for analysis of adrenal adenyl cyclase activity[9].

A summary of the adrenal corticosterone data is shown in Figure 1. In the normal rat there is a diurnal rhythm in adrenal corticosterone content with the lowest daily value occurring at 07.00 h and the highest value at 19.00 h. In contrast to the corticosterone rhythm the adenyl cyclase rhythm (Figure 2) in normal animals shows a low level at 04.00 h and then rises rapidly to a peak at 22.00 h. Sham operated animals do not differ significantly from normals. The rise in adrenal adenyl cyclase in the morning suggests that ACTH stimulates the enzyme in this time period. It is known that pituitary ACTH content is highest in the morning[3,10], and that plasma ACTH levels peak between 07.00 h and 13.00 h[3]. Thus, the findings in the normal animal are in accord with the view that central neural events trigger the

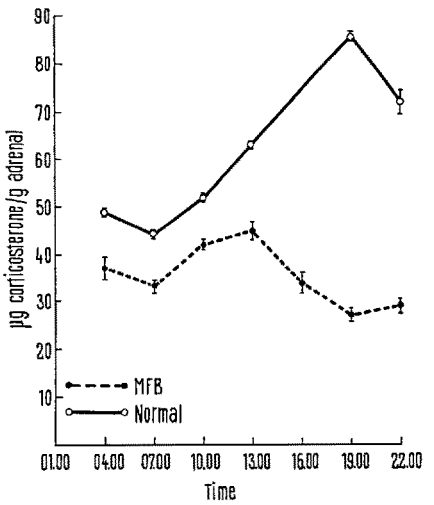

Fig. 1. Adrenal corticosterone rhythm in the normal rat (continuous line) and the rat with bilateral transection of the medial forebrain bundle (MFB interrupted line). The vertical bars above and below each point represent the standard error of the mean.

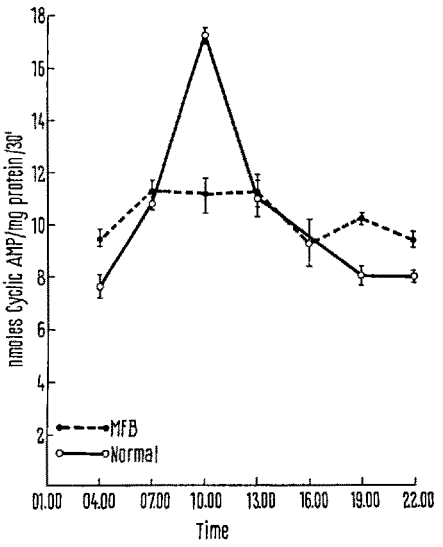

Fig. 2. Adrenal adenyl cyclase rhythm in the normal rat (continuous line) and in the rat with bilateral transection of the medial forebrain bundle (MFB, interrupted line). The vertical bars above and below each point represent the standard error of the mean.

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release of ACTH in the morning and that ACTH stimulates the activity of adenyl cyclase to produce cyclic AMP which, in turn, stimulates corticosterone production. This is further supported by observations that the adenyl cyclase rhythm, like the corticosterone rhythm[1], has the properties of a circadian rhythm and, in addition, is eliminated by hypophysectomy[11].

The animals with hypothalamic lesions provide further information on the central pathways controlling the rhythms. Both the corticosterone (Figure 1) and adenyl cyclase (Figure 2) rhythms are abolished by bilateral transection of the MFB. In these operated animals the corticosterone levels are all below the normal levels but the differences between the two groups are only significant at the points around the peak in the normal curve. The MFB animals appear to show an attenuated curve but the difference between the low (19.00 h) and high (13.00 h) points only approaches statistical significance ($p < 0.10 > 0.05$; two-tailed t-test). The adenyl cyclase levels in the MFB animals show minor fluctuations in a range around the mid-point in the normal cycle but the curve is nearly flat. Thus, both rhythms are eliminated by section of the MFB; an effect identical to that of bilateral MFB lesions on pineal rhythms of serotonin and the melatonin-forming enzyme, hydroxyindole-*O*-methyltransferase (HIOMT)[12]. The alteration of the HIOMT rhythm can be attributed to section of a visual pathway, the inferior accessory optic tract, which runs among the fibers of the MFB in the rat[13], but this will not account for the effects of MFB section on the pineal serotonin or the adrenal rhythms, since elimination of visual input should not abolish these circadian rhythms but only cause them to become free-running[1,11]. Lesions transecting the medial forebrain bundle are known to produce substantial decreases in brain monamines[14] and should destroy, in particular, serotonin-containing axons arising in the brainstem raphe nuclei to traverse the medial forebrain bundle before innervating the suprachiasmatic nuclei[15]. It has been suggested recently that the circadian corticosteroid rhythm is mediated by serotonergic neural mechanisms[16]. If this is the case, and the serotonergic innervation to the suprachiasmatic area is essential, raphe lesions should be equivalent to MFB lesions in their effects on adrenal rhythms. The MFB lesion effect on the adrenal corticosterone rhythm is very similar to that of anterior deafferentiation of the medial hypothalamus[10], suggesting that both lesions might interrupt a critical pathway to the tuberal hypothalamus. Regardless of the mechanism of the MFB lesion effect, it is evident that this pathway participates in the neural regulation of circadian rhythms in the rat adrenal gland[17].

Zusammenfassung. Nachweis, dass die Nebennierenrinde von Ratten nicht nur eine rhythmische Tagesschwankung in ihrem Gehalt an Corticosteron, sondern auch eine Schwankung im Gehalt an Adenyl-Cyclase aufweist. Diese Rhythmen können durch eine stereotaktisch ausgeführte Trennung des medianen Vorderhirnbündels, welches zum Hypothalamus führt, aufgehoben werden.

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[17] This work was supported by research grants No. NS-05002 and No. HD-04581 from the National Institutes of Health, USPHS.

A Comparison of L-Asparaginase from *Erwinia aroideae* and from *Escherichia coli*: Biochemical and Biological Properties

In search of L-asparaginases from sources, other than *E. coli*, which were suitable for a large scale production WADE[1-3] and PETERSON[4,5] found several strains of the plant pathogens *Erwinia* producing L-asparaginase in high yield. Treatment of mice bearing 6 H3 HED tumors with L-asparaginase from *Erwinia* caused regression of the tumors. NORTH[6] described the crystallization of the enzyme and some physical data.

Since there is no immunological cross-reaction between L-asparaginases from *Erwinia* and from *E. coli* (Figure 1), their exchange during leukaemia therapy might be favorable when allergic reactions are to be expected.

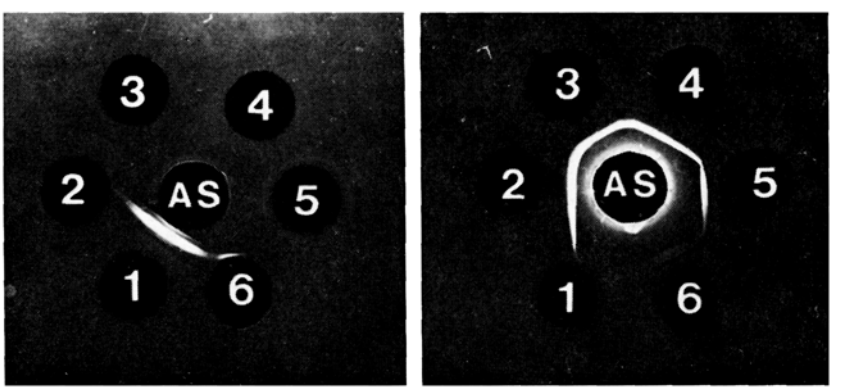

Fig. 1. Immunodiffusion according to Ouchterlony. Antisera from rabbits are applied to the center wells. The left exposure shows the precipitation with antiserum against L-asparaginase from *Erwinia aroideae*, the right exposure the precipitation with antiserum against L-asparaginase from *E. coli*. In both exposures sample 1 represents L-asparaginase from *E. aroideae*, sample 2–6 are EC2 L-asparaginases from various strains of *E. coli*. There is no immunological crossreaction between the 2 L-asparaginases.