6. This new species of the worldwide genus *Actinostola* has been identified by C. E. Cutress of the Institute of Marine Biology, University of Puerto Rico. Until it has been formally described, a specific name cannot be given. We thank Dr. Cutress for permission to use his unpublished identification of this species in reporting our observations of its behavior, observations that were meaningless while the animal continued to be wrongly identified as *Stomphia coccinea*.
7. D. M. Ross and L. Sutton, in preparation.
8. E. A. Robson, *Symp. Zool. Soc. London* **16**, 333 (1966).
9. This work was carried out at the Friday Harbor Laboratories of the University of Washington. We thank the director, Dr. R. Fernald, for hospitality and help. Supported by the National Research Council of Canada (operating grant A-1445).

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Circadian Pattern of Plasma 17-Hydroxycorticosteroid: Alteration by Anticholinergic Agents

Abstract. *Atropine, administered to cats just prior to the time of the expected circadian rise in levels of 17-hydroxycorticosteroid in plasma, blocks this rise. Atropine does not alter this circadian pattern when administered at other times in the circadian cycle. Results similar to those obtained with atropine have been observed with short-acting barbiturates. Dibenzyline administered just prior to the time of the expected circadian rise is ineffective in blocking this rise. These findings support the hypothesis that the circadian pattern of plasma 17-hydroxycorticosteroid levels reflects activation, by the central nervous system, of the hypothalamic-pituitary-adrenal axis during a "critical time period" in the circadian cycle.*

The occurrence of a circadian pattern in levels of plasma 17-hydroxycorticosteroids (17-OHCS) and in other parameters of adrenal cortical function has been well documented in man (*1*), monkeys (*2*), rats (*3, 4*), and mice (*5*). The constancy of this pattern in man during illness (*6, 7*), night work (*1*), and total bed rest (*8*), as well as the difficulty of altering this pattern with change in activity-sleep routine (*9*), have been commented upon. Alterations in this pattern have been reported in patients with chronic, diffuse, central nervous system disease accompanied by impairment of consciousness and by delirium or restlessness (*2*). Alterations in this pattern have also been reported in our studies (*10*) which described such abnormalities in patients with central nervous system disease localized in the temporal lobe or in pretectal or hypothalamic areas. These observations suggest that the central nervous system plays a dominant role in determining this daily variation in plasma 17-OHCS levels.

The phenomenon of sleep, and its accompanying rapid-eye-movement periods (*11*), suggest circadian function of the nervous system (*12*), as does the circadian variation of the human electroencephalogram (*13*). Central nervous system regulation of pituitary adrenocorticotropin (ACTH) secretion is well established (*14*). Integrity of some portion of the hypothalamus seems to be a necessary factor in this regard (*10, 15*). There is preliminary evidence from the mouse (*5*) and from the rat (*3*) of circadian variation in the hypothalamic content of pituitary corticotrophic stimulatory factor.

The nature of the mechanisms initiating the release or elaboration, or both, of hypothalamic releasing factors has still to be elucidated. Some workers (*16*) have introduced the concept of a "variable set point" component in the central nervous system. It is possible that this set point, in addition to being "reset" by steroid levels, may also be reset by a variety of changes in the internal or external milieu acting on the central nervous system, as well as by the state of synaptic activity at the hypothalamic secretory cell involved. We have demonstrated (*17*) that implantation of minute amounts of cholinergic and adrenergic agents in certain areas of the hypothalamus is capable of eliciting an abrupt, prompt rise in plasma 17-OHCS levels in the cat. Similar results have also been reported by Endroczi (*18*).

Our hypothesis in the present investigation is that the observed circadian pattern in levels of plasma 17-OHCS reflects the release of corticotrophic-releasing factor, and consequently ACTH, at one *critical period* in the 24-hour cycle, immediately preceding the period when levels of plasma 17-OHCS begin their circadian rise. If we accept the findings that activity of the nervous system is circadian, and that pituitary adrenal activation can be effected by synaptically active agents, then it should be possible to abolish the circadian rise in plasma 17-OHCS levels by administering, just prior to the postulated "critical period," drugs which can block synaptic transmission and thereby block release of corticotrophic-releasing factor.

This concept is analogous to the "critical period" described for release of luteinizing hormone in the rat (*19*). Such release can be blocked by the administration of anticholinergic and anti-

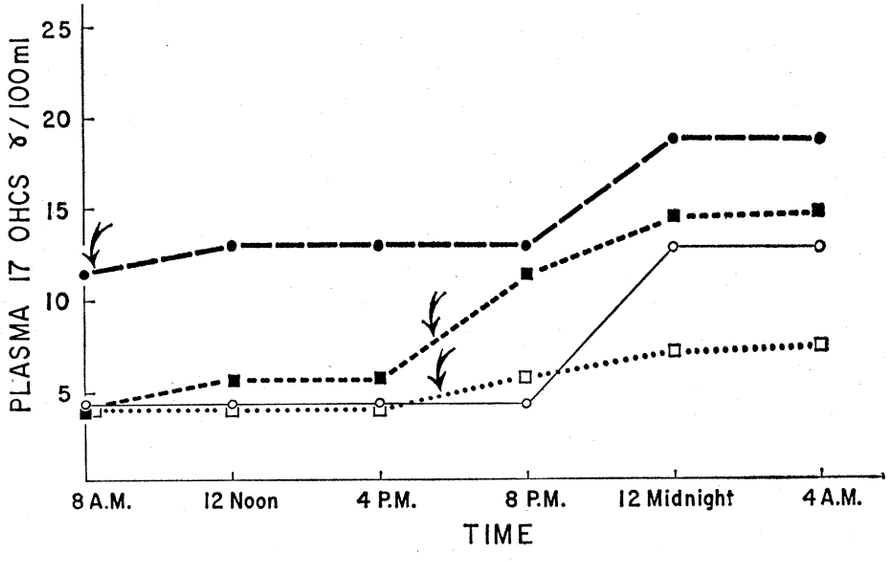

Fig. 1. Effect of atropine administered in different dosages and at different times of day on circadian pattern of plasma 17-OHCS in a cat (No. 224). Arrows indicate time of administration of drug. Key: ○——○, control day; □ · · · · □, atropine, 1.2 mg subcutaneously (0.4 mg/kg) at 6 p.m.; ■- - - - -■, atropine, 0.6 mg subcutaneously (0.2 mg/kg) at 6 p.m.; ●— — —●, atropine, 1.2 mg subcutaneously at 8 a.m.

adrenergic drugs, as well as by barbiturates, just prior to or during this period (*20*).

To test our hypothesis, we first demonstrated the occurrence and constancy of a circadian pattern in plasma 17-OHCS levels in ten cats. Figures 1 and 2 (solid line) show this pattern to consist of a peak level occurring between 8 p.m. and 4 a.m., with lower levels occurring during the remainder of the 24-hour cycle.

Atropine was selected for its anticholinergic action. Six animals received atropine subcutaneously at 6 p.m. in amounts varying from 0.4 to 0.6 mg/kg. Systemic manifestations of atropine effect were present in all instances. Figures 1 and 2 show that such a dose abolished the nocturnal rise in plasma steroid levels. Doses of 0.2 mg/kg, administered at a similar time, were ineffective (Figs. 1 and 2) in suppressing this nocturnal rise. Administration of 0.4 to 0.6 mg/kg at 8 a.m. was also ineffective in suppressing the nocturnal rise in plasma 17-OHCS levels.

Dibenzyline was used as an antiadrenergic drug. Six animals received 10 to 20 mg/kg in the form of an intravenous infusion over a 1-hour period from 6 to 7 p.m. During the infusion, all animals developed prominence of the nictitating membranes. In the animals receiving the higher doses, flaccid weakness of the posterior extremities occurred. Figure 2 illustrates the ineffectiveness of dibenzyline (administered at 6 p.m.) in blocking the evening rise in plasma steroid levels. This is in marked contrast to the effect of atropine when administered at a similar time.

Sodium pentobarbital (30 mg/kg intraperitoneally), administered to four cats at 6 p.m., completely suppressed the circadian rise in plasma 17-OHCS levels. A similar suppression was observed, however, when this dose was administered at 8 a.m. These observations, therefore, could not be used to determine whether or not barbiturates blocked at a "critical period" prior to release of ACTH. For this reason, sodium thiamylal, a much shorter acting barbiturate, was studied. Intravenous administration (from 6 to 7 p.m.) of 10 to 20 mg/kg of freshly prepared solutions of sodium thiamylal was effective in blocking the circadian rise in plasma 17-OHCS levels. In contrast, the same, as well as larger (25 mg/kg), doses were ineffective when similarly administered at 8 a.m.

In discussing drug blockage of release of luteinizing hormone, Sawyer (*20*) suggested that this phenomenon might be secondary to the increase in the threshold of electroencephalographic arousal in cortex and brain stem, on direct stimulation of the midbrain reticular formation, brought about by these drugs. The reticular formation has been postulated to be part of the central nervous system pathway regulating release of ACTH (*21*). His results and ours might also be understood in terms of alterations in synaptic transmission in the pathways converging in the hypothalamus, as well as in the neurons of the hypothalamus itself.

Our findings lend credence to the hypotheses that (i) cholinergic mechanisms are involved in release of ACTH, and (ii) these are effective over a relatively discrete, brief period in the 24-hour cycle in initiating the circadian rise in levels of plasma 17-OHCS. The ability of short-acting barbiturates in this regard also provides evidence for central nervous system mechanisms in the regulation of the circadian pattern of levels of plasma 17-OHCS. Further studies with drugs affecting levels of other postulated central neurotransmitters would help to delineate the nature of the pathways involved.

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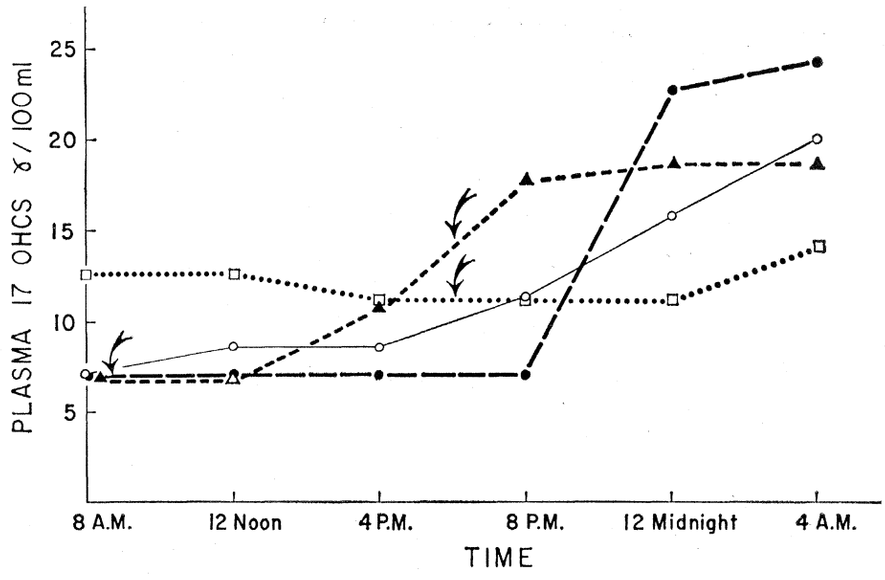

Fig. 2. Comparison of effects of atropine and dibenzyline on circadian pattern of plasma 17-OHCS in a cat (No. 232). Arrows indicate time of administration of drug. Key: ○———○, control day; ●———●, atropine, 1.2 mg subcutaneously (0.4 mg/kg) at 8 a.m.; □ · · · · □, atropine, 1.2 mg subcutaneously at 6 p.m.; ▲- - - -▲, dibenzyline, 50 mg (17 mg/kg) intravenously at 6 p.m.

References and Notes

1. C. J. Migeon, F. H. Tyler, J. P. Mahoney, A. A. Florentin, H. Castle, E. L. Bliss, L. T. Samuels, *J. Clin. Endocrinol. Metab.* **16**, 622 (1956).
2. J. W. Mason, *Am. J. Physiol.* **190**, 429 (1957).
3. U. K. Rinne and V. Sonninen, *Acta Anat.* **56**, 131 (1964).
4. V. Critchlow *et al.*, *Am. J. Physiol.* **205**, 807 (1963).
5. F. Ungar, *Ann. N.Y. Acad. Sci.* **117**, 374 (1964).
6. G. T. Perkoff, K. Eik-Nes, C. A. Nugent, H. L. Fred, R. A. Nimer, L. Rush, L. T. Samuels, F. H. Tyler, *J. Clin. Endocrinol. Metab.* **19**, 432 (1959).
7. L. J. Sholiton, E. E. West, Jr., R. T. Marnell, *Metabolism* **10**, 632 (1961).
8. D. Cordus, C. Vallbona, F. B. Vogt, W. A. Spencer, H. S. Lipscomb, K. Eik-Nes, *Aerospace Med.* **36**, 524 (1965).
9. G. W. G. Sharp, S. A. Slorach, H. J. Vipond, *J. Endocrinol.* **22**, 377 (1961).
10. D. T. Krieger and H. P. Krieger, *J. Clin. Endocrinol. Metab.* **26**, 929 (1966).
11. E. Aserinsky and N. Kleitman, *Science* **118**, 273 (1953).
12. G. Moruzzi, *Active Processes in the Brain Stem during Sleep* (Harvey Lectures) (Academic Press, New York, 1963), p. 233.
13. G. S. Frank, in *Report of the Thirty-Ninth Conference on Pediatric Research*, S. J. Fomon, Ed. (Ross Laboratories, Columbus, Ohio, 1961), p. 48.
14. G. W. Harris, in *Handbook of Physiology*, Section I, *Neurophysiology*, J. Field, W. H. Magoun, V. E. Hall, Eds. (American Physiological Soc., Washington, D.C., 1960), vol. 2, p. 1007.
15. M. A. Slusher, *Am. J. Physiol.* **206**, 1161 (1964).
16. F. E. Yates and J. Urquhart, *Physiol. Rev.* **42**, 359 (1962).
17. D. T. Krieger and H. P. Krieger, *Proc. 2nd. Intern. Congr. Endocrinol. London* (1964), p. 640.
18. E. Endroczi, G. Schrieberg, K. Lissak, *Acta Physiol. Acad. Sci. Hung.* **24**, 211 (1963).
19. J. H. Everett and C. H. Sawyer, *Endocrinology* **47**, 198 (1950).
20. C. H. Sawyer, B. V. Critchlow, C. A. Barraclough, *ibid.* **57**, 345 (1955).
21. W. J. H. Nauta, in *Advances in Neuroendocrinology*, A. J. Nalbandov, Ed. (Univ. of Illinois Press, Urbana, 1963), p. 912.
22. Supported by PHS grant No. NB-02893.

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