reaction[12]. Finally, the increase in cellular lysolecithin might play an important role in the regulation of guanylate and adenylate cyclase activities[13].

1 This work has been partly supported by a grant from Consiglio Nazionale delle Ricerche.
2 To whom reprint requests should be addressed.

3 J.C. Mac Giff, N.A. Terragno, K.U. Malik and A.J. Lonigro, Circulation Res. *31*, 36 (1972).
4 J. Colina-Chourio, J.C. Mac Giff, M.P. Miller and A. Nasjletti, Br. J. Pharmac. *58*, 165 (1976).
5 H.S. Margolius, R.G. Geller, W. De Jong, J.J. Pisano and A. Syoerdsma, Circulation Res. *31*, 125 (1972).
6 R.G. Geller, H.S. Margolius, J.J. Pisano and H.C. Keiser, Circulation Res. *31*, 857 (1972).
7 H. Kunze and W. Vogt, Ann. N.Y. Acad. Sci. *180*, 123 (1971).
8 O.H. Lowry, N.J. Rosebrough, A.L. Farr and R.J. Randall, J. biol. Chem. *193*, 265 (1951).
9 B. Baggio, S. Favaro, A. Antonello and A. Borsatti, Res. exp. Med. *169*, 77 (1976).
10 A. Adetuyibi and J.H. Mills, Lancet *2*, 203 (1972).
11 A. Nasjletti, J.C. Mac Giff and J. Colina-Chourio, Circulation Res. *43*, 799 (1978).
12 S. Sen, R.B. Smeby and F.M. Bumpus, Biochemistry *6*, 1572 (1967).
13 W.J. Shier, J.H. Baldwin, M. Nelson-Hamilton, R.T. Hamilton and N.M. Thanassi, Proc. nat. Acad. Sci. USA *73*, 1586 (1976).

Circadian variation of adrenocortical cyclic nucleotides (cyclic AMP and cyclic GMP) in hypophysectomized rats

J. Guillemant, S. Guillemant and A. Reinberg[1]

Faculté de Médecine Pitié-Salpêtrière, 91 bld. de l'Hôpital, F-75634 Paris cedex 13, and E.R. Chronobiologie Humaine CNRS No. 105, Fondation A. de Rothschild, 29, rue Manin, F-75940 Paris cedex 19 (France), 27 June 1979

Summary. The existence of a circadian variation in the adrenocortical concentrations of cyclic AMP and cyclic GMP in male adult Wistar rats examined 10 days after hypophysectomy is demonstrated. The results suggest that the circadian variations of adrenocortical cyclic nucleotides observed previously in intact rats might not entirely depend upon pituitary corticotrophin.

A previous report[2] has examined the circadian rhythms in adrenocortical cyclic AMP(cAMP) and cyclic GMP (cGMP) as compared to the circadian variation of blood corticosterone. The question then arose whether the circadian variations of the adrenocortical cyclic nucleotides observed in intact rats were completely under the control of the pituitary. The present experiment is designed to answer that question.

Materials and methods. 52 hypophysectomized male Wistar rats weighing around 160 g each were supplied by IFFA-CREDO (Saint-Germain-sur-l'Arbresle, France). They had been hypophysectomized using the auricular technique under ether anaesthesia. The effectiveness of hypophysectomy was assessed by the following criteria: no weight gain, adrenal atrophy and undetectable blood corticosterone (below 0.2 µg/100 ml). The animals were used 10 days subsequent to operation. During this period they were maintained at a temperature of 22 °C with dry rat pellets and salt water available ad libitum. They were synchronized with natural day/light alternation. On the day of killing, sunrise occurred at 05.49 and sunset at 21.56 (local time = GMT + 2). The animals were sacrificed one by one at 10 min. intervals in series of 8-9 subjects. Each adrenal was decapsulated and its zona medullaris removed. The efficiency of this procedure in isolating only the fasciculata-reticularis zona has been assessed by histological examination[1]. The adrenocortical tissue was then lyophilized and weighed. The lyophilized tissues were extracted and the cyclic nucleotides determined by radioimmunoassay according to Cailla et al.[3,4].

The excellent specificity of the antibodies used (cross-reactivity between succinyl cAMP and succinyl cGMP was less than 0.01%) allowed direct measurement of the 2 cyclic nucleotides without their initial separation. Both conventional and Cosinor methods 5 were used for statistical analysis of the time series. Chronograms were drawn by plotting the studied variables (mean ± SEM for each group of animals) as a function of time (clock hours).

The differences between peaks and troughs have been checked using Student's t-test. The Cosinor method was used for both rhythm validation and quantification. The latter method involves computer programs in combination with the least-squares method in order to find the best fitting cosine function which approximates all data. With this method, a rhythm is validated when its amplitude (half of the peaktrough difference) differs from zero where $p < 0.05$. Furthermore, the Cosinor method takes the actual time of the death of each animal into account.

Results. Chronograms are shown in figures 1 and 2. It appears from the patterns of these chronograms that a circadian variation is present for adrenocortical cAMP and cGMP, since both curves are roughly a sine function.

Cosinor summary circadian rhythms in adrenocortical cAMP and cGMP in hypophysectomized rats

Variable tested	Rhythm detection	Mesor M ± SEM	Amplitude Mean	Range*	Acrophase h/min Mean	Range*
Cyclic AMP	$p < 0.005$	26.4 ± 2.8	2.5 [9.5]	1.6-3.5 [6.1-13.3]	05.24	03.57-06.54
Cyclic GMP	$p < 0.005$	0.46 ± 0.03	0.063 [13.7]	0.034-0.093 [7.4-20.2]	08.00	06.00-10.00

52 mature male Wistar rats hypophysectomized 10 days before. Synchronization with natural day-light. Sunrise at 05.49 and sunset at 21.56 (local time) on the day of killing. Sampling intervals $\Delta t = 4$ h. Trial period $\tau = 24$ h. The mesor or 24 h rhythm adjusted average is given in pmole/mg dry weight. The amplitude or half of the peak-trough difference is given in pmole/mg dry weight and as a percentage of the mesor in brackets. The acrophase or peak time is expressed in h and min. Phase reference is 00.00 (local midnight). * 95% confidence limits.

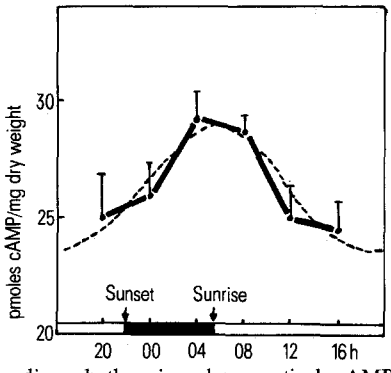

Fig. 1. Circadian rhythm in adrenocortical cAMP (fasciculata-reticularis zona) of mature male Wistar rats hypophysectomized 10 days before. Synchronization with natural day-light. Temperature 22±1 °C. Food and water available ad libitum. Sampling interval ≃4 h (8-10 animals). ·—· Raw data (chronogram) with time point mean±SEM in pmole cAMP/mg dry weight. --- Cosinor analysis: best sine function with period $\tau = 24$ h.

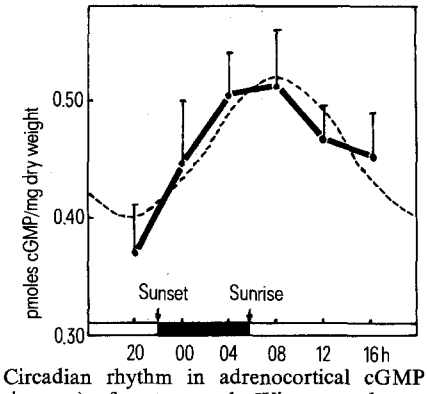

Fig. 2. Circadian rhythm in adrenocortical cGMP (fasciculata-reticularis zona) of mature male Wistar rats hypophysectomized 10 days before (cf. legend to figure 1). ·—· Raw data (chronogram) with time point mean±SEM in pmole cGMP/mg dry weight, --- Cosinor analysis: best fitting sine function with period $\tau = 24$ h.

Peak-trough differences are statistically significant with $p < 0.02$ for cAMP and $p < 0.05$ for cGMP. The results obtained from raw data were confirmed by Cosinor analysis (table and figures 1 and 2). Statistically significant circadian rhythms were validated for both variables (amplitude differing from zero with $p < 0.005$). The peaks are located at about 05.30 and 08.00 for cyclic AMP and cyclic GMP respectively, that is, at the end, or just after the end of the darkness period. The amplitudes expressed as a percentage of the mesor are comparable for both nucleotides, equalling 9.5% for cAMP and 13.7% for cGMP. Since the cyclic nucleotide concentrations have been expressed as a function of dry weights, these were verified to be constant throughout the experiment.

Discussion. As the adrenocortical dry weights remain constant throughout the nycthemere, it can be inferred that the circadian variations observed reflect a true modification of the cyclic nucleotide concentrations. Expressing the results as a function of adrenocortical protein concentrations (not shown here), gives comparable results. The somewhat surprising finding of a circadian variation of the adrenocortical cyclic nucleotides in hypophysectomized rats raises some unresolved questions about the origin and role of these cyclic nucleotides in the adrenal cortex. The persistence of rhythmic adrenal activity has been noted in hypophysectomized rats implanted with pellets of ACTH and thyroxine[6]. Although not much attention has generally been paid to the relationship between the nervous system and the adrenal cortex, some experiments indicate such a link. Denervation of the rat adrenal has been shown to diminish the elevation of cAMP induced by stress[7]. In rats with adrenal autotransplants, daily variations in plasma corticoids are no longer observed[8]. Following these observations, some authors have postulated the existence of a neural pathway to the adrenal which would regulate the circadian variation of adrenal steroidogenic activity[8]. On the other hand, persistence of circadian rhythms has been demonstrated in adrenal tissue cultures[9,10]. Whatever the mechanism involved in the present findings, the demonstration of a circadian variation of adrenocortical cyclic nucleotides in hypophysectomized rats is an additional proof of at least a partially extrapituitary regulation of the circadian rhythm in adrenocortical function.

1 Acknowledgment. Thanks are due to Prof J. M. Racadot and Dr Y. Drouet for their assistance with the histological examinations.

2 S. Guillemant, J. Eurin, J. Guillemant and A. Reinberg, Annls Endocr. *39*, 49 (1978).
3 H.L. Cailla, M.S. Racine-Weisbuch and M.A. Delaage, Analyt. Biochem. *56*, 394 (1973).
4 H.L. Cailla, C.J. Vannier and M.A. Delaage, Analyt. Biochem. *70*, 195 (1976).
5 F. Halberg, Y.L. Tong and E.A. Johnson, in: The Cellular Aspects of Biorhythms, p.20. Ed. H. v. Mayersbach. Springer, Berlin 1967.
6 A.H. Meier, Endocrinology *98*, 1475 (1976).
7 M.I. Paul, R. Kyetnansky, H. Cramer, S. Silbergeld and I.J. Kopin, Endocrinology *88*, 338 (1971).
8 J.E. Ottenweller, A.H. Meier, B.R. Ferrell, N.D. Horseman and A. Proctor, Endocrinology *103*, 1875 (1978).
9 R.V. Andrews and G.E. Folk, Jr, Comp. Biochem. Physiol. *11*, 393 (1964).
10 R. Shiotsuka, J. Jovonovich and J.A. Jovonovich, in: Chronobiological Aspects of Endocrinology, p.255. Ed. J. Aschoff, F. Ceresa and F. Halberg. Schattauer, Stuttgart, New York 1975.

High affinity binding of labeled androgens in the androgen-target tissues of the male rhesus monkey[1]

T.N.R.V. Thampan[2], N. Dinakar and M.R.N. Prasad

Department of Zoology, University of Delhi, Delhi 110007 (India), 27 April 1979

Summary. High affinity testosterone (T)-specific and dihydrotestosterone (DHT)-specific binding sites exist in a 1:2 ratio in the cytosol fractions of the caput epididymidis, prostate, seminal vesicles and the ductus deferens of the rhesus monkey. The number of androgen-binding sites in the caput epididymidis is 3 times greater than that of the other 3 tissues.

The interaction of a steroid hormone with a cytoplasmic macromolecular receptor in the target cell is one of the earliest events recognized in the mechanisms of steroid hormone action. In an earlier study on the androgen metabolism in the monkey[3,4], we had reported that after in vivo treatment with ^{3}H-testosterone, the tissue-bound an-