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CIRCADIAN RHYTHMS IN ADRENOCORTICAL ACTIVITY DURING AND AFTER A 36 HOUR 4-HOURLY-SUSTAINED ADMINISTRATION OF METYRAPONE IN HUMANS

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SUMMARY

The circadian variations of plasma cortisol, plasma 11-deoxycortisol and their urinary metabolites, 17-hydroxycorticosteroids and tetrahydro-11-deoxycortisol, have been investigated on two healthy young human males before, during and after a 36 h sustained oral administration of metyrapone (total dose: 7.50 g). Circadian changes were analysed by the cosinor method. Subjects were synchronized with diurnal activities from 0715 h to 2300 h and nocturnal rest.

A circadian rhythm is likely to be present for plasma cortisol before, and plasma 11-deoxycortisol after metyrapone administration. A prominent peak has been shown at a trial period of 24 h. A circadian rhythm seems to be present in 17-hydroxycorticosteroids excretion before during and after metyrapone administration. In tetrahydro-11-deoxycortisol excretion it was found only during and after, and in the estimated portion of 11-hydroxylated tetrahydrocorticosteroids before and after, but not during metyrapone administration. The acrophases of validated rhythms in urinary steroids are located between 1130 h and 1630 h.

INTRODUCTION

A fall in the production of cortisol and 11-hydroxylated steroids usually results from metyrapone* (2-methyl-1,2-bis[3-pyridyl]-1-propanone) administration. Changes in the effects of metyrapone on the adrenal steroidogenesis as a function of the timing of its administration have been reported by several authors [1–9].

However, these studies did not take into consideration time series of either plasma 11-deoxycortisol or urinary tetrahydro-11-deoxycortisol in attempting to document circadian rhythms.

The aim of the present investigation was to explore the effects of a 36 h sustained administration of metyrapone on the circadian variations of steroids, especially plasma 11-deoxycortisol and its urinary metabolite tetrahydro-11-deoxycortisol. It was of interest to test the hypothesis of a circadian bioperiodicity of these two variables.

Since the spontaneous physiological secretion of 11-deoxycortisol and the physiological output of tetrahydro-11-deoxycortisol are extremely low, time series were documented during and after metyrapone administration. Such circadian changes, if any, can be useful to investigate the variability of both metyrapone activity and adrenal secretion.

More precisely, it was interesting to learn whether or not a precursor of cortisol has a circadian rhythm.

* Metyrapone = Metopirone ® (Ciba).

EXPERIMENTAL

Subjects. Two adult men (16 and 16½) volunteered for this study during a stay at the Centre Héliomarin de Roscoff, France. They had recovered from scoliosis (n° 1) and poliomyelitis (n° 2). From both clinical and biological routine examination they were considered healthy at the time of the study (July 1972).

For at least three weeks prior to the beginning of the experiment and during it, the subjects were synchronized with diurnal activities from 0715 h to 2300 h and nocturnal rest. Meals were taken at 0730 h, 1130 h, 1530 h, 1830 h.

The study lasted 72 h: plasma steroids were measured in the first 24 h span (A) and again during the last 24 h span (C). The three parts of the study were: A = 24 h control span; B = 24 h span with oral administration of metyrapone every 4 h beginning at 2000 h; C = 24 h span including 12 h with drug administration (2000 h–0800 h) and 12 h without any drug administration (0800–2000 h) (Fig. 1).

MATERIALS AND METHODS

During spans A and C venous blood was sampled in the recumbent position every hour without disrupting the night's sleep. The butterfly needle was changed every 24 h. At each sampling time, 4.5 ml blood was taken into a heparinized tube and centrifuged (10 min at 3500 rev/min at + 4°C).

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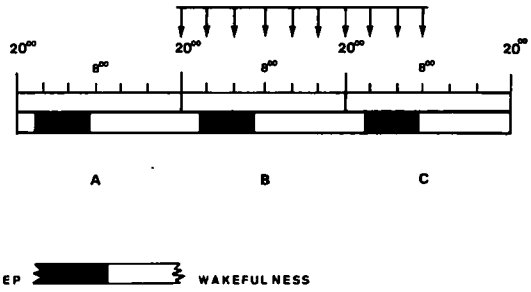

Fig. 1. Experimental protocol. Timing of administration of metyrapone: each arrow ↓ represents 0.75 g oral administration (total intake 7.50 g). A is a 24 h control span, B a 24 h span with oral administration of metyrapone every 4 h and C a 24 h span including 12 h with drug administration and 12 h without any drug administration.

The plasma was kept frozen at −20°C until analysed. Carbon tetrachloride was used for selective extraction of 11-deoxycortisol from plasma. Both cortisol and extracted 11-deoxycortisol were determined by competitive protein binding [10]. The corticosteroid-binding globulin (CBG) isotope solutions, the incubation and the separation of free from bound corticoids by Dextran–florisil powder were done as described by Meikle *et al.* [11].

[1,2-^{3}H]Cortisol (44 Ci/mmol) and [1,2-^{3}H] 11-deoxycortisol (35 Ci/mmol) were obtained from New England Nuclear Corp. and were checked for purity by paper chromatography in the Bush B_5 system. The mean recovery for cortisol was 79 ± 5% and 82 ± 4.2% for 11-deoxycortisol. Results were corrected for these values. The interassay coefficient of variation and the intra-assay coefficient of variation were 12% (n = 18) and 8.9% (n = 10) respectively for a sample with a 11-deoxycortisol concentration of 12 μg/100 ml. For cortisol these values were 11.2% (n = 18) and 10.1% (n = 10) respectively (plasma cortisol value of 8 μg/100 ml). The minimum amount detectable was 1 μg/100 ml for cortisol and 0.8 μg/100 ml for 11-deoxycortisol.

Integrated 4-h urine samples were collected for 72 h at fixed clock hours beginning at 2000 h. They were frozen at −20°C until analysed; sodium mercurithiolate was used as preservative. Tetrahydro-11-deoxycortisol was selectively extracted by carbon tetrachloride according to Henke's method [12]. Tetrahydro-11-deoxycortisol and 17-hydroxycorticosteroids were determined by the Silber–Porter reaction [13]. In any one sample the estimated portion of the 11-hydroxylated steroids, part of the 17-hydroxycorticosteroids, can be roughly appreciated by substracting tetrahydro-11-deoxycortisol value from that of total 17-hydroxycorticosteroids, thus giving an index of the persisting 11-β hydroxylase activity.

The cosinor method has been proposed by Halberg *et al.* [14] to validate bioperiodic phenomena (statistically significant rhythm detection from time series, e.g.: noise–signal ratio < 0.3) and to characterize the detected rhythms by the estimation of several parameters: the period τ (duration of one complete cycle in a rhythmic variation), the acrophase ϕ (crest time estimate of the variable in the 24 h scale), the amplitude A (½ of the total 24 h variability) and the mesor M (24 h rhythm adjusted mean).

Usually a bioperiodic phenomenon can be approximated by a cosine function in the form $Y(t) = M + A \cos [(2\pi/\tau)\ t + \phi]$ in which t = time. Special computer programs and the least squares method are used to find the best fitting cosine function approximating all data in a given experimental situation.

Since this statistical method allows both the detection and the quantification of biological rhythms, it is extensively used in chronobiology [15] when the considered time series can be roughly assimilated to a cosine function, which is here the case. Moreover the period τ can be approximated by the subject's synchronization (in man the rest-activity period) and the prominent period resulting from the spectral analysis of time series [16].

RESULTS AND DISCUSSION

As is usually the case it may be difficult to validate a circadian rhythm only from the inspection of row data plotted as a function of time (Fig. 2). The cosinor method was used to solve this problem of rhythm detection and quantification.

For both subjects the peak of plasma cortisol during span A was located between 0700 h and 0800 h. Plasma 11-deoxycortisol determinations during span C showed a peak located at 0800 h for both subjects (Fig. 2).

The cosinor analysis (Table 1) of the same time series shows that: (1) the noise–signal ratio is lower than 0.3; thus a circadian rhythm is likely to be present; (2) the acrophases of the studied circadian rhythms do not coincide in time with the peaks obtained when plotting row data. This difference between figures resulting from the used methods is not surprising. The acrophase is the peak of the cosine function used to approximate all data, while the curves obtained from row data show that the considered circadian rhythm can be only roughly assimilated to a cosine function.

A spectral analysis has been performed according to Halberg's method [16]. It shows a prominent peak at a trial period τ = 24 h with amplitudes of 2.43 μg/100 ml for cortisol and 4.19 μg/100 ml for 11-deoxycortisol. The method could not be used to explore the ultradian rhythm of cortisol and 11-deoxycortisol, taking into consideration the size of the sample.

A circadian rhythm seems to be present (noise–signal ratio < 0.3) in total 17-OHCS excretions during spans A, B and C (Table 1). A circadian rhythm seems to exist in urinary tetrahydro-11-deoxycortisol during spans B and C but not during control span A (Table

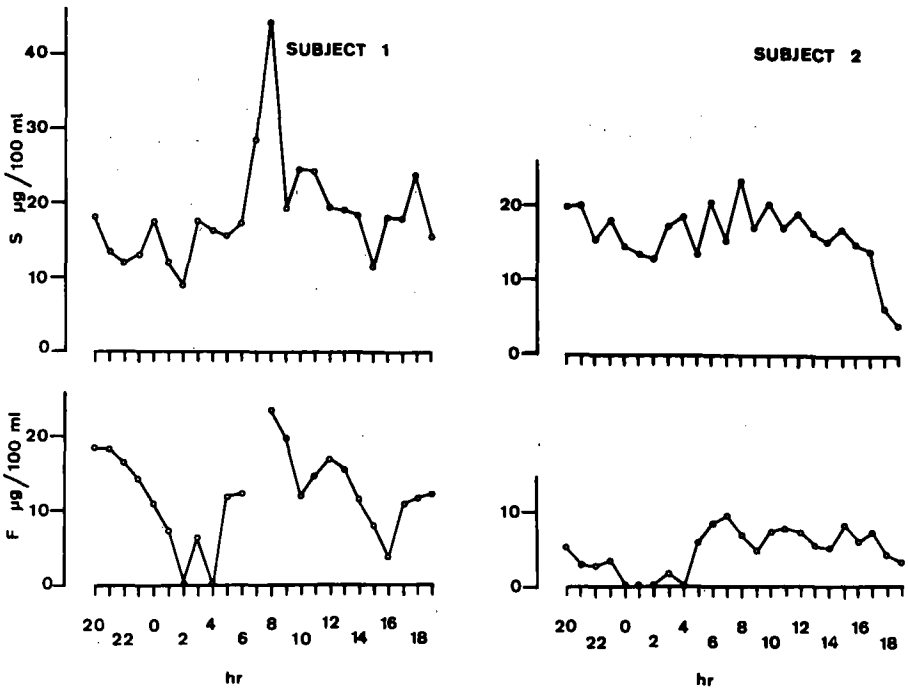

Fig. 2. Individual data for plasma cortisol (F) and plasma 11-deoxycortisol (S) are plotted as a function of time:fixed hours of sampling; plasma cortisol was measured before and plasma 11-deoxycortisol after a sustained administration of metyrapone.

Table 1. Circadian rhythms in plasma steroids before (A = 24 h control span) and after (C = 24 h span including 12 h with and 12 h without drug administration) a sustained administration of metyrapone (A = plasma cortisol determination; C = plasma 11-deoxycortisol determination); and circadian rhythms in urinary excretion of steroids before (A), during (B) and after (C) a sustained oral administration of metyrapone. Two young human male subjects synchronized with light-on 0715 h and light-off 2300 h. Sampling interval Δt = 1 h: cortisol and 11-deoxycortisol. Sampling interval Δt = 4: urinary steroids

Determined steroid	Experimental situation	Subject n°	Cosinor summary Mesor M mg/4 h	Amplitude mg (% of M)	Acrophase h, min	Noise–signal ratio
Total 17 OH-corticosteroids	A	1	0.59	0.29 (49.1%)	13^{44}	0.17
		2	0.71	0.31 (43.7%)	15^{20}	0.10
	B	1	0.75	0.33 (43.2%)	15^{18}	0.20
		2	1.44	0.83 (58%)	15^{15}	0.16
	C	1	1.91	0.85 (44.5%)	13^{55}	0.22
		2	1.54	0.65 (42.2%)	14^{29}	0.11
Tetrahydro-11-deoxy-cortisol	A	1	traces	—	—	—
		2	traces	—	—	—
	B	1	0.56	0.29 (51.7%)	16^{03}	0.09
		2	1.28	0.61 (47.6%)	14^{23}	0.15
	C	1	1.16	0.64 (55.1%)	15^{55}	0.24
		2	0.94	0.54 (57.4%)	16^{35}	0.18
Estimated portion of 11-OH-corticosteroids	A	1	0.59	0.27 (45.7%)	13^{44}	0.17
		2	0.71	0.31 (43.6%)	15^{20}	0.10
	B	1	0.16	—	—	0.34
		2	0.15	0.04 (26.6%)	15^{10}	0.28
	C	1	0.82	0.35 (42.6%)	14^{46}	0.15
		2	0.61	0.28 (45.9%)	11^{37}	0.20
			Mesor: M µg/100 ml	Amplitude µg (% of M)		
Plasma Cortisol	A	1	12.21	2.97 (24.3%)	11^{54}	0.10
		2	6.20	2.27 (36.6%)	09^{01}	0.16
Plasma 11-Deoxycortisol	B	1	18.71	5.76 (30.8%)	09^{54}	0.06
		2	16.12	2.66 (16.5%)	07^{41}	0.05

1). The estimated portion of 11-hydroxylated tetrahydrocorticosteroids seems to have circadian changes during spans A and C (Table 1), but probably not during span B (noise–signal ratio $\simeq 0.3$). The acrophases of validated rhythms are located between 1130 h and 1630 h.

Thus for both plasma cortisol and urinary 17-OHCS the results reported here are in excellent agreement with those published by many others [see 17].

The partial replacement of a physiologic secretion (cortisol) by a forced secretion of a cortisol precursor (11-deoxycortisol) does not seem to alter both the circadian rhythm of involved secretions and the timing of their acrophases. This study shows that sustained administration of metyrapone is followed by a circadian rhythm in 11-deoxycortisol secretion. The estimated portion of 11-hydroxylated tetrahydrocorticosteroids does not show a validated rhythm during metyrapone administration (noise–signal ratio $\simeq 0.3$; low mesor and decreased amplitude). Such findings are compatible also with the hypothesis of circadian changes in the activity of metyrapone: the amplitude being expressed as a percentage of the mesor, one would expect, if metyrapone activity was constant, an unchanged relative amplitude of 11-hydroxylated tetrahydrocorticosteroids. The fact that this relative amplitude, when detectable, is decreased almost to half its original value is in favour of a greater activity of metyrapone at the time of the acrophase of cortisol.

Thus our results and those obtained by Angeli *et al.* [8] lead to similar conclusions despite differences in experimental protocols: the pituitary-adrenal circadian periodicity is not affected by metyrapone sustained administration. Moreover this study indicates that fluctuations in the secretion of the precursor of cortisol i.e. 11-deoxycortisol are similar to, but preceed those of cortisol.

The following trivial names have been used: Cortisol: 11β,17,21-trihydroxy-4-pregnene-3,20-dione 11-deoxycortisol: 17,21-dihydroxy-4-pregnene-3,20-dione Tetrahydro-11-deoxycortisol: 3α,17,21-trihydroxy-5β-pregnan-20-one.

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