DIURNAL FLUCTATION OF PLASMA CORTISOL LEVELS IN THE GUINEA PIG

By

David R. Garris1)

ABSTRACT

Diurnal changes in the basal levels of plasma cortisol were studied in the female guinea pig. Plasma cortisol levels were determined at 8 bleeding times during the entrained 24 h photoperiod (14 h light/10 h dark: lights on 06.00 h). Plasma cortisol levels remained low during the dark phase of the cycle ranging between 6.4 and 9.0 μg/100 ml. However, at 4 h prior to the onset of the light phase of the photoperiod, a dramatic rise in the plasma cortisol level was measured, which peaked between 04.00 and 08.00 h at 12.3 μg/100 ml. A subsequent decline in plasma cortisol levels was measured throughout the light phase of the cycle, reaching basal levels before the onset of the dark phase. These data indicate that a diurnal fluctuation in plasma cortisol occurs in the guinea pig which is very reminiscent of that seen in the human and in contrast with that observed in the rat.

Cortisol is the major corticoid in the guinea pig, the adrenal gland reportedly secreting six times as much cortisol as corticosterone into the systemic circulation (Dalle & Delost 1974). Basal cortisol levels are known to change during the process of maturation (Greiner et al. 1976), temperature regulation (D'Angelo 1960), stress (Viru & Akke 1969; El Hani & Delost 1976) and parturition (Donovan & Peddie 1974) in this species suggesting that the adrenal gland, via its secretion of cortisol, participates in a number of homeostatic mechanisms. How-

Supported by NIH Grant RR 65387-13

1) Present address and reprint requests:
   Department of Reproductive Biology, Case Western Reserve University,
   School of Medicine, Cleveland, Ohio 44106 USA

692
ever, the effect of photoperiod on cortisol secretion has not been investigated in this species. This study was undertaken to determine if a diurnal rhythm exists in the basal plasma levels of cortisol in the female guinea pig and if short exposure to methoxyflurane anaesthesia causes an alteration in plasma cortisol levels.

**MATERIALS AND METHODS**

Adult female guinea pigs weighing between 600 and 700 g were used in this study. Animals were maintained in individual cages under a controlled photoperiod of 14 h light/day (lights on: 06.00 h). Food and water were available *ad libitum.*

Animals were housed undisturbed for 24 h prior to the start of the experiment. Dioestrous animals were randomly assigned to one of the 8 bleeding groups; bleedings were performed at 02.00, 04.00, 06.00, 08.00, 10.00, 14.00, 18.00 or 22.00 h. In all cases, animals were removed from their cages, lightly anaesthetized by a 1 min exposure to methoxyflurane and 2 ml of blood collected in a heparinized syringe by intracardiac puncture. The collection of blood samples within any one group occurred within a 15 min period. Blood was also collected from animals decapitated within 30 seconds of cage removal at various bleeding times in order to compare "non-anaesthetized" with "anaesthetized" plasma samples to assure that the short exposure to methoxyflurane had not altered plasma cortisol levels. All experiments were repeated twice. Plasma was collected and stored at −10°C until assayed. Plasma cortisol was analysed using a competitive protein-binding assay previously described (*Murphy* 1967). Serum rich in corticosteroid-binding globulin (CBG) was used. Briefly, each plasma sample was extracted with petroleum ether (20:1 v/v) to remove endogenous P and other circulating steroids which bind to CBG (> 98% of interfering compounds removed based on recovery of [3H]progesterone from ether extracted material). Plasma was then extracted with methylene chloride (20:1 v/v). Each sample was extracted by shaking vigorously for 45 min in capped tubes on a horizontal shaker; the solution was subsequently quick frozen in a dry ice-ethanol bath and the methylene chloride fraction decanted into 12 × 27 mm test tubes and dried under nitrogen.

To each dried sample and standard (range 0 to 100 µg), 1 ml of a [3H]cortisol-1% CBG solution was added to each tube at 4°C. Each tube was subsequently vortexed and incubated at 4°C for 20 min. At 30 second intervals, 60 mg of florisil was added to each tube, the samples vortexed for 30 seconds, the florisil allowed to settle out and 0.5 ml of the supernatant removed and placed in 10 ml of Fisher Scintiverse. Radioactivity was measured in a Packard Spectrophotometer.

Assay linearity ranged between 2 and 30 µg/100 ml serum. Extraction recovery averaged 98% and all values are expressed uncorrected for procedural loss. All values are expressed as the daily means ± SEM and the statistical difference between means assessed by Student's *t*-test.

**RESULTS**

Basal cortisol levels ranged between 6.4 and 9.0 µg/100 ml of plasma in this study. Basal levels of cortisol in anaesthetized animals compared favourably with those collected from decapitated animals (Fig. 1). Thus, the 1 min ex-
Fig. 1.

Mean (± sem) plasma cortisol levels during the 24 h photoperiod. (N) denotes the number of animals per group; the dark bar denotes the dark phase of the photoperiod. Dashed-line indicates cortisol levels from decapitated animals (N = 2 per group).

Exposure to methoxyflurane did not alter basal cortisol levels and thus all values from plasma collected by intracardiac puncture were taken to represent the normal basal levels of the steroid.

A diurnal fluctuation was noted in circulating cortisol levels (Fig. 1). Near the mid-point of the light phase (14.00 h), basal cortisol levels fluctuated between 6.5 and 9.0 μg/100 ml plasma. At the onset of dark, no significant change from baseline was noted in circulating cortisol concentrations. In contrast, starting 4 h before the onset of light (i.e. 02.00 h), a significant increase in the mean of plasma cortisol levels was observed as compared to 14.00 h levels (P < 0.01). Plasma levels continued to rise, peaking between 04.00 and 08.00 h (12.3 ± 0.47 μg/100 ml; P < 0.01 vs. basal levels). Plasma cortisol levels declined and subsequently returned to basal levels between 08.00 and 14.00 h.

**Discussion**

These data indicate that a definite diurnal rise in plasma cortisol occurs near the beginning of the light phase of the entrained photoperiod and that the elevation in basal cortisol levels lasts for approximately 8 h. Also, short ex-
posure to methoxyflurane anaesthesia does not alter basal cortisol levels supporting the results of others that stress or muscular fatigue do not significantly affect adrenal corticoid secretion in this species (Viru & Akke 1969). The observation that the diurnal elevation in cortisol levels corresponds to the onset of light resembles the pattern of cortisol reported for the human (Nichols & Tyler 1967) and contrasts with the circadian rhythm of corticosterone in the rat (Critchlow et al. 1963). This rhythm also explains the differences in plasma cortisol levels previously described; Gala & Westphal (1967) reported low cortisol levels in samples collected in the afternoon which contrast to the higher cortisol levels reported by Dalle & Delost (1974) from samples collected in the early portion of the daily light cycle. The results of the present study confirm the results of both studies in that cortisol levels collected late in the light period cycle are low compared to values from samples obtained near the onset of the light phase. Also, the present study supports the contention that the guinea-pig adrenal gland does not respond to stress by anaesthetization with an increased secretion of cortisol. These results are in marked contrast to those reported for the rat (Zimmerman & Critchlow 1967). Thus, this study indicates that experiments concerned with guinea-pig adrenal function must take into account the daily fluctuation in cortisol secretion rates in order to properly evaluate changes in adrenal activity.

REFERENCES


Received on July 14th, 1978.