Plasma and Adrenal Corticosterone Levels During the Different Phases of the Sexual Cycle in Normal Female Rats

Variations in the concentration of corticosteroids (mainly corticosterone) in the plasma and the adrenal glands of female rats during the different phases of the sexual cycle have been the subject of several publications. No significant difference in corticosterone concentration during the various phases of the cycle could, however, be found in adrenal vein blood (TELEGDY et al.[1]) or in blood collected from the abdominal aorta (GUILLEMIN et al.[2]) of animals in which the cycle phases were determined from vaginal smears taken on 7 (GUILLEMIN et al.[2]) or 14 (TELEGDY et al.[1]) consecutive days, prior to barbiturate anaesthesia and the collection of blood samples. CRITCHLOW et al.[3], on the other hand, found significant differences in the corticosterone concentrations in plasma collected during the morning hours in the various phases of the cycle, the levels being highest in pro-oestrus. Since only single determinations were made, however, no curves could be plotted. Vaginal smears were taken at least 3 days before each experiment as well as immediately after the collection of blood by decapitation.

We have attempted to determine the nychthemeral rhythm of corticosterone concentration in the plasma and adrenals of rats during the different phases of the sexual cycle. As it is known (GUILLEMIN et al.[2]), slight changes in environment as well as in handling may lead to marked variations in the plasma corticosterone levels, care was taken to avoid disturbances as far as possible.

Materials and methods. The experiments were carried out in female Ivanovas rats weighing 180–220 g, all of which had been obtained from the same source. The animals were housed in cages in a room kept at a constant temperature of 23 °C and subjected to rhythmic lighting, 14 h of light (06.00–20.00), being followed by 10 h of darkness (20.00–06.00). They were fed a standard diet with water ad libitum for at least 4 days before the experiment. 24 h before the start of the experiment the animals were weighed and were devided at random into groups of three. The determinations were performed at the following times: 08.00 h, 12.00 h, 16.00 h, 20.00 h, 24.00 h and 04.00 h. The animals were exsanguinated by decapitation, all the rats in each group being killed within a few minutes of each other. After the collection of blood, vaginal smears were taken and the phase of the cycle was registered according to a three-point classification: 1. Resting phase (met-oestrus and di-oestrus). 2. Pro-oestrus. 3. Oestrus.

As the rats were sacrificed at random, the number of animals on the various cycle phases differed. Plasma and adrenal corticosterone was extracted with chloroform, separated chromatographically and determined according to the method of NEHER[4]. Mean values, standard errors and their significance were calculated according to Student's *t*-test.

Results. Under these experimental conditions the concentration of corticosterone in the plasma (Table I and Figure) of normal female rats appears to be subject to 2 different sources of variation, the one nychthemeral, the other related to the different phases of the sexual cycle.

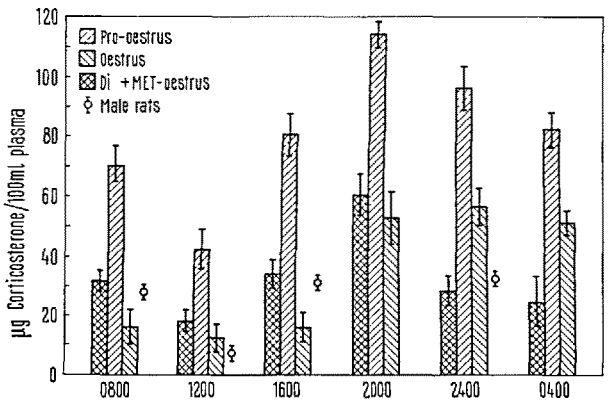
The nychthemeral rhythm due to the experimental set-up is characterized by a minimum plasma concentration at about midday, and maximum concentrations between 20.00 h and 24.00 h, the levels declining slowly thereafter. This pattern is only slightly affected by the different phases of the cycle.

The phases of the sexual cycle have essentially a quantitative influence on the plasma corticosterone concentration. The values are by far the highest in pro-oestrus; maximal levels amounting to 100 µg/100 ml plasma and more are attained between 20.00 and 24.00 h on the day of this phase of the cycle. These concentrations are significantly higher than those found during the resting phase. The onset of oestrus is accompanied

Table I. Nychthemeral variations in plasma corticosterone concentration during different phases of the sexual cycle in female rats

Time	Resting phase µg/100 ml	Pro-oestrus µg/100 ml	Oestrus µg/100 ml
08.00	31.9 ± 4.3 $n = 27$ $p < 0.001$	70.2 ± 7.2 $n = 14$	16.4 ± 6.4 $n = 9$ $p < 0.001$
12.00	18.3 ± 3.6 $n = 31$ $p < 0.01$	42.1 ± 7.2 $n = 9$	12.5 ± 5.3 $n = 9$ $p < 0.01$
16.00	34.3 ± 4.6 $n = 19$ $p < 0.001$	80.9 ± 7.3 $n = 13$	15.9 ± 5.4 $n = 13$ $p < 0.001$
20.00	61.4 ± 6.6 $n = 9$ $p < 0.001$	114.6 ± 4.4 $n = 9$	53.2 ± 8.5 $n = 9$ $p < 0.001$
24.00	29.1 ± 4.9 $n = 10$ $p < 0.001$	97.0 ± 6.8 $n = 11$	57.4 ± 6.0 $n = 4$ $p < 0.01$
04.00	25.0 ± 8.9 $n = 7$ $p < 0.001$	83.4 ± 6.2 $n = 12$	53.4 ± 4.4 $n = 8$ $p < 0.01$

Significance is calculated by reference to the pro-oestrus values. n = number of animals per group (see Figure).



Nychthemeral variations in plasma corticosterone concentration during the different phases of the sexual cycle in female rats, in comparison with the variations in plasma corticosterone in male rats (number of animals per group > 24).

[1] G. TELEGDY, L. HUSCAR, E. ENDROCZI and K. LISSAK, Acta physiol. hung. *22*, 171 (1962).

[2] R. GUILLEMIN, G. W. CLAYTON, J. D. SMITH and H. S. LIPSCOMB, Endocrinology *63*, 349 (1958).

[3] V. CRITCHLOW, R. A. LIEBELT, M. BAR-SELA, W. MOUNTCASTLE and H. S. LIPSCOMB, Am. J. Physiol. *205*, 907 (1963).

[4] R. NEHER, *Steroid Chromatography* (Elsevier, Amsterdam 1963).

with a sharp decline in the corticosterone concentration in plasma, the decrease being particularly marked between 08.00 and 20.00 h on this day. At this time, plasma corticosterone levels in oestrus are comparable with or lower than those noted in the resting phase; this situation is reversed if values between 20.00 and 04.00 h are considered. The corticosterone concentrations are then higher than during the resting phase.

Adrenal corticosterone concentrations (Table II) follow a similar, if not so distinct a pattern. The levels in pro-oestrus are at most times significantly higher than those in the other phases of the sexual cycle. This is not the case, however, at 12.00 h, when neither the values in the resting phase nor those in oestrus are significantly lower, or, as far as the oestrus values are concerned, at 24.00 h or 04.00 h, when the levels are comparable. The cause of this discrepancy needs further elucidation.

Table II. Nychthemeral variations in adrenal corticosterone concentration during the different phases of the sexual cycle in female rats

Time	Resting phase µg/100 mg adrenal	Pro-oestrus µg/100 mg adrenal	Oestrus µg/100 mg adrenal
08.00	4.0 ± 0.43 $n = 27$ $p < 0.01$	6.5 ± 0.56 $n = 14$	3.3 ± 0.95 $n = 9$ $p < 0.01$
12.00	2.7 ± 0.42 $n = 31$ $p < 0.1$	4.3 ± 0.75 $n = 9$	2.4 ± 0.70 $n = 9$ $p < 0.1$
16.00	3.3 ± 0.52 $n = 19$ $p < 0.01$	5.9 ± 0.68 $n = 13$	3.0 ± 0.77 $n = 13$ $p < 0.01$
20.00	3.7 ± 0.74 $n = 9$ $p < 0.01$	7.1 ± 0.6 $n = 9$	4.7 ± 0.78 $n = 9$ $p < 0.05$
24.00	3.8 ± 0.71 $n = 9$ $p < 0.001$	7.4 ± 0.45 $n = 11$	6.1 ± 1.02 $n = 4$ $p < 0.2$
04.00	2.6 ± 0.72 $n = 7$ $p < 0.01$	6.2 ± 0.56 $n = 12$	5.1 ± 0.60 $n = 8$ $p < 0.3$

Significance is calculated by reference to the pro-oestrus values. n = number of animals per group.

Discussion. Corticosterone concentration has been shown to be affected in the normal female rat by nychthemeral rhythm and by the different phases of the sexual cycle. The nychthemeral rhythm of corticosterone in the female rat is fully comparable with that observed in the male rat under similar experimental conditions (see Figure), with the exception that the minimum values in the latter are as a rule lower than with values determined in females during the resting phase.

Sexual activity does not seem to affect the regularity of the nychthemeral rhythm. On the other hand, the pronounced and sustained increase in corticosteroid secretion during the day of the pro-oestrus phase, culminating in very high values in the late afternoon, is noteworthy. It appears that during this critical phase the thalamo-pituitary-adrenal system is subjected to a marked stimulation, which immediately precedes or coincides with ovulation in the rat. It remains to be shown how this burst of adrenocortical activity is related to the different functional changes occurring during this period.

Zusammenfassung. Es wird das physiologische Verhalten von Corticosteron in den einzelnen Zyklusphasen im Plasma und in den Nebennieren normaler weiblicher Ratten untersucht. Die Corticosteronkonzentrationen in Plasma und Nebennieren sind während der Proöstrusphase signifikant höher als während den anderen Zyklusphasen. Der normale Tag-Nacht-Rhythmus bleibt in allen Zyklusphasen aufrechterhalten.

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Experimental Evidence of Inhibitory Control of Pars Intermedia Function and the Rate of Recovery of Function After Denervation in the Teleost *Ictalurus melas*

It is generally agreed that the colour changes in elasmobranchs and amphibians are regulated by the pars intermedia (PI) of the pituitary gland which itself is under the inhibitory control of the brain. Thus any interruptions of the hypothalamo-hypophyseal innervation or transplantation of the gland, i.e. its release from the central control, leads to its hypertrophy and uncontrolled liberation of MSH[1]. Consequently pigment in the skin melanophores becomes either permanently dispersed or remains so for a long time and the ability of the animal to adapt to a white background is totally impaired. The nature of the central control of the PI (meta-adenohypophysis)[2] function in teleosts is, however, obscure. In the only case so far reported (*Poecilia formosa*)[3] the PI, in contrast to elasmobranchs and amphibians, was found to be markedly atrophied in ectopic pituitary transplants. This indicates that in this species the brain has a stimulatory control on PI function. However, the role of MSH in the pigmentation in *Poecilia* has not been experimentally established.

In the present paper the effects of denervation of the PI on the melanophores in *I. melas* (in which MSH plays an important role in the colour changes) are reported with a view to elucidating the nature of the innervation controlling PI function.

Material and method. Denervation of the PI was effected by causing ca. 4 mm deep cuts and lesions by means of a dental drill and a fine probing needle in the exposed hypothalamo-hypophyseal region in between the optic chiasma and the pituitary; in some cases lesions were also made around the gland. In most cases thin rectangular pieces of black plastic (about 2.0×0.7 mm) were vertically inserted into the lesions to prevent the reestablishment of vascular connections. In all, 20 animals (average length 7.0 cm) were operated. Out of these 9 did not survive beyond 14 days (1st casualty after 5 days), 5 lived 27–38 days, 3 50–62 days and 3 were still alive over 143 days. The fish were white-adapted for 3–4 weeks, their mean of melanophore index (MI) before the operation being 1.4, and were replaced on an