

Estrogens Modulate the Circadian Rhythm of Hypothalamic Beta-Endorphin Contents in Female Rats

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Key Words. Estrous cycle · β -Endorphin · Estrogens · Circadian rhythm · Hypothalamus

Abstract. The aim of the present study was to evaluate the changes in the diurnal rhythm of the hypothalamic β -endorphin (β -EP) contents in female rats as a function of circulating estrogens. With this purpose we evaluated the diurnal hypothalamic β -EP changes (1) during the estrous cycle, and (2) in ovariectomized rats with and without acute and chronic estrogen replacement. Ovariectomized rats were treated either acutely with 10 μ g of estradiol benzoate (EB) or chronically with 2 μ g/day of EB for 15 days. β -EP concentrations were measured in acid extracts of medial basal hypothalamus by a specific radioimmunoassay. During the estrous cycle, hypothalamic β -EP concentrations showed a significant nocturnal increase, with no difference between the 4 days of the cycle. On the day of estrus, β -EP concentrations between 12.00 and 18.00 h resulted significantly lower than in the other days of the cycle. After ovariectomy, the night-related changes in hypothalamic β -EP disappeared. The acute administration of EB induced a significant increase in hypothalamic β -EP after 21 h (18.00 h). On the other hand, the chronic replacement restored the nocturnal peak of hypothalamic β -EP (18.00, 21.00, 24.00 h). The present data emphasize the role of central β -EP in regulating the reproductive functions. Moreover, the effect of estrogen in modulating the circadian changes in hypothalamic β -EP supports the important role of estrogens in brain function.

β -Endorphin (β -EP) is one of the most important and biologically active endogenous opioid peptides. Initially investigated for its role in the modulation of pain perception and of a broad range of behavioral activities, it was further shown that β -EP plays a relevant role in the control of pituitary function, particularly in the stimulation of prolactin release and in the inhibition of luteinizing hormone (LH) and follicle-stimulating hormone secretion [1–4]. The degree of inhibition of the gonadotropin-releasing hormone-gonadotropic axis appears to depend on the reproductive state, and to change from puberty to aging in rats and humans [5].

Although results are variable, the hypothalamic β -EP concentration has also been reported to vary during the estrous cycle in the rat, and particularly on afternoon of proestrus and morning of diestrus 2. These changes are likely related to variations in plasma levels of gonadal steroids [6, 7].

The hypothalamic β -EP concentration has also been shown to fluctuate with a diurnal rhythm, demonstrating a significant nocturnal increase [8–10], abolished by the suppression of the dark phase of the day-night cycle [10].

Received: October 17, 1989

Accepted after revision: February 9, 1990

The aim of the present study was to evaluate the effects of gonadal hormones, particularly of estrogens, on the diurnal rhythm of the hypothalamic β -EP concentration in female rats. Accordingly, the levels of β -EP in the medial basal hypothalamus (MBH) were measured every 3rd hour during the different phases of the estrous cycle, after ovariectomy (OVX), and after acute and chronic estradiol replacement treatment in castrated rats.

Materials and Methods

The animals used were 60-day-old female virgin Wistar rats weighing 150 \pm 10 g. Before initiating the experiments, the animals were housed under controlled temperature (22 \pm 2 °C) with a 14:10 h light:dark cycle (lights on at 06.00 h) and fed Purina rat chow and water ad libitum for 30 days. Vaginal smears were daily checked after this date. Only rats which exhibited at least 3 consecutive 4-day vaginal cycles were used for further experiments.

Experiment 1

4 groups of 80 rats each were selected on the basis of the phases of the vaginal cycle: estrus, diestrus 1, diestrus 2 and proestrus. 10 rats per group were killed by decapitation every 3rd hour, starting at 09.00 h

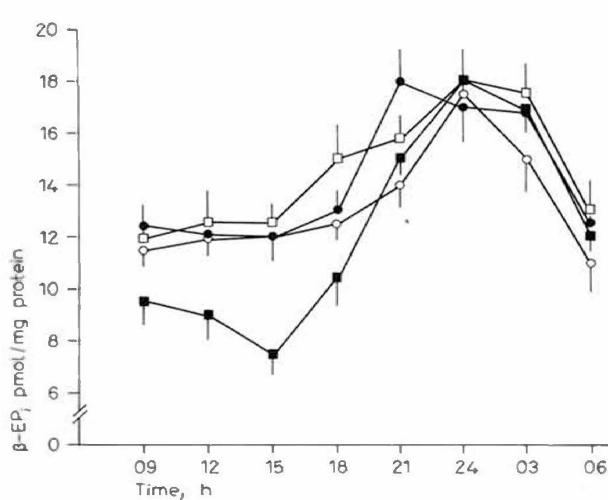


Fig. 1. Hypothalamic immunoreactive β -EP contents (pmol/mg protein; mean \pm SEM) were measured every 3 h for 24 h during the 4-day estrous cycle. ● = Diestrus 1; ○ = diestrus 2; □ = proestrus; ■ = estrus.

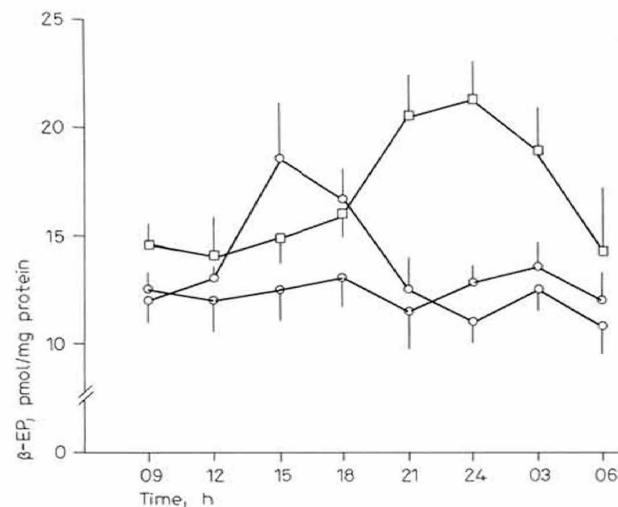


Fig. 2. Hypothalamic immunoreactive β -EP contents (pmol/mg protein; mean \pm SEM) were measured every 3 h for 24 days in OVX rats with or without acute (1 day) or chronic (15 days) EB replacement. ● = OVX; ○ = OVX + acute EB; □ = OVX + chronic EB.

along the 24-hour period. After decapitation, the brain was immediately removed and MBH dissected under a dissecting microscope according to the previously described limits [11]. The samples were boiled (10 min) and homogenized in 1 ml 0.01 N acetic acid. Supernatants were stored at -20°C until assayed for β -EP content.

Experiment 2

250 rats were subjected to bilateral OVX under pentobarbital (30 mg/kg, i.p.) anesthesia. 3 weeks later, the animals were divided randomly into 3 groups and subjected to either acute or chronic estradiol replacement treatment or to vehicle injection. Acute estradiol treatment consisted of a single subcutaneous injection of 10 μg of estradiol benzoate (EB) in sesame oil at 18.00 h done 5 weeks after OVX. The chronic treatment consisted of an equally timed daily injection of 2 μg of EB, initiated 3 weeks after OVX and continued for 2 weeks. The control treatment consisted either of a single or of a 2-week daily injection of the vehicle. On the day of the experiment, 10 rats per group were decapitated every 3rd hour starting at 09.00 h, namely 15 h after the estradiol injection, and processed as reported in experiment 1.

β -EP Extraction and Assay

Tissue extract β -EP content was measured by radioimmunoassay (RIA), using camel β -EP (Peninsula, Belmont, Calif., USA) as standard. The antcamel β -EP serum (supplied by Dr. A.E. Panerai, Milano, Italy) was used at a final dilution of 1:130,000, thus allowing detection of 10 pg camel β -EP standard. The antiserum, the labeled hormone [^{125}I] and the standard curve were dissolved with 0.5% bovine serum albumin-phosphate buffer (pH 7.4). The recovery of [^{125}I]-c β -EP added to an MBH sample corresponded to 85% of the total amount after acetic acid extraction. The characteristics of the RIA have been described previously [12]. The protein content was determined on the whole homogenate by the Bradford method [13].

Statistical Analysis

Data (corrected for recovery losses) were expressed as picomoles β -EP per milligram protein. Data were analyzed statistically using either Dunnett's or Duncan's t test or analysis of variance, where appropriate [14].

Results

Experiment 1

As depicted in figure 1, the daily rhythmic changes in MBH content of β -EP did not show substantial differences between the various phases of the estrous cycle. MBH β -EP concentrations appeared to be characterized by a significant nocturnal increase ($p < 0.01$ at 24.00 and 3.00 h), whose timing and peak did not differ on the 4 phases of the reproductive cycle (fig. 1).

A significant difference was detected during the light period of the day of estrus, when β -EP concentrations appeared significantly lower ($p < 0.01$ at 12.00, 15.00 and 18.00 h) than those observed at the same hours of diestrus 1, diestrus 2 or proestrus.

Experiment 2

The results are summarized in figure 2. OVX plus vehicle administration was followed 5 weeks later by the complete loss of the daily changes in hypothalamic β -EP content. MBH β -EP levels were found to remain constant throughout the 24-hour period, with no difference between the light and dark phase. The nocturnal peak shown by the intact female rats was absent and the values registered along the 24-hour period did not differ from those presented at the diurnal hours by the control rats.

The acute EB administration at 18.00 h was followed by a significant increase ($p < 0.01$ at 15.00 and 18.00 h) of the MBH β -EP content. Instead, an almost control-like profile of the 24-hour β -EP concentrations was shown by the daily curve of MBH β -EP content in rats receiving chronic EB replacement treatment. In this group, hypothalamic β -EP concentrations appeared significantly higher during the night than during the day ($p < 0.01$ at 21.00, 24.00 and 3.00 h) and showed the nocturnal peak typical of the normal rats.

Discussion

Based on previous studies in female [10] and male [9] rats and in male Syrian hamsters [8], the present work shows that the β -EP content in MBH of female rats fluctuates during the day with a peak level at about the middle of the scotophase. Moreover, the present data also show that this rhythm is strictly related to the internal gonadal steroid milieu. The loss of diurnal changes in MBH concentrations of β -EP following OVX and the restoration of their nocturnal increase by chronic EB replacement treatment support the importance of estrogens in regulating the hypothalamic β -EP content.

No significant differences were detected in the diurnal MBH β -EP concentrations following castration. However, in comparison to control rats, rats treated with chronic steroid replacement showed basal β -EP values higher than in controls, in agreement with previous observations [12]. This result is also in agreement with the observation that the number or the affinity of opiate receptors following steroid replacement increases consistently [15–17]. As far as we know, no study has examined changes in opiate receptors during the 24-hour period. In recording the results obtained of control β -EP changes during the estrous cycle, there are discrepant observations. Opposing modifications in discrete hypothalamic areas were demonstrated by Barden et al. [6] and Knuth et al. [7] at 15.00 h of diestrus 2 and proestrus, in studies restricted to evaluate possible variations at the time of the preovulatory LH surge. On the other hand, the strict chronobiological protocol of the present study failed to demonstrate any difference in MBH concentrations and diurnal rhythm of β -EP between proestrus and diestrus 2. The only detected difference was represented by the significant decrease in β -EP levels on the diurnal time points of estrus. In addition to confirming that the brain concentration of this peptide is influenced by the reproductive events, these results might suggest the possible involvement of hypothalamic β -EP in the regulation of the sexual behavior typical of this phase of the estrous cycle. The reduction on the day of estrus of the β -EP inhibitory activity on sexual behavior [18] could be simultaneous with the appearance of the copulatory behavior. The significant increase in β -EP content observed 21 h after the single injection of EB in OVX animals shows that gonadal steroids are able to influence β -EP levels in rat MBH. However, the lack of the nocturnal increase indicates that the chronic

presence of estrogens promotes the re-appearance of the diurnal β -EP rhythm, and not just a single exposure.

In previous studies [10], the suppression of the nightly increase in β -EP content in MBH by lighting protraction during the dark phase of the light-dark cycle, and the reappearance of an almost normally timed circadian rhythm in chronically reversed lighting conditions, led us to hypothesize the endogenous nature of the diurnal rhythm in rat hypothalamic β -EP content. The results of the present study appear to support this possibility. In fact, the loss of daily variations in hypothalamic β -EP content in OVX rats and their restoration following chronic estrogen replacement appear to indicate that ovarian steroids exert a priming effect on this endogenous neuronal rhythm, on which photoperiods should play a synchronizing effect. However, the mechanism by which steroids and photoperiods influence the daily pattern of hypothalamic β -EP content is yet unclear. Gonadal steroids have been shown to affect the activity of opiateergic nerve terminals in the hypothalamus, probably by altering the diurnal rhythm of serotonin activity [19–23]. At the same time, there is evidence for a link between serotonergic and endorphinergic neurons [24, 25], suggesting a possible role of serotonin in regulating the circadian endorphinergic activity in the hypothalamus. On the other hand, the pineal gland conveys photoperiodic changes by secreting melatonin, a hormone increasingly involved in the mediation of β -EP effects [26–28] and in the modulation of the activity of the hypothalamic serotonergic system [29, 30]. Thus, a final common pathway may be operative in mediating gonadal and photoperiodic messages regulating the circadian organization of the opiate system activity in the hypothalamus.

Although it is difficult to ascribe a physiological significance to the diurnal changes in tissue β -EP levels (even for the diversity of physiological and behavioral effects reported for this neuropeptide), the present results emphasize a possible implication of hypothalamic β -EP changes in reproductive physiology. Moreover, the finding that a neuropeptide with great biological activity, i.e. β -EP, is modulated in its diurnal hypothalamic concentrations by the presence of gonadal steroids reinforces the major role of estrogen in the functional organization of the brain.

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