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Effects of melatonin infused into the III ventricle on prolactin, β-endorphin and luteotropin secretion in ewes during the different stages of the reproductive cycle

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Mini-review

Abstract. Secretion of all the pituitary hormones undergoes marked circadian and seasonal changes. The rhythmicity of these changes is controled by the circadian pacemaker system and the pineal gland transmitting daylength information to the neuroendocrine axis *via* the secretion of melatonin. This article presents data on the effects of the short-term melatonin administration into the third brain ventricle on prolactin, β-endorphin and luteotropin secretion in ewes kept under the increasing and decreasing daylength conditions. Additional emphasis is given to dopamine and LHRH release in the mediobasal hypothalamus under the melatonin treatment by the push-pull method. The long-term and short-term actions of melatonin on the hormonal status in ewes is also discused.

Key words: melatonin, prolactin, β-endorphin, luteotropin, LHRH, dopamine

INTRODUCTION

In the eighties it has become clear, that the pineal gland and the circadian pacemaker system are essential for the regulation of photoperiodic responses (Reiter 1982), and that melatonin is the pineal hormone responsible for transmitting daylength information. The synthesis and secretion of melatonin is closely related to the dark/light cycle and the highest concentrations of this hormone in the pineal gland and in blood are observed during hours of darkness. In almost all mammals so far studied, the duration of melatonin secretion varies with the length of the night and thus provides an endocrine index of nightlength/daylength.

The sheep is a seasonal breeder that shows well characterized endocrine responses to changes in photoperiod. Decreasing daylength or short days are accompanied by increased release of pituitary gonadotropins and marked gonadal recrudescence leading to enhanced steroid production (Karsch et al. 1984, Bittman et al. 1985). Conversely, as daylength becomes progressively longer a decrease in gonadotropin and steroid secretion is observed. Long photoperiods are marked by high circulating prolactin concentrations, while low prolactin concentrations characterize short photoperiods (Pelletier 1973, Lincoln et al. 1978). The concentration of β-endorphin, an endogenous opioid peptide, also undergoes seasonal variations in sheep (Ebling and Lincoln 1987). Studies performed in our laboratory on Polish Lowland ewes (Misztal et al. 1994b), have revealed increases in circulating concentrations of β-endorphin during the period of high sexual activity from September to December.

All these seasonal cycles can be modified by treatment with artificial photoperiod regimes (Ebling and Lincoln 1987, Poulton and Robinson 1987), or egzogenous melatonin administration (Lincoln and Ebling 1985, Poulton et al. 1986, Lincoln and Maeda 1992b). Pinealectomy, performed at various times of a year, also modifies long-term rhythms of prolactin and luteotropin (LH) secretion (Barrel and Lapwood 1978). Particularly, little is known whether the twenty-four hour rhythms of those hormones secretion may also be modulated by melatonin. We investigated therefore, whether melatonin infused into the III ventricle affects prolactin, β-endorphin and LH secretion in ewes, under the natural short and long day conditions. We tried to answer either the question whether responsiveness to direct melatonin administration is similar to seasonal responses of these hormones to the changes in photoperiod.

The studies were carried out on 18 Polish Lowland ewes during the period of anestrus, (from April to May), and during the breeding season, (from September to December). All ewes were infused twice: with Ringer-Locke solution (control group), and with melatonin solution 100 μg/100 μl/1 h (experimental group), from 02.00 to 06.00 p.m. Blood samples were collected from 12.00 a.m. to 10.00 p.m. and the hormone concentrations were determined by the radioimmunoassay methods.

PROLACTIN (PRL)

The opinions about the existence of 24-hour rhythms in prolactin secretion in sheep are controversial. Ravault and Ortavant (1977) showed that a circadian rhythm in prolactin secretion, with a major peak occurring coincidently with the onset of the dark phase, could be found throughout the whole year, whereas Walton et al. (1980), could only show the night-time rise during the periods of high secretion in summer. The results of our previous studies demonstrate that the circadian patterns of prolactin secretion alter throughout a year (Misztal et al. 1994a). The night-time rise of prolactin concentration in blood plasma was observed during the longest days in summer, while there were only distinct peaks of prolactin associated with dawn, during the period of short day in winter (Fig. 1). In spring and autumn, a major increase in prolactin occurred coincidently with the onset of the dark phase. It would appear that, as in the case of the seasonal, either the circadian rhythm of prolactin secretion in sheep is controlled by the mechanism mediated through the pineal gland. However, Brown and Forbes (1980), demonstrated that pinea-

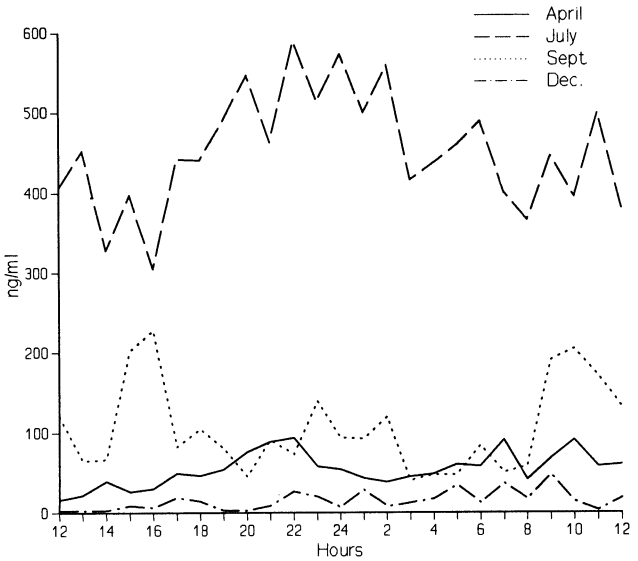

Fig. 1. Mean 24-h patterns of prolactin secretion at four times of the year in ewes ($n = 4$), kept under the natural photoperiod at 52°N.

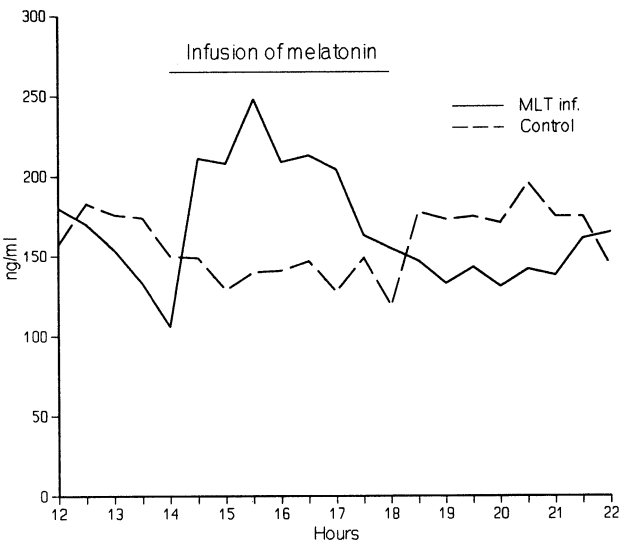

Fig. 2. Mean concentrations of prolactin in the blood plasma in anestrous ewes ($n = 8$), on the days of the melatonin (MLT) and control infusions.

lectomy did not block the marked rise in prolactin, that occurred in sheep at dusk.

In our study, the mean daily concentration of plasma melatonin in anestrous ewes ($n = 9$), during the control infusion was 94.3 ± 14.5 pg/ml (mean ± SD) and all animals demonstrated a natural rise in this hormone secretion up to 300 pg/ml after sunset. Increase in prolactin concentration in blood plasma to 177.9 ± 8.9 ng/ml also occurred around the time of sunset. Melatonin infused into the III ventricle evoked an abrupt increase in prolactin concentration after 30 min in 7 out of 9 ewes (Fig. 2). In one ewe, the rise of prolactin occurred after one hour. The mean concentration of prolactin (215 ± 16 ng/ml; mean ± SD) was maintained during three hours of the melatonin infusion and was significantly higher than in the control infusion (138 ± 11 ng/ml; $P \leq 0.01$).

During the breeding season, infusions were performed in ewes being in the late follicular ($n = 5$) and mid-luteal phases ($n = 4$) of the estrous cycle. The evening increase in prolactin concentration in the blood plasma of these ewes was not so well-marked as in anestrous ewes during the period of increasing day length. However, in 4 out of 5 ewes being in the late follicular phase, an increase in prolactin concentration occurred during the melatonin infusion (Fig. 3), and the mean prolactin concentration was significantly higher, than during the control infusion (231 ±22 vs. 151 ± 17 ng/ml; $P \leq 0.01$). This effect was maintained throughout the four hours, until the end of the melatonin administration.

Fig. 3. Mean concentrations of prolactin in the blood plasma in ewes ($n = 4$) in the follicular phase of the estrous cycle on the days of the melatonin (MLT) and control infusions.

In ewes in the mid-luteal phase of the estrous cycle, the concentrations of prolactin were lower than during the late follicular phase. There were no significant differences in prolactin concentrations between the melatonin and control infusions (21 ± 6 vs. 19 ± 9 ng/ml; NS).

The regulation of prolactin secretion is a complex process which involves various hypothalamic factors. It is well known that catecholamines influence the secretion of prolactin and that dopamine (DA) is a major prolactin-inhibiting factor. In view of this fact, our further studies concerned the direct effects of melatonin on the dopamine release from the tuberoinfundibular dopaminergic system in the mediobasal hypothalamus (MBH) which is postulated to play an important role in the regulation of prolactin secretion (Ben-Jonathan 1985). The afternoon perfusions of melatonin (20 µg/ml) into the MBH were performed in ewes both during the period of increasing ($n = 4$) and decreasing ($n = 6$) day-length. The flow rate of Ringer-Locke or melatonin solutions was 7 µl/min and the volume of the perfusates collected at 30 min intervals was about 200 µl.

In 2 out of 4 anestrous ewes, concentrations of DA were lower in perfusates collected from the MBH during the afternoon melatonin administration than in the perfusates collected during the control period before melatonin treatment (102.8 ± 31.1 vs. 26.1 ± 17.8 pg/30 min and 67.7 ± 67.9 vs. 24.4 ± 10.3 pg/30 min; mean ± SD; Fig. 4). In one ewe, DA concentrations in perfusates were similar during both perfusions of Ringer-Locke solution and melatonin (161.1 ± 55.4 vs. 124.3 ± 35.7 pg/30 min). An afternoon decrease in the DA concentration was also observed in two ewes not treated with melatonin (158.6 ± 50.8 vs. 90.5 ± 37.5 pg/30 min and 127.3 ± 66.4 vs. 33.7 ± 15.2 pg/30 min; Fig. 5).

In 2 out of 6 ewes in the luteal phase of the estrous cycle, DA concentrations were below the sensitivity of the assay. In 3 out of 4 ewes in which DA concentrations were assayed, the level of this neurotransmitter noted before melatonin administration (in range from 28.5 ± 12.1 to 75.4 ± 21.2 pg/30 min.), decreased during the infusion of melatonin below the sensitivity of the assay (Fig. 6). In one ewe, a decrease in the DA concentration below the sensitivity level occurred just 1 h before melatonin treatment.

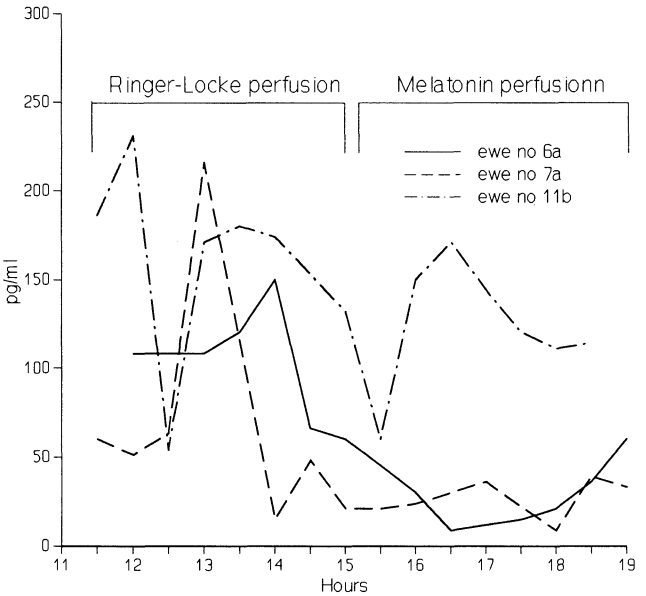

Fig. 4. Concentrations of DA in the perfusates collected from the MBH during the perfusion of the Ringer-Locke and melatonin solutions in anestrous ewes ($n = 3$).

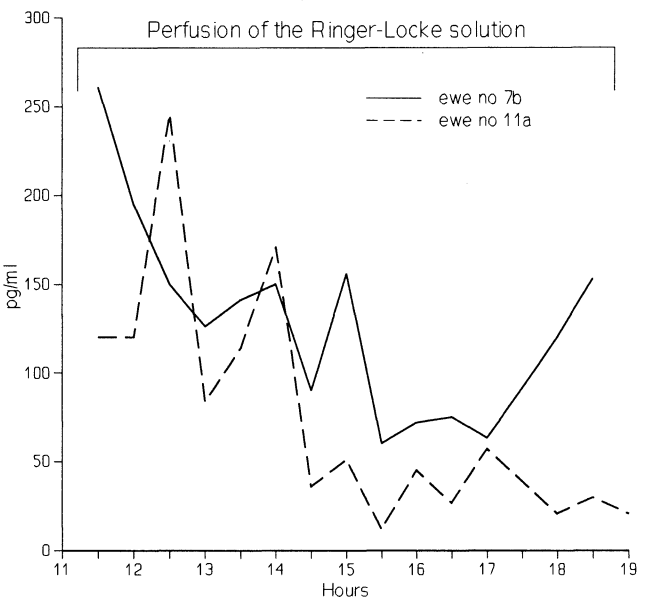

Fig. 5. Concentrations of DA in the perfusates collected from the MBH during the perfusion of the Ringer-Locke solution in anestrous ewes ($n = 2$).

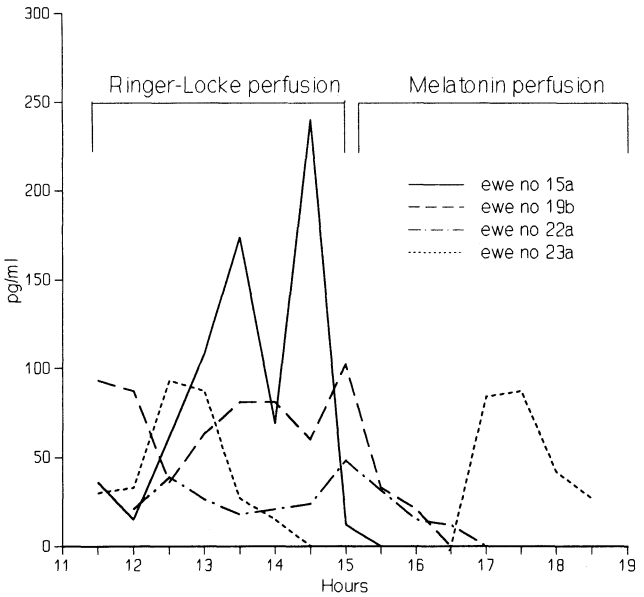

Fig. 6. Concentrations of DA in the perfusates collected from the MBH during the perfusion of the Ringer-Locke and melatonin solutions in ewes ($n = 4$) in the luteal phase of the estrous cycle.

The interpretation of the stimulatory effect of melatonin on prolactin secretion presented in our study is difficult. First, it is well known that long--term melatonin administration decreases prolactin secretion in sheep and other photoperiodic mammals. Second, studies on rats (Koulu et al. 1989), revealed changes in DA levels in the median eminence with low levels at night and high in the middle of the light period. The presented changes of DA concentrations in perfusates received from the control ewes indicate, that DA release from the median eminence in sheep also undergoes diurnal changes. The 24-h rhythm of prolactin secretion in ewes with a peak coincident with the dark phase could therefore directly reflect an endogenous rhythm of DA release. Similar changes in DA concentrations were also observed in perfusates collected during the melatonin perfusion from ewes being both in anestrus and in the luteal phase of the estrous cycle. However, there was no stimulatory effect of melatonin infused into III ventricle on prolactin secretion in ewes during the luteal phase. Thus, the question whether melatonin can exert its short-term stimulatory effect on daily prolactin secretion *via* the dopaminergic system located in the MBH remains unresolved. There is also the possibility that other, unrecognized prolactin regulatory factors, other than DA are involved and vary with the reproductive cycle. Nevertheless, if melatonin is not a major factor generating an evening or nightly surge of prolactin, there is a base to suppose that this pineal indolamine modulates activity of these factors in the ewes.

β-ENDORPHIN (β-END)

It is well established that endogenous opioid peptides (EOP) modulate gonadotropin secretion in ewes, essentially exerting a suppresive effect on the gonadotropin-releasing hormone (LHRH). This effect is the most pronounced in cycling animals under the shortening daylength conditions. Studies conducted in our laboratory on ewes, based on the hourly samples collected for 24 h on ten days of the year showed, that both an increase in β-END secretion and the marked circadian changes with nocturnal increase in β-END level occur in autumn (Misztal et al. 1994b). Marked changes in the plasma levels of β-END were demonstrated also in rams (Ebling and Lincoln 1987), kept under the artificial photoperiod regimen of alternating 12- to 16 week periods of long days (16L:8D) and short days (8L:16D). Transfer from long to short days led to a greater than 20-fold increase in the levels of β-END, reaching a maximum after 4-8 weeks. The reverse switch in photoperiod led to a rapid decrease in the levels. However the authors did not observe diurnal rhythm in the plasma levels of β-END in rams neither under conditions of long nor short days.

In the current study, periods preceding the melatonin or control infusions were characterized by great fluctuations of β-endorphin concentrations in blood plasma in ewes being both in anestrus and in the breeding season. The direct administration of melatonin into the III ventricle induced in 6 out of 9 anestrous ewes an abrupt increase in β-endorphin concentration during the first hour of the infusion (Fig. 7). After that, a gradual decrease in β-endor-

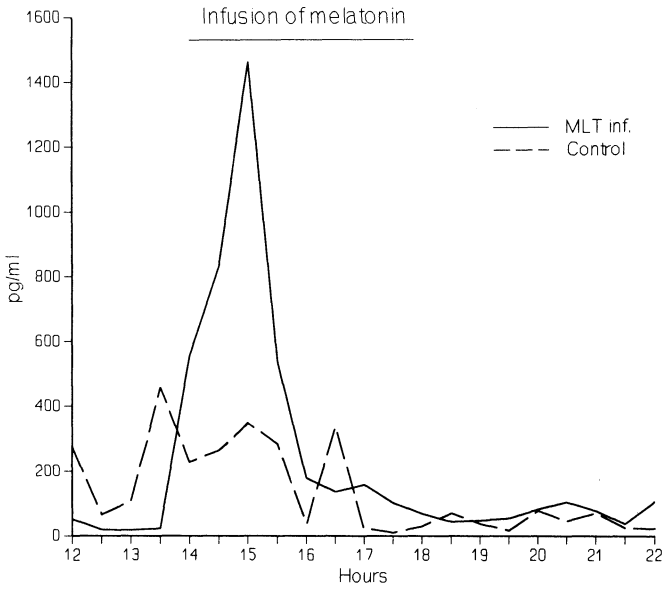

Fig. 7. Mean concentrations of β-endorphin in the blood plasma in anestrous ewes (n = 6), on the days of the melatonin (MLT) and control infusions.

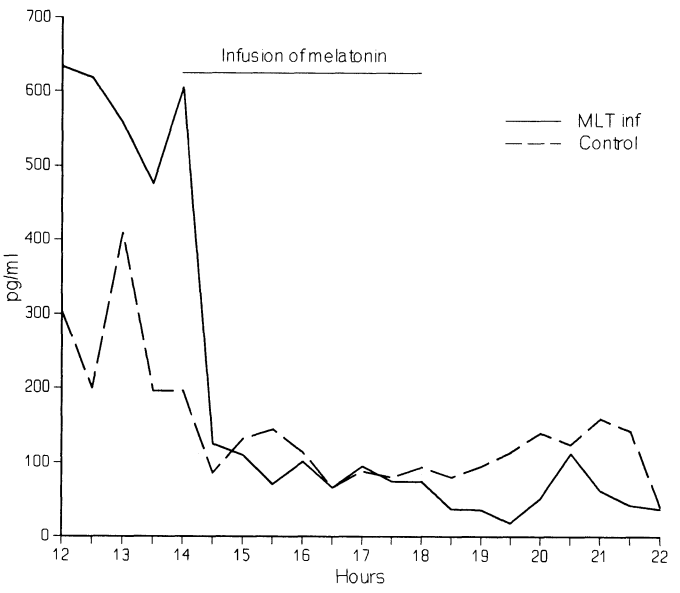

Fig. 8. Mean concentrations of β-endorphin in the blood plasma in ewes (n = 4) in the follicular phase of the estrous cycle on the days of the melatonin (MLT) and control infusions.

phin concentration was observed. The mean β-endorphin concentration during the melatonin infusion was 434 ± 495 pg/ml (mean ± SD; range from 68 ± 17 to 1463 ± 2394 pg/ml), and this was significantly higher, than for the control infusion (167 ± 154 pg/ml; range from 10 ± 10 to 349 ± 495 pg/ml; $P \leq 0.05$).

During the breeding season two ewes in the late follicular phase showed the estrous LH surge, one ewe on the day when melatonin was infused, and the other during the control infusion. The concentrations of β-endorphin from these two ewes were not used in the statistical analysis.

Both in ewes being in the late follicular and in the mid-luteal phase of the estrous cycle, the concentrations of β-endorphin decreased during the melatonin or control infusion. There were no significant differences between the mean β-endorphin concentration during the melatonin infusion, comparatively to the control infusion in ewes of these experimental groups: 145 ± 98 pg/ml (range from 49 ± 29 to 350 ± 253 pg/ml) vs. 160 ± 57 pg/ml (range from 108 ± 60 to 265 ± 237 pg/ml) in the late follicular phase (Fig. 8) and 89 ± 21 pg/ml (range from 66 ± 20 to 125 ± 102 pg/ml) vs. 100 ± 27 pg/ml (range from 66 ± 25 to 145 ± 116 pg/ml) in the mid-luteal phase (Fig. 9). However, the level of β-endorphin in the blood plasma observed during the next four hours, after the melatonin infusion was significantly lower, than the level noted after the control

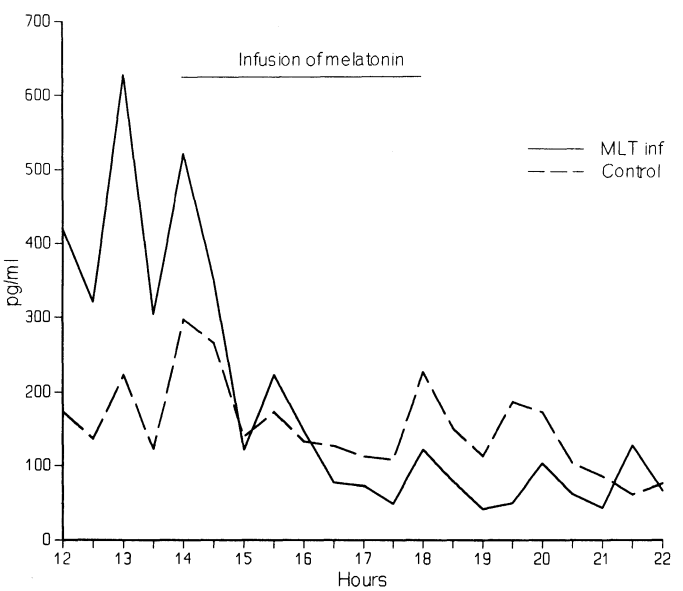

Fig. 9. Mean concentrations of β-endorphin in the blood plasma in ewes (n = 4) in the luteal phase of the estrous cycle on the days of the melatonin (MLT) and control infusions.

infusion both in ewes in the late follicular and mid-luteal phase of the estrous cycle: 71 ± 30 vs. 118 ± 46 pg/ml ($P \leq 0.05$) and 49 ± 28 vs. 111 ± 39 pg/ml ($P \leq 0.01$), respectively.

The presented effects of short-term melatonin administration into the III ventricle on β-END secretion were not as expected. Stimulation of β-END was observed in the anestrous ewes, when the physiological level of this peptide in blood plasma (Misztal et al. 1994b), and a degree of inhibition of the gonadotropic axis (Brooks et al. 1986) are low. The inhibitory effect of melatonin was obtained during the breeding season in ewes during either the late follicular or mid-luteal phase of the estrous cycle. It is worth of notice, that the β-endorphin circulating in the peripheral blood is largely derived from the pituitary gland and this may play no role in the central regulation of LHRH/LH secretion. The opioidergic pathways which apparently modulate the LHRH neurones are likely to be quite separate and localized in the MBH. There is the possibility that they are a key site for the action of melatonin.

Studies on rats (Genazzani et al. 1990), show that the circadian rhythm of the hypothalamic β-END is strictly related to the internal gonadal steroid milieu. The diurnal changes in the MBH concentrations of β-END that disappeared following ovariectomy were restored by chronic estradiol benzoate replacement. Thus, a final common pathway may operate through the mediation of gonadal and photoperiodic messages regulating the organization of the EOP activity. If an increase in the EOP activity in ewes during the shortening daylength conditions results from the increased gonadal function then indeed, melatonin may exert an inhibitory action on the β-END secretion. This phenomenon is in agreement with the hypothesis relating to the role of melatonin in sustaining the genetically encoded length of the breeding season (Malpaux and Karsch 1990), nevertheless requires further investigations.

LUTEOTROPIN (LH)

The secretion of LH from the pituitary gland is characterized in sheep by low frequency and high amplitude LH pulses under the increasing daylength conditions (decreasing duration of melatonin secretion) and by high frequency with low amplitude pulses in the breeding season under decreasing daylength (increasing duration of melatonin signal). The exposure of ewes and rams to various regimes of melatonin administration during the non-reproductive period results in an increase of gonadotropin secretion and in a change of the parameters of secretion typical for the short days. The earliest studies on the circadian rhythm of LH and follicle-stimulating hormone (FSH) in rams showed that an increase in gonadotropin secretion occurs in the early dark phase of the 24-h cycle (Lincoln et al. 1977). A similar nocturnal rise in gonadotropin secretion has been reported to occur in the laboratory rat (Haar et al. 1974).

In our study, the mean LH concentrations on the days of the melatonin and control infusions varied both in anestrous (5.7 ± 0.2 vs. 4.8 ± 0.2 ng/ml; mean ± SD, respectively) and in ewes in the mid-luteal phase of the estrous cycle (2.3 ± 0.5 vs. 1.5 ± 0.3 ng/ml, respectively). Treatment with melatonin in the III ventricle evoked no significant changes in the LH secretion in ewes of both of these groups. Similarly, melatonin infused into the III ventricle had no effect on the LH secretion in ewes in the late follicular phase of the estrous cycle, comparatively to the LH concentrations noted during the control infusion (4.8 ± 0.4 vs. 4.6 ± 0.1 ng/ml; NS). As in the case of β-endorphin, the concentrations of LH from ewes, in which the estrous surge occurred, were omitted from the statistical analysis.

At the same time we studied an effect of melatonin, perfused into the MBH by the push-pull method, on the luteinizing hormone - releasing hormone (LHRH) secretion in ewes in anestrus ($n = 6$) and mid-luteal phase of the estrous cycle ($n = 5$).

The LHRH concentration in the MBH decreased significantly during the melatonin perfusion, comparatively to the period of the preceding control perfusion in one ewe out of each group: 144 ± 49 vs. 81 ± 24 pg/ml of perfusate (mean ± SD; $P \leq 0.05$) and 163 ± 44 vs. 66 ± 33 pg/ml of perfusate ($P \leq 0.01$), in anestrous and cycling ewes, respec-

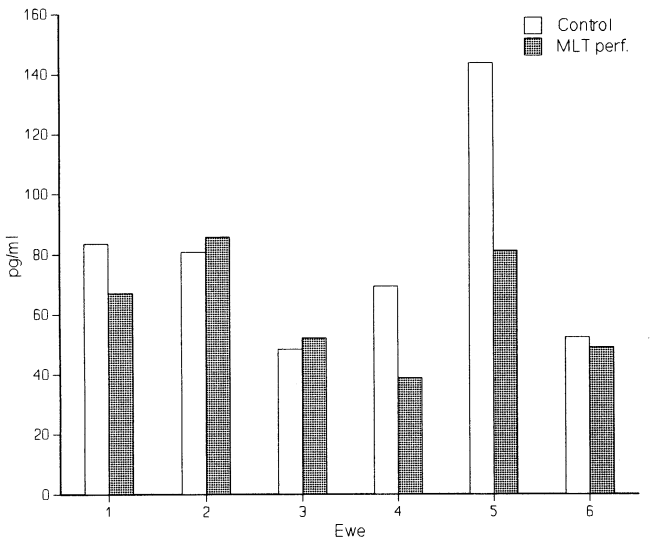

Fig. 10. Mean concentrations of LHRH in the perfusates collected from the MBH during the perfusion of the Ringer-Locke and melatonin (MLT) solutions in anestrous ewes (n = 6).

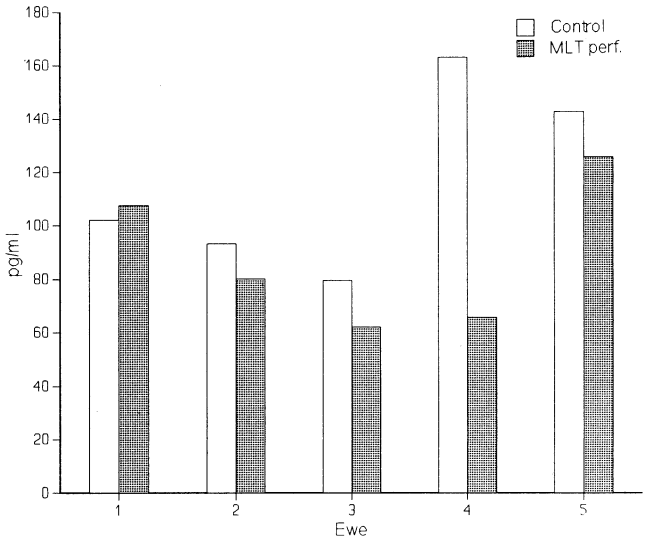

Fig. 11. Mean concentrations of LHRH in the perfusates collected from the MBH during the perfusion of the Ringer-Locke and melatonin (MLT) solutions in ewes (n = 5) in the luteal phase of the estrous cycle.

tively (Figs. 10 and 11). In the other ewes in these groups, melatonin perfused into the MBH had no significant effect on the LHRH secretion. The mean LHRH concentrations in perfusates collected during the control and melatonin perfusion in anestrous ewes were in range from 48 ± 21 to 84 ± 42 pg/ml and from 39 ± 21 to 86 ± 28 pg/ml respectively. In perfusates collected from ewes in the mid-lutel phase, the values were in range from 80 ± 53 to 143 ± 48 pg/ml and from 62 ± 55 to 126 ± 69 pg/ml, during the control and melatonin treatment respectively.

The results of our study clearly show that melatonin infused into the III ventricle does not affect the daily pattern of LH secretion in ewes. Moreover, the short-term melatonin administration into the MBH does not affect LHRH release. This lack of a short--term effect of melatonin is different from the long--term effect obtained by placing the melatonin micro-implants in the III ventricle or in the MBH which resulted in an increase in the secretion of LH in ewes and rams (Lincoln and Maeda 1992a; Malpaux et al. 1993). An absence of a short-term effect of melatonin on LHRH/LH release both in anestrus and in breeding season also raises objections to a hypothesis that there are seasonal changes in the sensitivity of the pituitary gonadotrophic cells or the LHRH neurones in the MBH to melatonin. It rather supports a view that the action of melatonin on LHRH neurones involves a more complicated mechanism. In particular, dopaminergic neurones seem to be implicated in this regulation. The modification of DA content and tyrosine hydroxylase (the rate-limiting enzyme of catecholamine synthesis) activity in the median eminence by photoperiod has been reported by Thiery (1991) and Viguie et al. (1993). Furthermore, systemic injection or local hypothalamic administration of a DA antagonist (pimozide), and neurotoxic lesions of a dopaminergic cell group (A15) in photoperiodically inhibited ewes, have been shown to stimulate LH secretion (Meyer and Goodman 1985, Thiery et al. 1989, Havern et al. 1991). Neuroexcitatory amino acids could also be implicated in the regulation of LHRH secretion by melatonin since the injections of NMDA stimulate LHRH secretion in sheep differentially according to the season (Lincoln and Wu 1991). A recent study by Viguie et al. (1995) shows that action of melatonin on LHRH/LH secretion requires a delay of 40-60 days. This period seems

to be necessary for transformation of the LHRH regulating mechanism to relay the effects of the melatonin signal. The identification of all steps of this process is the most important challenge to an understanding of photoperiodic regulation of reproduction.

CONCLUDING REMARKS

The presented stimulatory or inhibitory effects of the short-term melatonin administration on prolactin and β-END secretion and the lack of such effects on LH secretion allows for a wider look at melatonin as a modulator of endogenous hormonal rhythms. There is evidence that the seasonal change in prolactin secretion may result at least in part from changes in the circadian secretion. Thus, the high level of prolactin during the long-day period may be a function of the nightly rises of prolactin enhanced by melatonin. The opposite effect observed in ewes receiving long-term treatment with melatonin indicates that rather different mechanisms are involved in regulation of the circadian and seasonal rhythms of prolactin secretion.

According to EOP activity, a paradoxical inhibitory effect of melatonin at the time of seasonally increased secretion of β-END during a reproductive period may be a part of the mechanism by which melatonin sustains the length of the breeding activity in normal duration.

Finally, the lack of an effect of short-term melatonin administration on LH and LHRH release raises objections to a hypothesis that there are seasonal changes in the sensitivity of the pituitary gonadotrophic cells or LHRH neurones in the MBH to melatonin, and supports the suggestions that the action of melatonin on the neuroendocrine reproductive axis is a long-term process implicating a more complicated mechanism controlling the LHRH secretion.

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