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# Daily GnRH and LH secretion in ewes is not modified by exogenous melatonin during seasonal anestrus

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**Abstract.** The effect of central, short-term melatonin administration on daily GnRH and LH secretion was studied in ewes during seasonal anestrus. Melatonin, in a total dose of 32 μg and the vehicle were perfused for 4 hours into the mediobasal hypothalamus/median eminence (MBH/ME). The mean GnRH concentration during perfusion with melatonin decreased significantly ($P<0.05$), as compared to the concentration during the preceding perfusion with the vehicle only. This change resulted from high variations in GnRH concentration noted during the initial phase of perfusion rather than from an action of melatonin. Melatonin perfused into the MBH/ME did not significantly affect LH secretion. A higher dose of melatonin and vehicle were also infused intracerebroventricularly (icv.) in either intact (300 μg for 3 hours) or ovariectomized (OVX) ewes (400 μg for 4 hours, 100 μg/100 μl/h). In the intact animals, melatonin did not significantly affect LH secretion. Interestingly, melatonin significantly decreased ($P<0.05$) the number of LH peaks in OVX ewes. These results demonstrate that melatonin delivered for a few hours directly into the central nervous system did not affect either daily hypothalamic GnRH release or pituitary LH secretion in intact ewes during seasonal anestrus, but did modify pulsatile LH secretion in ewes deprived of the negative feedback of estradiol.

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**Key words:** melatonin, GnRH, LH, anestrus

## INTRODUCTION

Chronic administration of exogenous melatonin in anestrous ewes leads to an increase in the secretion of LH during treatment (Kenneway et al. 1982, Bittman and Karsch 1984, Webster et al. 1991, Viguie et al. 1995). Exactly how melatonin exerts this effect is, however, not well understood. In the last decade, it was found that the mediobasal hypothalamus (MBH) is the primary site of melatonin's action on reproductive activity in sheep. Placement of micro-implants of melatonin in the MBH of anestrous ewes (Malpaux et al. 1993) and rams (Lincoln and Maeda 1992a) evoked an increase in the secretion of gonadotropins and in sexual behavior, while their withdrawal restored anestrus. More recently, Malpaux et al. (1998) have found that a discrete area of high melatonin binding in the sheep hypothalamus is detectable in the premammillary hypothalamic area (PMH) and melatonin, given by the insertion of micro-implants, is able to stimulate LH secretion if it is delivered into this part of the hypothalamus.

Activation of the GnRH/LH system in anestrous ewes by melatonin leading to the early onset of estrus requires several weeks of exposure to melatonin (Viguie et al. 1995). Relatively little data is available on the short-term effect of this pineal hormone on daily LH secretion in sexually inactive ewes. It is possible that pulsatile secretion of this gonadotropin will also be modulated during or shortly after a single administration of melatonin. Considering that the site of melatonin's action on reproductive activity in sheep is located in the hypothalamus (Lincoln and Maeda 1992a, Malpaux at al. 1993, Malpaux et al. 1998) and a subset of GnRH neurons is present in the MBH (Lehman et al. 1986), the purpose of this study was to find out whether changes in GnRH/LH secretion would appear after a brief administration of melatonin into this structure. Thus, in experiment 1, one dose of melatonin was perfused in ewes directly into the MBH/ME, where many GnRH axons have terminals (Silverman et al. 1994). A second, higher dose of melatonin was infused into the third ventricle to act on the neighboring areas in the hypothalamus, including the PMH, both in intact (experiment 2) and in OVX ewes (experiment 3). All the experiments were performed during seasonal anestrus; perfusate GnRH and plasma LH concentrations were monitored.

## METHODS

### Animals, Management and Experimental Procedure

Twenty 2- to 3-year-old Polish Lowland ewes were used in the experiments which lasted two years. In the first year, during the winter and following experimental periods, the animals were maintained indoors under natural lighting conditions (52°N, 21°E) and fed a constant diet of commercial concentrates, with hay and water available ad libitum. Ewes appropriated for the experiment in the second year spent the summer grazing on pasture.

All procedures were approved by the Ethics Committee at the Kielanowski Institute of Animal Physiology and Nutrition in Jabłonna, according to the Polish Guide for the Care and Use of Animals (August 2, 1997). Stainless steel guide cannulae (1.6 mm OD) were implanted under stereotaxic control bilaterally into the MBH/ME ($n = 6$) or into the third ventricle ($n = 14$) through a drill hole in the skull (Traczyk and Przekop 1963) one month before the experiments. The guide cannula were fixed to the skull with stainless steel screws and dental cement. The external opening to the canal was closed with a stainless steel cap. The placement of the guide cannula was confirmed by the outflow of a small amount of cerebro-spinal fluid during surgery (in case of the third ventricle) and by the infusion of a small volume of blue ink after slaughtering.

Melatonin (Sigma Chemical Co. Ltd.) was dissolved in ethanol and stored at -20°C as a stock solution (10 mg/500 µl) for no longer than three days. Immediately before the experiment it was dissolved in Ringer- -Locke solution and a similar solution, without melatonin, was prepared as the control vehicle.

All perfusions and infusions of melatonin into the MBH/ME and into the third ventricle, respectively, were performed during the period of seasonal anestrus, from March to June, with calibrated 1.0-ml gas-tight syringes and a CMA/100 microinjection pump. During the experiments, animals were kept in comfortable cages where they could lie down and had unrestrained access to hay.

### Experiment 1: Effect of melatonin perfused into the MBH/ME on GnRH and LH secretion

Six ewes were subjected to perfusion of the MBH/ME with melatonin (20 µg/ml, total 32 µg) from 15.00 to

19.00 h preceded by perfusion with the vehicle alone (Ringer-Locke solution, RL) from 9.00 to 15.00 h and to control perfusion with RL alone from 9.00 to 19.00 h at 10-12-day intervals. The perfusions were performed by the push-pull method, alternately in each side of the head. The flow rate was 7 µl/min and the volume of perfusates collected at 30-min intervals was about 200 µl. The tubes for perfusates contained 5 µl of aprotinine (250 IU, a proteolytic enzyme inhibitor) and were kept in an ice bath during sampling. Immediately after filling, they were frozen in liquid nitrogen and stored at -80°C until assayed for GnRH. The perfusates collected from 9.00 to 11.00 h were excluded from the assay to eliminate the changes in GnRH release caused by insertion of the push-pull cannula into the MBH/ME. Simultaneously with perfusates, blood samples were collected from 11.00 to 19.00 h at 20-min intervals through a catheter inserted into the jugular vein a day before the experiment. After centrifugation in heparinized tubes, plasma was stored at -20°C until assayed for LH.

### Experiment 2: Effect of melatonin infused icv. on LH secretion in intact ewes

Eight ewes were infused icv. twice: with melatonin (100 µg/100 µl/h, total 300 µg) and with Ringer-Locke solution (control) alternately, at two-week intervals. The dose of melatonin used in this experiment was about ten times higher than that perfused into the MBH/ME and, as we showed previously, this dose of melatonin stimulated the secretion of prolactin in anestrous ewes (Misztal et al. 1997). All infusions were performed in the afternoon from 14.00 to 17.00 h 3.5 to 5.5 h before sunset. Blood samples were collected from 11.00 to 20.00 h at 30-min intervals through a catheter inserted into the jugular vein a day before the experiment. After centrifugation in heparinized tubes, plasma was stored at -20°C until assayed for LH.

### Experiment 3: Effect of melatonin infused icv. on LH secretion in OVX ewes

Six ewes were ovariectomized two weeks before the experiment and then infused twice: alternately with melatonin (100 µg/100 µl/h, total 400 µg) and with Ringer-Locke solution (OVX control) at weekly intervals. The infusions were performed from 14.00 to 18.00 h and blood samples were collected from 10.00 to 24.00 h as described above. After centrifugation in heparinized tubes, plasma was stored at -20°C until assayed for LH.

### Analytical Techniques

The concentration of GnRH in hypothalamic perfusates was measured by a double-antibody radioimmunoassay (RIA) according to Kerdelhue et al. (1973) using an antiserum produced in our laboratory in rabbits. The full characterization of this antiserum and the procedures of the method are given by Domański et al. (1991). The assay sensitivity was 5 pg/tube.

Plasma LH concentration was assayed by a double-antibody RIA, using antiovine-LH and antirabbit-gammaglobulin antisera and bovine LH standard (NIH-LH-B6), according to Stupnicki and Madej (1976). The assay sensitivity was 0.3 ng/ml, and the intra- and interassay coefficients of variations were 8.2 and 12.5%, respectively.

### Statistics

Perfusate GnRH and plasma LH concentrations are expressed as a mean ± SE. The significance of differences between means at given times before, during and after infusions were assessed by ANOVA followed by the least significant differences (LSD) test (STATISTICA). The number of LH pulses was determined by the PC-PULSAR computer program according to the method of Merriam and Wachter (1982) with the following G parameters: G1 = 3.98; G2 = 2.40; G3 = 1.68; G4 = 1.24 and G5 = 0.93. Analysis was performed for all ewes individually, including the entire sampling period. The frequency of LH pulses before, during and after the infusion was defined as the number of identified pulses per collecting period (Viguie et al. 1995) and is expressed as a mean ± SE. Differences in LH pulse frequency among these times, in the vehicle and melatonin infused groups, were analyzed by a Wilcoxon test and between groups by a Mann-Whitney test.

## RESULTS

### Effect of melatonin perfused into the MBH/ME on GnRH and LH secretion

The perfusate concentrations of GnRH were assayed in five of six ewes, in which the placement of the push-pull cannula, confirmed by *post mortem* study of

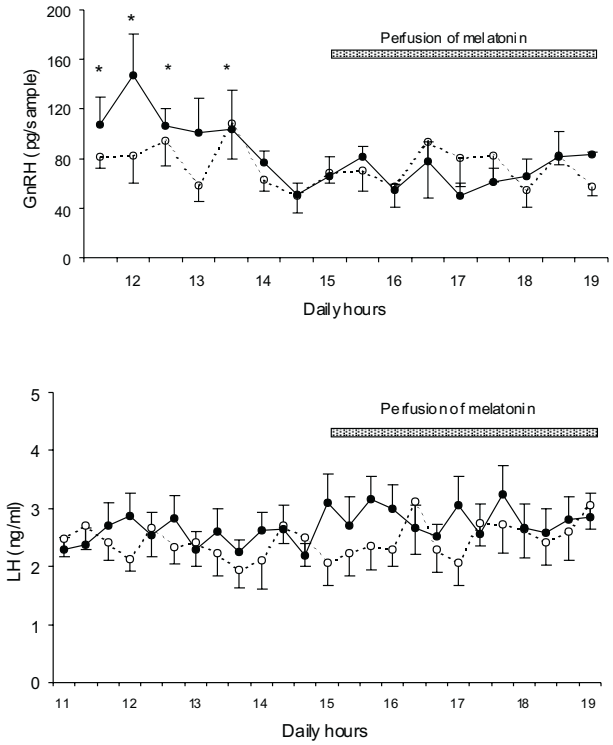


Fig. 1. Hypothalamic perfusate GnRH (top) and plasma LH (bottom) concentrations in intact anestrous ewes perfused with a vehicle alone from 11.00 to 19.00 h (mean ± SE, $n$ = 5, dashed line) and perfused with a vehicle from 11.00 to 15.00 h and with melatonin (20 μg/ml) from 15.00 to 19.00 h (*, $P$<0.05, mean ± SE, $n$ = 5, solid line).

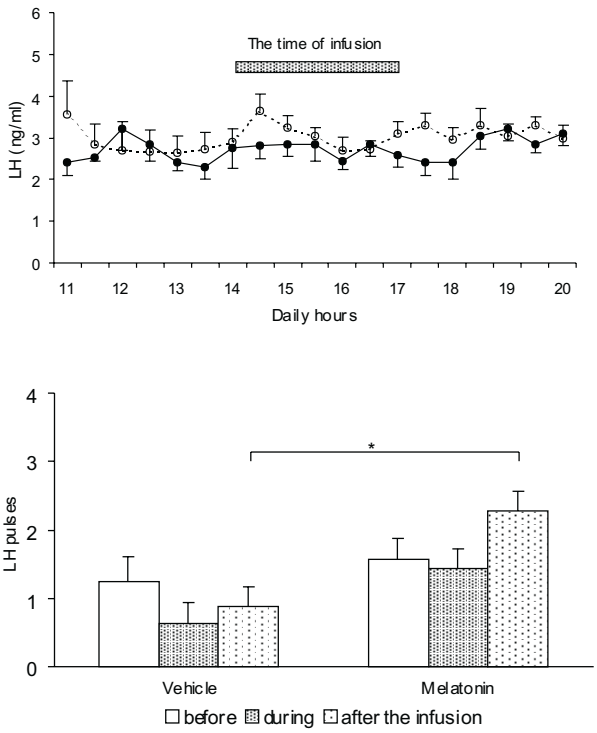


Fig. 2. Mean (± SE) plasma LH concentration (top) in intact ewes infused with a vehicle ($n$ = 8, dashed line) and with melatonin (100 μg/100 μl/h, $n$ = 8, solid line) from 14.00 to 17.00 h during seasonal anestrus and mean (± SE) LH pulse frequency (bottom) before, during and after the infusions of a vehicle (left panel) and melatonin (right panel), defined as the number of identified pulses per these collecting periods (*, $P$<0.05, See the text for additional statistical comparisons).

the brain, was correct as shown by a blue dot in the area of the ME. During the control perfusion, the mean perfusate GnRH concentrations did not differ significantly throughout the entire period of sampling and ranged from 49.6 ± 13.7 to 108.2 ± 28.5 pg/sample and from 54.8 ± 14.5 to 93.5 ± 45.5 pg/sample during the first (11.00 - 15.00 h) and second (15.00 - 19.00 h) phase of the perfusion, respectively. During the perfusion of melatonin into the MBH/ME, perfusate GnRH concentration which ranged from 50.3 ± 9.7 to 83.5 ± 1.8 pg/sample (15.00 - 19.00 h) was significantly ($P$<0.05) lower than that noted before melatonin treatment: from 51.2 ± 9.2 to 147.5 ± 33.0 pg/sample (11.00 - 15.00 h). The highest GnRH concentration was noted, however, in the perfusates collected during the 2.5-hour period from the beginning of vehicle perfusion (11.00 – 13.30 h). At this time, a very high concentrations of GnRH occurred in ewes No. 2 and 9. Patterns of GnRH secretion in control and melatonin perfused ewes are shown in Fig. 1 (top).

Melatonin treatment had no significant effect on the LH concentration as compared with the values noted before the melatonin perfusion (Fig. 1 bottom) and with the control during the same time. Melatonin also had no significant effect on frequency of LH pulses compared with the preceding vehicle treatment: 2.83 ± 0.17/4 h vs. 3.33 ± 0.33/4 h, respectively.

### Effect of melatonin infused icv. on LH secretion in intact ewes

The mean plasma LH concentration in control ewes ranged from 2.65 ± 0.40 to 3.55 ± 0.81 ng/ml, from 2.71 ± 0.29 to 3.65 ± 0.42 ng/ml and from 2.95 ± 0.35 to 3.31 ± 0.39 ng/ml before, during and after infusion of the vehicle, respectively (Fig. 2 top). Melatonin infused icv. did not significantly affect the secretion of LH, which ranged from 2.59 ± 0.28 to 2.85 ± 0.27 ng/ml, as com-

pared with the concentrations noted before, from 2.30 ± 0.26 to 3.21 ± 0.51 ng/ml, and after the infusion, from 2.40 ± 0.28 to 3.42 ± 0.38 ng/ml (Fig. 2 top). Treatment with melatonin also had no significant effect on plasma LH concentrations as compared with the values noted during the control infusion. Similarly, infusion of melatonin did not significantly affect the frequency of LH pulses compared with the period before and after treatment: 1.43 ± 0.30/3 h vs. 1.57 ± 0.30/3 h and 2.28 ± 0.28/3 h, respectively (Fig. 2 bottom). A significantly higher frequency of LH pulses was observed in ewes after the infusion of melatonin when compared only with the controls after vehicle treatment: 2.28 ± 0.28/3 h vs. 0.88 ± 0.29/3 h, respectively ($P<0.05$, $n = 8$, Fig. 2 bottom).

### Effect of melatonin infused icv. on LH secretion in OVX ewes

The secretion of LH in OVX ewes was about 3 times higher than that noted in the intact animals. In control OVX ewes, the plasma LH concentration before the infusion of the vehicle ranged from 8.82 ± 1.15 to 10.67 ± 2.84 ng/ml and was significantly ($P<0.05$) higher than the concentrations noted during and after the infusion: from 7.22 ± 0.68 to 9.05 ± 1.78 ng/ml and from 7.18 ± 0.67 to 8.55 ± 0.85 ng/ml, respectively (Fig. 3 top). This concentration was also significantly ($P<0.05$) higher than these noted in melatonin treated animals during and after the infusion. Melatonin infused icv. did not significantly affect the LH concentration, which ranged from 6.55 ± 0.72 to 9.78 ± 1.61 ng/ml, as compared with the level noted before, 7.50 ± 0.79 to 9.75 ± 1.55 ng/ml, or after the infusion, 6.60 ± 0.95 to 10.22 ± 1.74 ng/ml (Fig. 3 top). There was also no significant effect of melatonin treatment on plasma LH concentration as compared with the values noted during control infusion. Interestingly, a significant decrease was observed in frequency of LH pulses during melatonin infusion as compared with the preceding period: 1.17 ± 0.31/4 h vs. 2.83 ± 0.17/4 h, respectively ($P<0.05$, $n = 6$, Fig. 3 bottom). LH pulse frequency noted before the infusion of melatonin was also significantly higher than that observed in the controls before infusion of the vehicle, 1.67 ± 0.33/4 h ($P<0.05$, $n = 6$, Fig. 3 bottom). There was no significant difference between frequency of LH pulses in ewes after infusion of melatonin and the vehicle: 2.17 ± 0.40/6 h vs. 2.33 ± 0.61/6 h, respectively.

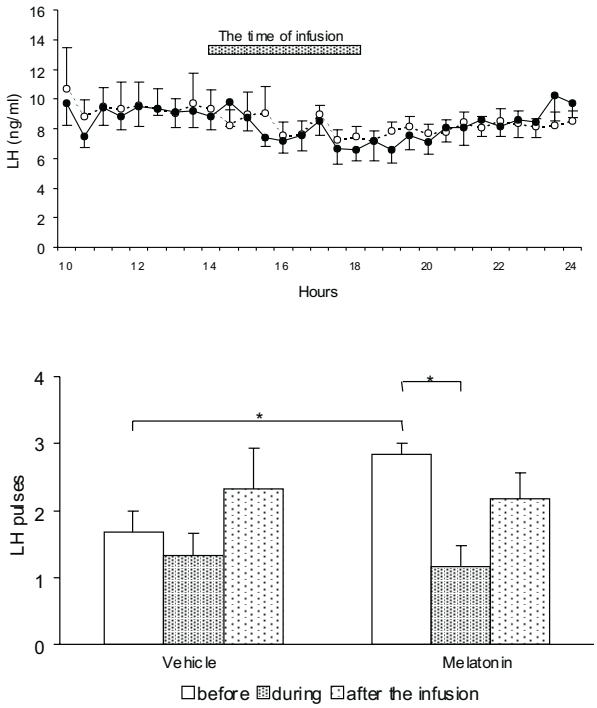


Fig. 3. Mean (± SE) plasma LH concentration (top) in OVX ewes infused with a vehicle ($n = 6$, dashed line) and with melatonin (100 μg/ 100 μl/h, $n = 6$, solid line) from 14.00 to 18.00 h during seasonal anestrus and mean (± SE) LH pulse frequency (bottom) before, during and after infusions of a vehicle (left panel) and melatonin (right panel), defined as the number of identified pulses per these collecting periods (*, $P<0.05$, See the text for additional statistical comparisons).

## DISCUSSION

The results of this study demonstrate that several-hour long administration of melatonin directly into the central nervous system does not affect either hypothalamic GnRH release or pituitary LH secretion in intact ewes during the period of seasonal anestrus. Surprisingly, in OVX ewes, melatonin infused icv. decreased frequency of LH pulses without affecting the mean LH concentration.

The pattern of pulsatile LH secretion in ewes during seasonal anestrus is characterized by a low frequency of LH pulses, which in turn reflects the frequency of GnRH release into the hypothalamo-hypophysical portal vessels (Barrel et al. 1992, Viguie et al. 1995). Previous studies connecting melatonin to the reproductive functions in sheep showed a stimulatory effect of this pineal hormone on the secretory activity of the GnRH/LH axis

(Kenneway et al. 1982, Bittman and Karsch 1984, Webster et al. 1991, Viguie et al. 1995). Recently, Boukhliq et al. (1999) suggested that a subset of GnRH neurons responsible for episodic GnRH, and hence LH secretion in ewes, is located within the MBH. Moreover, much of the GnRH axons have terminals in the ME, a structure located on the ventral part of the MBH (Silverman et al. 1994). Following demonstration that hypothalamic micro-implants of melatonin stimulated reproductive activity in ewes and rams (Lincoln and Maeda 1992a, Malpaux et al. 1993), the hypothalamus was proposed to be the primary site of melatonin's action on GnRH/LH secretion. In addition, Malpaux et al. (1998) have shown that a discrete area of high melatonin binding is detectable in the PMH and that melatonin, given by the insertion of micro-implants, is able to stimulate LH secretion if it is delivered into this site of the hypothalamus. In contrast to the long-term action of melatonin in the sheep hypothalamus (Lincoln and Maeda 1992a, Malpaux et al. 1993, Malpaux et al. 1998), the results of our study demonstrate that melatonin delivered for a few hours into the MBH/ME or into the third ventricle in intact anestrous ewes has no effect on either hypothalamic GnRH or pituitary LH secretion during and shortly after administration, a period which is often not considered during long-term studies. The significantly lower GnRH level noted during perfusion of melatonin into the MBH/ME seems not to result from action of this hormone. The great variations in the perfusate GnRH concentration occurred during 2.5-hour period from the beginning of vehicle perfusion. At this time, a very high concentrations of GnRH were noted in two animals. Since insertion of the push-pull cannula into the MBH may cause some disturbances in the hypothalamic function (Chomicka et al. 1996), perfusates collected from 9.00 to 11.00 h were excluded from the assay. In two ewes, stabilization of the GnRH system evidently took by far more time.

Although the lower dose of melatonin would act only locally in, or close to the MBH/ME, as confirmed by *post mortem* study, the higher dose spread to the all areas neighboring the third ventricle, which may be at least in part involved in the control of the GnRH/LH system, including the PMH. As we have shown, the only important difference in the secretory activity of this system in intact ewes was observed after the icv. infusion of a higher dose of melatonin as compared with that in the control. This is in agreement with the early results of Lincoln et al. (1977), who described a greater frequency of episodic LH pulses in rams in the beginning of the dark phase of the 24 h cycle. The rams in the cited study were subjected to an artificial light regime of alternating periods of long and short days and such a pattern of pulsatile LH secretion predominated during the short-day period (Lincoln et al. 1977). It has been suggested that a daily rhythm of LH, with greater secretion at night, exists also in sexually active ewes (Currie et al. 1993). In the natural short-day conditions, plasma concentration of melatonin in ewes is higher than during the summer time (Misztal et al. 1996a). Our present observation would then confirm a stimulatory effect of melatonin on the frequency of LH pulses, what could be typical for both sexes. However, the mentioned difference in pulsatile secretion might not result from a direct response of LH to melatonin since there were no differences in the frequency of LH pulses during the whole period of sampling in ewes treated with this pineal hormone. The difference in LH pulse frequency noted in this study resulted rather from the higher total number of LH peaks observed in ewes treated with melatonin than in ewes during control infusions. In some animals the number of LH peaks was increased during the day of the experiment reflecting a variability within the same animal which is often found in this kind of study. Thus an assumption that melatonin affects the daily pattern of pulsatile LH secretion in anestrous ewes requires further clarification.

That a dose of melatonin administered into the third ventricle is sufficient to direct brain stimulation is evidenced by an abrupt increase in prolactin secretion observed in anestrous ewes during infusion, as described in our previous work (Misztal et al. 1997). It is noteworthy that the response of prolactin to infused melatonin also varied from that observed in ewes receiving long-acting implants (Lincoln and Ebling 1985, Lincoln and Maeda 1992b). Moreover, plasma melatonin concentration, monitored during the infusion of this hormone, was only 3- to 4-times higher than that noted in this breed during winter nights (Misztal et al. 1996a, 1997). The above arguments together suggest that during seasonal anestrus, daily secretion of GnRH/LH in ewes is strongly stabilized and insensitive to a brief administration of melatonin.

The low secretion of LH during anestrus results mainly from increased negative estradiol feedback (Legan et al. 1977). The sites at which estradiol exerts its inhibitory effect on the activity of the hypothalamo-pituitary GnRH/LH axis in ewes have recently been extensively studied (Herbison et al. 1993, Lehman and Karsch

1993, Blache et al. 1994, Havern et al. 1994). Removing the main source of estradiol by ovariectomy destroys the inhibitory mechanism and leads to increased LH secretion, which was observed in the present study. Interestingly, our results demonstrate that melatonin infused into the third ventricle decreased the frequency of LH pulses in ewes deprived of the negative feedback of estradiol, without affecting mean plasma LH concentration. These observed changes in pulsatile LH secretion were transient and occurred only during a period of the infusion. Although the inhibitory effect of melatonin on LH secretion in OVX ewes was not as strong as that of estradiol (intact ewes), this phenomenon is significant and shows a possible close relation between melatonin, estradiol and LH secretion in the ewe. An inhibitory action of the pineal hormone in OVX ewes was first documented by Roche et al. (1970) who determined LH concentration in sera and pituitaries in ewes subjected to continuous, intrajugular infusion of melatonin from the 14th through the 30th day after OVX. By this method, a rise in LH concentration, which escalated during this period in non-infused ewes, was stopped in melatonin treated animals. In contrast, an increased number of LH peaks, noted in our study before the infusion of melatonin, could occur as a result of any stress in the initial phase of the experiment. In control OVX ewes plasma LH concentration was significantly increased during this phase. It has been recently demonstrated that corticotrophin-releasing hormone, which activates the hypothalamo-pituitary-adrenal axis under the influence of stress, stimulates the secretion of LH in the sheep (Caraty et al. 1997, Tilbrook et al. 1999).

Neural control of GnRH/LH secretion is a complex process also involving an interaction between various hypothalamic neurotransmitters, where dopamine (DA) and β-endorphin (β-END) are the most important (Tortonese 1999). Both DA and β-END participate in the suppressive mechanism of the hypothalamo-pituitary GnRH/LH axis in ewes and rams (Tortonese 1999, Jackson and Kuehl 2000). As we have previously demonstrated, the perfusion of the same, lower dose of melatonin into the MBH/ME in anestrous ewes did not affect DA release as compared with the control (Misztal et al. 1997). In turn, higher dose of melatonin used in the icv. infusion abruptly increased the secretion of prolactin and β-END (Misztal et al. 1996b, 1997). This suggests that infused melatonin might suppress DA release, so it should also promote stimulation of LH secretion. The increased release of β-END in response to melatonin (Misztal et al. 1996b) may, however, be why there are no changes in the secretion of LH in intact anestrous ewes and also why the frequency of LH pulses decreased in the OVX. Further studies examining these relationships in melatonin-treated animals are in progress.

In summary, melatonin administered for a few hours directly into the CNS did not affect either daily hypothalamic GnRH release or pituitary LH secretion in intact ewes during seasonal anestrus, but modified pulsatile LH secretion in ewes deprived of the negative feedback of estradiol. Given the increased prolactin secretion in response to the central infusion of melatonin (Misztal et al. 1997), the present data suggest that secretory activity of the GnRH/LH axis in anestrous ewes is strongly stabilized and insensitive to brief administration of this pineal hormone.

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## REFERENCES

Barrell G.R., Moenter S.M., Caraty A., Karsch F. J. (1992) A marked seasonal change in GnRH secretion in the ewe. Biol. Reprod. 46: 1130-1135.

Bittman E.L., Karsch F.J. (1984) Nightly duration of pineal melatonin secretion determines the reproductive response in inhibitory day length in the ewe. Biol. Reprod. 30: 585-593.

Blache D., Batailler M., Fabre-Nys C. (1994) Oestrogen receptors in the preoptico-hypothalamic continuum, immunohistochemical study of the distribution and cell density during induced oestrous cycle in ovariectomized ewe. J. Neuroendocrinol. 6: 329-339.

Boukhliq R., Goodman R.L., Berriman S.J., Adrian B., Lehman M.N. (1999) A subset of gonadotropin-releasing hormone neurons in the ovine medial basal hypothalamus is activated during increased pulsatile luteinizing hormone secretion. Endocrinology 140: 5929-5936.

Caraty A., Miller D.W., Delaleu B., Martin G.B. (1997) Stimulation of LH secretion in sheep by central administration of corticotrophin-releasing hormone. J. Reprod. Fertil. 111: 249-257.

Chomicka L.K., Barcikowski B., Snochowski M. (1996) Neural and endocrine changes associated with push-pull perfu-

sion of the nucleus infundibularis/median eminence in ewes. In: Stress: molecular genetic and neurobiological advances (Eds. R. McCarty, G. Aguilera, E. Sabban and R. Kvetnansky). Gordon and Breach Science Publishers S.A., New York, p. 541-548.

Currie W.D., Medhamurthy R.J., Cook S.J., Rawling N.C. (1993) Seasonal fluctuation in diurnal rhythms of luteinizing hormone secretion in ewes during the mid-luteal phase of the oestrous cycle. J. Reprod. Fertil. 97: 71-74.

Domański E., Chomicka L.K., Ostrowska A., Gajewska A., Mateusiak K. (1991) Release of luteinizing hormone-releasing hormone, β-endorphin and noradrenaline by the nucleus infundibularis/median eminence during periovulatory period in the sheep. Neuroendocrinology 54: 151-158.

Havern R.L., Whisnant C.S., Goodman R.L. (1994) Dopaminergic structures in the ovine hypothalamus mediating estradiol negative feedback in anestrous ewes. Endocrinology 134: 1905-1914.

Herbison A.E., Robinson J.E., Skinner D.C. (1993) Distribution of estrogen receptor-immunoreactive cells in the preoptic area of the ewe - co-localization with glutamic acid decarboxylase but not luteinizing hormone-releasing hormone. Neuroendocrinology 57: 751-759.

Jackson G.L., Kuehl D.E. (2000) Interactions of photoperiod, testosterone and naloxone on GnRH and LH pulse parameters in the male sheep. Domest. Anim. Endocrinol. 18: 97-110.

Kenneway D.J., Gilmore T.A., Seamark R.E. (1982) Effect of melatonin feeding on serum prolactin and gonadotropin and the onset of seasonal estrous cyclicity in sheep. Endocrinology 110: 1766-1772.

Kerdelhue B., Jutisz M., Gillessen D., Studer R.O. (1973) Obtention of antisera against a hypothalamic decapeptide (luteinizing hormone, follicle stimulating hormone releasing hormone) which stimulates the release of pituitary gonadotrophin and development of its radioimmunoassay. Biochem. Biophys. Acta 297: 540-544.

Legan S.J., Karsch F.J., Foster D.L. (1977) The endocrine control of seasonal reproductive function in the ewe: a marked change in response to the negative feedback action of estradiol on luteinizing hormone secretion. Endocrinology 101: 818-821

Lehman M.N., Karsch F.J. (1993) Do gonadotropin-releasing hormone, tyrosine hydroxylase-immunoreactive, and beta-endorphin-immunoreactive neurons contain estrogen receptors? A double-label immunocytochemical study in the Suffolk ewe. Endocrinology 133: 887-895.

Lehman M.N., Robinson J.E., Karsch F.J., Silverman A.-J. (1986) Immunocytochemical localization of luteinizing hormone-releasing hormone (LHRH) in the sheep brain during anestrus and the mid-luteal phase of the estrous cycle. J. Comp. Neurol. 244: 19-35.

Lincoln G.A., Ebling F.J.P. (1985) Effect of constant release implants of melatonin on seasonal cycles in reproduction, prolactin secretion and moulting in rams. J. Reprod. Fertil. 73: 241-253.

Lincoln G.A., Maeda K.-I. (1992a) Reproductive effects of placing micro-implants of melatonin in the mediobasal hypothalamus and preoptic area in rams. J. Endocrinol. 132: 201-215.

Lincoln G.A., Maeda K.-I. (1992b) Effects of placing micro-implants of melatonin in the mediobasal hypothalamus and preoptic area on the secretion of prolactin and β-endorphin in rams. J. Endocrinol. 134: 437-448.

Lincoln G.A., Peet M.J., Cunningham R.A. (1977) Seasonal and circadian changes in the episodic release of follicle-stimulating hormone, luteinizing hormone and testosterone in rams exposed to artificial photoperiods. J. Endocrinol. 72: 337-349.

Malpaux B., Daveau A., Maurice F., Gayrard V., Thiery J.C. (1993) Short-day effects of melatonin on luteinizing hormone secretion in the ewe: evidence for central sites of action in the mediobasal hypothalamus. Biol. Reprod. 48: 752-769.

Malpaux B., Daveau A., Maurice-Mandon F., Duarte G., Chemineau P. (1998) Evidence that melatonin acts in the premammillary hypothalamic area to control reproduction in the ewe: Presence of binding sites and stimulation of luteinizing hormone by *in situ* microimplant delivery. Endocrinology 139: 1508-1515.

Merriam G.R., Wachter K.W. (1982) Algorithms for the study of episodic hormone secretion. Am. J. Physiol. 243: E310-E318.

Misztal T., Romanowicz K., Barcikowski B. (1996a) Seasonal changes of melatonin secretion in relation to the reproductive cycle in sheep. J. Anim. Feed Sci. 5: 35-48.

Misztal T., Romanowicz K., Barcikowski B. (1996b) 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. Acta Neurobiol. Exp. 56: 769-778.

Misztal T., Romanowicz K., Barcikowski B. (1997) Natural and melatonin-stimulated changes in the circadian rhythm of prolactin secretion in the ewe during seasonal anestrus. Neuroendocrinology 66: 360-367.

Roche J.F., Foster D.L., Karsch F.J., Dziuk P.J. (1970) Effect of castration and infusion of melatonin on levels of luteinizing hormone in sera and pituitaries of ewes. Endocrinology 87: 1205-1210.

Silverman A.-J., Livne I., Witkin J.W. (1994) The gonadotropin-releasing hormone (GnRH) neuronal systems: immunocytochemistry and *in situ* hybridization. In: The physiology of reproduction, (Eds. E. Knobil and J.D. Neill). II ed. Vol. 1. Raven Press Ltd, New York, p. 1683-1709.

Stupnicki R., Madej A. (1976) Radioimmunoassay of LH in blood plasma of farm animals. Endokrinologie 68: 6-13.

Tilbrook A.J., Canny B.J., Stewart B.J., Serapiglia M.D., Clarke I.J. (1999) Central administration of corticotrophin-

-releasing hormone but not arginine vasopressin stimulates the secretion of luteinizing hormone in rams in the presence and absence of testosterone. J. Endocrinol. 162: 301-311.

Tortonese D.J. (1999) Interaction between hypothalamic dopaminergic and opioidergic systems in the photoperiodic regulation of pulsatile luteinizing hormone secretion in sheep. Endocrinology 140: 750-757.

Traczyk W., Przekop F. (1963) Methods of investigation of the function of the hypothalamus and hypophysis in chronic experiments in sheep. Acta Physiol. Pol. 14: 217-226.

Viguie C., Caraty A., Locatelli A., Malpaux B. (1995) Regulation of luteinizing hormone-releasing hormone (LHRH) secretion by melatonin in the ewe. I. Simultaneous delayed increase in LHRH and luteinizing hormone pulsatile secretion. Biol. Reprod. 52: 1114-1120.

Webster J.R., Suttie J.H., Veenvliet B.A., Manley T.R., Littlejohn R.P. (1991) Effect of melatonin implants on secretion of luteinizing hormone in intact and castrated rams. J. Reprod. Fertil. 92: 21-31.

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