Short-Day Effects of Melatonin on Luteinizing Hormone Secretion in the Ewe: Evidence for Central Sites of Action in the Mediobasal Hypothalamus

BENOIT MALPAUX, AGNES DAVEAU, FRANCOISE MAURICE, VERONIQUE GAYRARD, and JEAN-CLAUDE THIERY

Physiologie de la Reproduction des Mammifères Domestiques, INRA, 37380 Nouzilly, France

ABSTRACT

Experiments were designed to localize the central sites of action of melatonin involved in the control of seasonal reproduction. Ewes were exposed to long days and received microimplants of melatonin in the preoptic area (n = 9), anterior hypothalamus (n = 8), or mediobasal hypothalamus (n = 12). The effects of implants were determined by comparison with control ewes (untreated or sham-operated, n = 10) and with ewes treated with an s.c. implant of melatonin (n = 8) or ewes subjected to short days (n = 8). All ewes were ovariectomized and treated s.c. with a silastic capsule of estradiol. Melatonin released in the preoptic area as well as in the anterior and lateral hypothalamus did not cause any difference as compared with the controls (no stimulation of LH secretion and no inhibition of prolactin secretion). In contrast, melatonin implanted in the mediobasal hypothalamus caused an increase in LH secretion in 7 of the 12 ewes on Day 53.0 ± 4.2 after implantation (mean ± SEM). Their response was not different compared with that of ewes treated s.c. with melatonin or exposed to short days either in terms of timing (Day 56.3 ± 6.2 and 59.5 ± 3.1, respectively, for controls) or of amplitude of the LH response. Similarly, melatonin caused only a reduction of prolactin secretion in the mediobasal, s.c., and short-day groups. It is concluded that the mediobasal hypothalamus or the surrounding tissues could be the sites of action of melatonin involved in the control of seasonal reproduction.

INTRODUCTION

In many species, reproduction is characterized by a seasonal pattern controlled by the photoperiod. In the ewe, annual changes in day length cause an alternation between anestrus in spring and summer and breeding in autumn and winter [1-4], and this regulatory effect of the photoperiod is thought to occur through a synchronization of an endogenous rhythm of reproduction [5-7]. Photoperiodic information is transduced by the pineal gland by means of a diurnal rhythm of melatonin secretion [8, 9]. This hormone is secreted only during the night, and the length of secretion in sheep is virtually identical to that of the dark period [10-12]. The duration of melatonin secretion varies therefore between long and short days, and this constitutes a neuroendocrine signal to the reproductive axis. The action of melatonin on reproductive activity implies changes in the pulsatile secretion of LH that probably reflect similar modifications in LHRH release [13-15]. Long daily durations of melatonin secretion typical of short days induce an increase in the frequency of the pulsatile discharges of LH. Very little is known about how melatonin regulates LH pulsatility; in particular, the sites of action of melatonin involved in this regulation have yet to be localized [16]. Autoradiographical binding studies have revealed that the pars tuberalis of the pituitary contains the largest concentration of melatonin receptors [16-19]. A lower density of receptors has been found in different brain areas [16, 20, 21]. The functional significance of binding at these different sites is not known. However, the modification of LHRH pulse frequency, which probably results from the action of melatonin, suggests an effect at the level of the central nervous system. The objective of the present study, therefore, was to determine the central sites of action of melatonin in the ewe. The approach was to identify sites where local and space-limited delivery of melatonin could induce the same changes in LH secretion seen when melatonin is given peripherally or when animals are subjected to short days.

MATERIALS AND METHODS

General

Experiments were performed with sexually mature Ile-de-France ewes. They were maintained outdoors in Nouzilly, France (48°N) before the study and in light-sealed rooms during the experiments. In the latter case, day length was regulated by electronic timers that operated fluorescent bulbs providing approximately 300 lx at the level of the animals' eyes. A dim red light producing less than 2 lx was used to facilitate nighttime collection of blood. Ewes were fed daily with hay, straw, and corn. They had free access to water and mineral licks. All ewes were ovariectomized at least 1 mo before the beginning of the experiment, and each received a 20-mm silastic (Dow Corning, MI) capsule containing estradiol that had been shown to maintain a physiological serum estradiol level of 3-5 pg/ml [22, 23]. Neuroendocrine reproductive activity was assessed from the concentration of LH in blood samples obtained twice weekly. In this model, pe-
riods of elevated LH are indicative of the breeding season, and those of low LH are associated with anestrus [23, 24].

**Design**

Before the experiments, ewes were first exposed to short days, either natural ones outside (until 24 January, experiment 1) or artificial (8L:16D for 90 days, experiment 2). Animals were then exposed to long days (16L:8D; lights-on at 0430 h). After 70 days (Day 0 of experiment), animals were allocated to one treatment group.

The objective of experiment 1 was to localize a brain site where melatonin is able to cause a stimulation of LH secretion. It was performed with 28 ewes (b.w. 62.9 ± 5.3 kg; age: 2.4 ± 0.1 yr; mean ± SEM); they all remained in the long-day treatment throughout the experiment. Twenty-two ewes received bilateral implants in four different brain areas: the dorsolateral hypothalamus (DLH, n = 4), mediobasal hypothalamus (MBH, n = 8), anterior hypothalamus (AH, n = 4), and preoptic area (POA, n = 6). The effect of these implants was determined by comparison with six control ewes that did not receive any melatonin treatment. Three controls received sham operations and empty implants; the other three were not operated on.

The objective of experiment 2 was to confirm the results of experiment 1 and to compare the response obtained with brain-implanted microimplants to that of s.c. implants and short-day treatment. It was performed with 27 ewes (63.6 ± 1.8 kg; 2.8 ± 0.3 yr). Seven ewes received bilateral implants of melatonin in the MBH (n = 4) or POA (n = 3). The effect of melatonin in those sites was determined by comparison with four ewes that received sham operations and empty implants in the MBH. Furthermore, the response to intracranial implants was compared to that of s.c. implants (n = 8) or to short days (8L:16D; n = 8). All but the last group remained exposed to long days.

In both experiments, the effect of treatments on LH secretion was assessed from single blood samples obtained twice weekly between 1400 and 1600 h. The effect of treatment also was assessed by the prolactin levels. Indeed, although prolactin does not seem to be involved in the regulation of seasonal breeding, its secretion is modulated by photoperiod, and this effect is transduced by melatonin [25, 26]. The effectiveness of treatments on prolactin secretion was determined from blood samples taken before and after the onset of treatments (experiment 1: Day −11 and Day +14; experiment 2: Day −10 and Day +16); on each occasion, samples were obtained hourly for 24 h starting at 0900 h. The first three samples were not assayed to avoid the interference of feeding and stress-associated initiation of bleeding on prolactin secretion. One out of every other sample also was assayed for melatonin.

**Surgery**

After premedication with atropine (40 mg; Meram, Melun, France), a brief general anesthesia was obtained by injection of barbiturates (12.5 mg/kg b.w. Neskonal; Rhône-Merieux, Lyon, France), and a deep anesthesia was maintained during surgery (Halothane, 3.5% in oxygen; Pitman-Moore France, Meaux, France). The head of the ewe was positioned in a stereotaxic frame (Précision Cinématographique, Paris, France) [27]. Radio-opaque liquid (Lipiodol, Guerbet, Aulnay sous Bois, France; 1 ml) was injected into the lateral ventricle (32 mm in front of the ear bars, 4 mm from the midline). Implantation of guide-cannulae was performed under radiographic controls using the thalamic mass intermedius and the infundibular recessus of the third ventricle as internal landmarks for the anteroposterior and vertical orientations, and the middle of the third ventricle for laterality [28, 29]. Guide-cannulae consisted of stainless steel tubing (o.d.: 1.20 mm; i.d.: 0.86 mm) welded onto a head (o.d.: 5 mm, length: 10 mm) threaded inside and outside. The length of the tubing varied according to the depth of the site (DLH: 30 mm; POA: 32.5 mm; AH: 35 mm; MBH: 40 mm). Cannulae were positioned such that the tip was located 3 mm above the target site. They were fixed to the skull of the ewe with acrylic cement and screws. A stainless steel mandrel consisting of a rod (diam.: 0.80 mm) welded onto a screw was placed inside each guide-cannula at the end of the surgery. The heads of the cannulae and mandrels were protected with a cylindrical teflon case (o.d.: 30 mm; i.d.: 22 mm; height: 22 mm) that was closed with a screwed nylon cap and anchored to the skull with four screws. After surgery, animals received a daily injection of antibiotics (5 ml Mixtencillin; Rhône-Merieux) for 5 days and of diuretics (3 ml Diurizone; Vetoquinol, Lure, France) for 3 days.

The microimplants consisted of stainless steel tubing (o.d.: 0.70 mm; i.d.: 0.45 mm) welded onto a head (o.d.: 3 mm) [28]. On Day 0 of the experiment (at least 30 days after implantation of the guide-cannulae), the teflon case was opened and cleaned with alcohol, the mandrels were removed from the guide-cannulae, and the microimplants were inserted in their place. Opposite the mandrels, the implants exceeded the tip of the cannulae by 3 mm. Animals remained awake during the insertion procedure.

**Melatonin Implants**

Melatonin was put into a beaker, which was immersed in an oil bath maintained at 120°C. When the melatonin was molten, the end of the implant was dipped into the solution, and by the other end melatonin was slowly aspirated into the tubing to fill it up. The beaker was then taken off the oil bath, and the implant was slowly removed from the beaker after crystallization. The outer surface of the implant was cleaned of any melatonin residue with pure ethanol. The implant was carefully examined under a dissecting microscope to verify the presence of melatonin as a flat surface. Before being inserted, the implants were left overnight in aldehyde vapor (Elan, Marseille, France). Thin-layer chromatographic analysis showed that melatonin was
not affected by that procedure. The release rate of melatonin from the microimplants was measured in vitro by incubation in 1 L of saline at 37°C. After an initial peak (> 10 μg/day), the rate of release stabilized after 3–5 days at 5.5 ± 0.4 μg/day (n = 4).

The s.c. implants used in experiment 2 were made and generously provided by CAMCO (Cambridge Animal Health Limited, Hauxton, UK). Implants were inserted in the left ear.

Histology

At the end of the experiment, animals were decapitated. Their heads were immediately perfused via the carotid arteries with 2 L of 4% formaldehyde in phosphate buffer. Brains were removed and stored in the same fixative for at least 4 days. They were transferred to a 15% sucrose and PBS solution, and 48 h later were cut into 40-μm thick frontal sections by means of a cryomicrotome. Every other section was stained by Küver and Barrera’s method [30] to localize the cannula. The site of implantation was determined by use of Richard’s atlas [27].

Diffusion of Melatonin from the Site of Implantation

To measure the diffusion of melatonin, microimplants were made as described above from a mixture of cold melatonin and 125I-labeled melatonin. Thirty microcuries of 125I-labeled melatonin (Dositek, Orsay, France) and 50 mg of melatonin were mixed into 0.5 ml of ethanol. Ethanol was evaporated and the mixture of melatonin was heated at 120°C to make the microimplants. Two microimplants were inserted into one ewe bearing guide-cannulae aimed at the MBH. After 14 days, the animal was decapitated, the brain was removed, and the pituitary stalk was carefully dissected. The brain was immediately frozen and then cut into 100-μm-thick frontal sections. One hundred and twenty-seven sections were obtained in the region ranging from the optic chiasma to the mammillary bodies. Each section was put in a tube, and the radioactivity was quantified with a gamma-counter. The amount of radioactivity was considered elevated when it exceeded background by more than two standard deviations of background. Every ten 100-μm sections, one 40-μm-thick section was used to determine the position of the microimplants; histology was performed as described above.

Blood Sampling and Assays

Blood samples were obtained by venipuncture of the right jugular vein; plasma was separated and stored at −20°C until assayed. LH was assayed in duplicate 100-μl aliquots of plasma using the RIA method of Pelletier et al. [31] modified by Montgomery et al. [32]. Sensitivity (two standard deviations from buffer controls) was 0.13 ± 0.01 ng/ml (mean ± SEM, 7 assays) of 1051-CY-LH (i.e., 0.27 ng/ml of NIH-LH-S1). The intraassay coefficient of variation (CV) for five plasma pools averaged 6.5% (7 assays); the interassay CV for these plasma pools averaged 12.8%.

Prolactin was assayed in duplicate 10–50-μl aliquots of plasma by means of the RIA method of Kari [33]. Sensitivity was 2.7 ± 0.4 ng/ml of NIHDI-oPRL-19 for 50 μl (6 assays). Intraassay CV for four plasma pools averaged 9.6% (6 assays); interassay CV for these plasma pools averaged 16.8%.

Melatonin was assayed in duplicate 100-μl aliquots of plasma by means of the RIA method of Fraser et al. [34], with an antibody first raised by Tallet et al. [35]. Sensitivity was 16 ± 0 pg/ml (2 assays). Intraassay CV for two plasma pools averaged 9.1% (2 assays); interassay CV for these plasma pools averaged 8.7%.

Analysis of Data

For each individual ewe, the time when circulating LH levels started to rise was determined by the first of at least three consecutive values exceeding 1 ng/ml. For the ewes that did not show an increase in LH secretion during the experiments, this time was set to 90 days (end of experiment). These times of increase were then analyzed by the Mann-Whitney two-sample rank test. In addition, the percentages of responding animals per brain structure were compared by Fisher’s Exact test.

After a logarithmic transformation of the prolactin data, a first ANOVA was run to test an “experiment” effect. Then, the data were analyzed by a three-factor repeated measures ANOVA (treatment as a between factor and time of day and time relative to treatment as within factors). Interaction between treatment and time relative to treatment were further analyzed by a one-factor (treatment) ANOVA bearing on the difference between mean values before and after treatment calculated for each animal. It was followed by Duncan’s New Multiple Range test for differences between treatment groups.

RESULTS

Effects of Melatonin on LH Secretion

In experiment 1, LH levels were low (0.4–0.6 ng/ml) at the beginning of the experiment. LH levels remained basal in all animals that were not treated or that received sham operations. Therefore, the data from those two groups were pooled. No animals that received microimplants in the POA, AH, and DLH showed any increase in LH secretion, and their LH profiles were not different from those of the controls (Fig. 1). In the MBH group, two types of profiles were observed. Four animals showed an increase in LH secretion starting on Day 58.0 ± 3.6, whereas the profiles of the other four did not differ from those of the controls (Fig. 1).

In experiment 2, LH levels remained basal throughout the experiment in all sham-operated animals that had received an empty implant in the MBH. As in the other experiment, animals from group POA displayed a similar LH profile to that of the controls. In group MBH, three animals
FIG. 1. Mean ± SEM plasma concentrations of LH in each group of experiment 1 (left panel) or experiment 2 (right panel). Shaded areas depict mean (± SEM) values of LH concentrations in the control group of each experiment (n = 6 in experiment 1 and n = 4 in experiment 2). In the MBH groups, LH values are plotted separately for ewes that displayed a significant increase in LH secretion and those that did not. All ewes were ovariectomized and treated with estradiol administered via an s.c. implant. Blood samples were obtained twice weekly. LH values were calculated after logarithmic transformations and plotted on a logarithmic scale. Melatonin or short-day treatment started on Day 0. n = Number of animals per treatment.
showed an increase in LH secretion on Day 46.3 ± 6.8, whereas LH levels remained basal in one ewe. In animals either treated with short days or with an s.c. melatonin implant, LH levels increased on Day 56.3 ± 6.2 and 59.5 ± 3.1, respectively (Fig. 1).

After data from both experiments were pooled, the statistical analysis revealed that the increase in LH secretion occurred only in MBH (all twelve animals included, p < 0.01), s.c., and short-day groups (p < 0.001). In addition, in the seven animals of the MBH group in which LH levels increased, the characteristics (timing, amplitude) of the rise were similar to those of ewes receiving an s.c. implant or treated with short days (Fig. 2).

The anatomical location of the implants in each animal is illustrated in Figure 3. The proportion of responding animals in the MBH group (7/12) was different from that in the POA group (0/9; p < 0.01) and in the DLH and AH groups (0/4 in each group; p < 0.07). Within the MBH group, the microimplants of the nonresponding animals appeared to be located more dorsally and caudally.

Effects of Melatonin on Prolactin Secretion

The ANOVA did not reveal any effect of experiment, or any interaction between experiment and any other factor on prolactin levels. Data from both experiments were therefore pooled and are illustrated in Figure 4. Prolactin levels remained fairly stable in the control group (182 ± 23 vs. 195 ± 21 ng/ml). The evolution of prolactin concentrations in the POA, DLH, and AH groups did not differ significantly from that in the controls, although in the latter two groups there was a tendency toward a decrease in prolactin levels after the animals received microimplants of melatonin (POA from 176 ± 17 to 157 ± 15 ng/ml; AH from 182 ± 25 to 119 ± 19 ng/ml; DLH from 269 ± 33 to 194 ± 30 ng/ml). A large decrease in prolactin levels occurred in the short-day group and in the s.c. group (from 154 ± 54 to 39 ± 7 ng/ml and from 121 ± 21 to 21 ± 2 ng/ml, respectively; p < 0.01 compared to controls). In the MBH group, the change in prolactin levels (from 177 ± 21 to 105 ± 13 ng/ml) was significantly different from that in controls (p < 0.05). This decrease was, however, smaller than in the short-day and s.c. groups (p < 0.01).

When the MBH group was divided into two subgroups according to the LH response of the animals, it appeared that prolactin secretion tended to decrease in the nonresponding animals (from 195.6 ± 35.6 to 138.5 ± 19.9 ng/ml), as it did in groups AHA and DLH. The decrease in the responding animals (from 162.8 ± 27.2 to 81.9 ± 12.6 ng/ml) was numerically larger than in the nonresponding ones, and it was a significant change relative to controls (Fig. 4, p < 0.01).

Plasma Levels of Melatonin

The presence of microimplants in the brain did not cause any detectable changes in the profile of melatonin. Levels remained low during the day and increased during the night in the same way as in the control group, as exemplified for experiment 2 (Fig. 5). Conversely, in the s.c. group, levels of melatonin were elevated throughout the 24-h period, and a nighttime increase could still be observed (Fig. 5).

Diffusion of Melatonin from the Site of Implantation

Microimplants containing 125I-labeled melatonin were located within the mediobasal hypothalamic area, where melatonin caused a stimulation of LH secretion in experiments 1 and 2. The amount of radioactivity was higher than background (10 ± 5 cpm, mean ± standard deviation) in 11 consecutive sections, with values ranging from 24 to 198 cpm. This represents an area of diffusion within the brain tissue of 1.1 mm. In addition, no radioactivity was detected in the pituitary stalk (including the pars tuberalis) or in the pituitary.

DISCUSSION

Our data show that microimplants of melatonin produced a stimulation of LH secretion only when they were located in the MBH and not in the POA, DLH, or AH; this increase in mean LH secretion in ovariectomized estradiol-treated ewes is known to reflect a higher frequency of LH pulses [36]. In sheep, it has been shown that melatonin im-
FIG. 3. Sites of melatonin delivery on a schematic sagittal representation. Each symbol represents the site of implantation in one animal, and its size reflects the area of diffusion of melatonin as estimated in one ewe with microimplants containing $^{3}H$-labeled melatonin. Closed and open circles indicate animals in which LH secretion was or was not stimulated by melatonin, respectively. Letters within the symbols indicate the group in which the animal was included in Figure 1 (P, preoptic area; A, anterior hypothalamus; D, dorsolateral hypothalamus; M, mediobasal hypothalamus). All microimplants were located medially (laterality less than 2.5 mm) except in the DLH where their laterality was between 3 and 5 mm. The thin lines delimit the third ventricle. AC, anterior commissure; SCN, suprachiasmatic nucleus; OCh, optic chiasma; VMH, ventromedial hypothalamic nucleus; PVH, paraventricular hypothalamic nucleus; ARC, arcuate nucleus; ME, median eminence; MB, mammillary bodies; PIT, pituitary; IIIV, third ventricle.
The present results demonstrating the effectiveness of MBH microimplants on LH secretion are complementary to those of Lincoln and Maeda [38] showing that melatonin microimplants, when placed in the MBH in Soay rams, can advance the reactivation of testicular activity and FSH secretion. However, in contrast to the present study, Lincoln and Maeda [38] also observed a minor effect in the POA of a small proportion of animals in their study. This difference may be due to the shorter distance between the POA and MBH in Soay rams (about 3 mm) compared to that in Ile-de-France ewes (about 6 mm); alternatively, it may be related to sexual differences in functions of some brain structures. The results of the present study are also in agreement with results obtained in other species indicating that the sites of action of melatonin are located within the hypothalamus. In the white-footed mouse, melatonin-beeswax implants cause a reduction of reproductive tract weight in the same way as s.c. implants if they are located in the suprachiasmatic area, the AH, or the MBH [39,40]. In the Syrian hamster, microimplants of melatonin located in the POA, the AH, or the MBH prevent the inhibitory effects of short days on testicular weight in contrast to microimplants located in the mid-brain, amygdala, or lateral hypothalamus [41]. Similarly, Siberian hamsters receiving a daily 10-h infusion of melatonin in the suprachiasmatic area by microdialysis and white-footed mice treated with a removable microimplant for 10 h every day in the POA/AH undergo gonadal regression as if treated with short days [42,43]. In addition, neurotoxic or electrolytic lesions of the AH in hamsters disrupt the photoperiodic response [44,45]. Because of the small size of the hypothalamus in rodents, these studies did not make possible a clear discrimination between the different regions of the hypothalamus. Our data enable us to state that the AH and POA area do not contain the sites of action of melatonin in sheep.

Binding sites of melatonin have been localized in different areas. All studies show a high density of binding in the pars tuberalis of the pituitary [16–19]. Within the brain, receptors have been found in many areas; two of these could be particularly relevant to the control of seasonal repro-

FIG. 4. Mean (± SEM) percentage of change in prolactin secretion relative to control period. Blood samples were obtained hourly for 21 h about 2 wk before (control period) and 2 wk after the onset of treatments. In each ewe, the percentage of change in prolactin secretion was defined as the ratio between the difference of prolactin levels in the two situations, on the one hand, and the prolactin levels during the control period, on the other hand. Animals from the MBH group were allocated to one of two subgroups according to their LH response (responders: animals displaying an increase in LH secretion, n = 7; non responders, n = 5). * and ** indicate means significantly different from controls with p < 0.05 and p < 0.01, respectively.

FIG. 5. Mean (± SEM) melatonin concentration in the animals of experiment 2. Samples were obtained every 2 h for 24 h on Day 16. "Intra Cerebral" values represent animals that received intracerebral microimplants either in the MBH or in the POA. n = Number of animals per group.
duction: the septo-preoptic region and the ventromedial hypothalamus [16, 20, 21, 46, 47]. However, our results indicate that in sheep melatonin does not act in the septo-preoptic area to control LH secretion, as no response was observed when microimplants were placed in that region; this allows us to exclude quite certainly a direct effect on the LHRH perikarya since most of them are located within that area in this species [48, 49]. Actually, our present data suggest that the binding observed in the hypothalamus, particularly in the vicinity of the ventromedial nucleus, could be important and are consistent with the hypothesis of an action of melatonin in that region; however, they do not allow complete rejection of the role of the pars tuberalis since a diffusion of melatonin from the mediobasal sites of implantation to that area cannot be excluded. This latter possibility, however, appears unlikely because no diffusion of $^{125}$I-labeled melatonin to the pars tuberalis was detected in the ewe in which melatonin diffusion was estimated.

The effects of microimplants on prolactin secretion are consistent with those observed on LH secretion, with an action in the MBH and no significant action in the POA, AH, or DLH. However, the difference in the reduction of prolactin secretion between sites of implantation was not as marked as it was for the stimulation of LH secretion, and the inhibition observed in the MBH group was smaller than with the s.c. implant. These results may be due to the large variability associated with prolactin determinations, which could have partly masked some effects. It may also be that the sites of action involved in the control of prolactin secretion are not the same as those involved in the control of LH secretion; the MBH implants could therefore have been located further from the former sites, and hence the resulting effect could have been smaller. Thus, these results suggest that the sites of action of melatonin involved in the control of prolactin secretion are located within or near the MBH. However, the absence of a clear-cut difference between the effect in that site and in the AH makes further studies necessary—both to obtain a better localization of the sites of action of melatonin involved in the control of prolactin secretion and particularly to determine whether they are the same as for the control of LH secretion.

The control of seasonal reproduction by melatonin involves changes in the pulsatile secretion of LH [13]. These changes most likely reflect similar modifications in LHRH pulse frequency, which implies events at the level of the central nervous system [14, 15]. Two hypotheses concerning the action of melatonin on seasonal reproduction should be considered. First, melatonin could act in the MBH, and its action could involve one or several relays before modulating the activity of the LHRH system, which has its perikarya in the POA and its terminals in the median eminence [48, 49]. One of these relays could be the dopaminergic neurons, whose involvement has been demonstrated during seasonal anestrus resulting from the action of melatonin [50–52]. The existence of a large lag time between melatonin action and the corresponding LH response suggests that the network between the sites of action and the LHRH system is complex and could involve morphological modifications. Second, melatonin could act in the pars tuberalis of the pituitary; thus there would have to be a signal from that tissue to the central nervous system [53]. This signal, probably humoral, must be identified. A critical step for gaining insight into the mode of action of melatonin is therefore to obtain data that would lead to rejection of one of these two hypotheses.

In conclusion, our results indicate that the sites of action of melatonin involved in the photoperiodic control of LH secretion are located within or in the vicinity of the MBH and exclude a direct action on the LHRH perikarya or AH. The next step will be to determine whether these sites are different from the binding sites located in the pars tuberalis.

ACKNOWLEDGMENTS

We wish to thank Drs. P. Chemineau, J. Pellietier, J.-P. Ravault, Y. Tillet, and M. Caldani for help in the design of the study and comments on the manuscript; Drs. D. Blache and C. Fabre for advice in making the microimplants; Mr. G. Durand, A. Locaselli, and F. Paulnier for assistance in the animal experimentation; and Mrs. K. Néra for revision of the English manuscript.

REFERENCES


