0013-7227/02/$03.00/0
Printed in U.S.A.

Endocrinology 143(1):84–90

Melatonin Enters the Cerebrospinal Fluid through the Pineal Recess

HÉLÈNE TRICOIRE, ALAIN LOCATELLI, PHILIPPE CHEMINEAU, AND BENOÎT MALPAUX

Unité Mixte de Recherche Institut National de la Recherche Agronomique–Centre National de la Recherche Scientifique (6073), Université de Tours "Physiologie de la Reproduction et des Comportements," 37380 Nouzilly, France

The pineal recess (PR), a third ventricle (IIIV) evagination penetrating into the pineal gland, could constitute a site of melatonin passage to the cerebrospinal fluid (CSF) and explain the high concentrations of melatonin in this fluid. To test this hypothesis, we characterized melatonin distribution in the IIIV of sheep by CSF collection in the ventral part of IIIV (vIIIV) and in PR. At 30 μl/min collection rate, melatonin concentrations were much higher in PR than in vIIIV (19,934 ± 6,388 *vs.* 178 ± 70 pg/ml, mean ± SEM, respectively, $P < 0.005$), and they increased in vIIIV when CSF collection stopped in the PR ($P < 0.05$). At 6 μl/min, levels increased to 1,682 ± 585 pg/ml in vIIIV and were not influenced by CSF collection in the PR. This concentration difference between sites and the influence of PR collection on vIIIV levels suggest that melatonin reaches the PR and then diffuses to the IIIV. To confirm the role of PR, we demonstrated that its surgical sealing off decreased IIIV melatonin levels (1,020 ± 305 pg/ml, compared with 5,984 ± 1,706 and 6,917 ± 1,601 pg/ml in shams or animals with a failed sealing off, respectively, $P < 0.01$) without changes in blood levels. Therefore, this study identified the localization of the main site of penetration of melatonin into the CSF, the pineal recess. (*Endocrinology* 143: 84–90, 2002)

THE CEREBROSPINAL FLUID (CSF), a clear, colorless liquid, fills the ventricles and external surfaces of the brain, its total cranial volume being about 60 ml in humans (1) and 15 ml in sheep (2). The major part of CSF is secreted by the choroid plexus, a vascular expansion found mainly in lateral ventricles. Then it circulates from the lateral to the third and fourth ventricle before entering the subarachnoidal space and flowing downward around the spinal cord. Several functions were attributed to the CSF, including buoyancy and protection of the brain, excretion of metabolites, and homeostasis of the brain chemical environment (3). More recently, it was suggested that it could also be an endocrine pathway for intracerebral transport between different brain areas (1, 3). Indeed, many substances have been detected in the CSF (4), some of which can penetrate the cerebral tissue as demonstrated by diffusion studies and by the physiological and behavioral effects following their intracerebroventricular injections. However, it is not clear whether their presence reflects a signal that uses the CSF circulation to distribute information far away from its release site or a by-product that spills over into the ventricles after accomplishing its function in the brain (1). To demonstrate that the presence of a molecule constitutes a signal in the CSF, several criteria must be met (1). First, the signaling substance should enter the CSF and be distributed within the brain by fluid movement. Second, the signal binding sites leading to the appropriate responses should be approachable by diffusion or by a specific trapping system. Third, the removal or replacement of the signaling substance should result in the correlative response. Considering this, there are already pieces of evidence for the existence of such signals in the CSF, for example, diffusible circadian signals delivered from suprachiasmatic nucleus transplants into the third ventricle (IIIV) that restore circadian rhythms (5) or IL-1β-increased levels accompanying sleep deprivation (6).

Melatonin, the pineal hormone secreted exclusively at night by the pineal gland, is a good candidate for being transported to its targets by the CSF for several reasons. First, many of its putative effects result from a central action, for example, the protection of the nervous system in neurodegenerative Alzheimer's or Parkinson diseases (7), the enhancement of sleep (8), and immunity (9). Most important, for one well-characterized effect of this hormone, the seasonal control of LH release, the target site of melatonin is localized in the premammillary hypothalamic area (10). In this structure, binding sites are very close to the CSF of the IIIV (0–1.5 mm), making them easily reachable by diffusion of melatonin from CSF. Second, melatonin is present in the ventricular system (11–14), particularly in the IIIV in which melatonin concentration is 20 times as high in the CSF as in blood (15).

One key question is how such a difference in concentrations of the same molecule between two liquid compartments, CSF *vs.* blood, can be obtained. Several hypotheses have been raised to explain the relative high concentration of CSF melatonin: active uptake of melatonin from peripheral blood or release from choroid plexus after retrograde transport from the Galen vein (15–18). However, none of these hypotheses has received convincing experimental support. Interestingly, the pineal gland is intimately related to the IIIV, of which an evagination, the pineal recess (PR), penetrates into the organ. This recess, in which the CSF circulates, separates two laminae forming the stalk and attaching the epiphysis to the brain (19). According to this, the PR could constitute a site of melatonin passage to the CSF, either by simple diffusion from pineal extracellular fluid or direct release of the molecule from CSF contacting-pinealocytes (20).

Abbreviations: CSF, Cerebrospinal fluid; F, failed sealed-off animals; IIIV, third ventricle; PR, pineal recess; S, sham animals; SO, sealed-off animals; vIIIV, ventral part of IIIV.

To test whether pineal melatonin enters the CSF via the PR, we performed two complementary experiments. In the first one, we measured in parallel CSF melatonin levels in the PR and in the ventral part of the third ventricle (vIIIV) (*i.e.*, a location in which melatonin should be diluted if it originates from the PR). In the second study, we sealed the pineal recess to determine whether it prevents melatonin release in the CSF. The study was performed in sheep because this species enables CSF collection for a prolonged period without physiological disturbance (21) and because the anatomical relationship between the pineal gland and the IIIV is identical in sheep and primates including humans (19).

Materials and Methods

General

Ile-de-France ewes were fed daily with hay, straw, and corn, and water was available *ad libitum*. In all experiments, ewes were restrained so that they were just able to move forward and backward but were always in contact with other sheep to prevent the stress of social isolation. Animals were kept in light-controlled rooms (Exp 1: 12 h of light per day, lights on 0000 h, lights off 1200 h; Exp 2: 16 h of light, lights on 0600 h, lights off 2200 h). All procedures were carried out in accordance with Authorization A37801 of the French Ministry of Agriculture.

Exp 1: does melatonin reach the CSF in the third ventricle directly from the pineal gland?

CSF melatonin concentrations in the PR and vIIIV were compared by simultaneous collection in these two sites, and then the influence of PR collection, the hypothetical source of CSF melatonin, on hormone levels in the vIIIV was assessed by stopping PR collection. In addition, this protocol of CSF collection was performed at two different flow rates to evaluate collection impact on measured concentrations.

Cannula implantation

Six Ile-de-France ewes were implanted stereotaxically with two guide-cannulae for CSF collection, as described previously (15). Briefly, radio opaque material (Omnipaque, Nycomed Ingenon SA, Paris, France) was injected in the lateral ventricle through a cannula, delimiting the ventricular system, particularly the IIIV. The first 40-mm-length × 1.5-mm-diameter guide cannula was placed to end at the basis of the PR and the second 55-mm-length, in the vIIIV (Fig. 1).

Study design

One week later, CSF was collected as previously described (15) during two consecutive 12-h nights. Each night was divided into an "experimental period" and a "control period" randomized among animals according to a cross-over design. The first one began at least 1 h after the onset of darkness and the last one finished 1 h before the end of darkness. CSF was collected throughout the two periods in the vIIIV. In the PR, it was collected throughout the control period and collection stopped after 2 h of the experimental period. The CSF fractions were collected every 15 min, except during the 30-min period following the cessation of CSF withdrawal in the PR, when 5-min CSF fractions were collected. This withdrawal design was performed at a flow rate of 30 μl/min during one night and 6 μl/min during the other night, randomized among animals according to a cross-over design. The flow rates of CSF used in this study were below the CSF formation rate in sheep of about 120 μl/min (2, 22). Collection of CSF samples in the PR was stopped on two animals before the end because the catheter stopped flowing. These animals were therefore excluded from the study and only the data of the other four animals were analyzed.

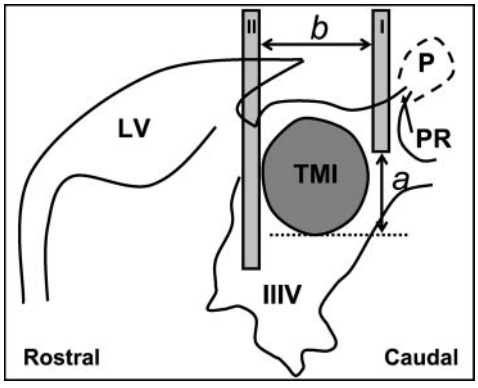

FIG. 1. Schematic representation of the ventricular system and the implantation of cannulae in Exp 1. I, Cannula in the PR; II, cannula in the ventral part of IIIV; a, distance between the TMI basis and PR cannula; b, distance between cannulae I and II. P, Pineal gland; LV, lateral ventricle; TMI, thalamus massa intermedia.

Exp 2: does surgical obliteration of pineal recess prevent melatonin release in the third ventricle?

The PR of ewes was surgically sealed off to prevent CSF exchange between the pineal gland and the ventricle. Jugular blood melatonin concentrations were measured before and after the sealing off as an index of melatonin synthesis and pineal gland integrity. Three months later, the nocturnal patterns of CSF and jugular blood melatonin concentrations were measured for each animal to evaluate sealing-off effects.

Surgical sealing off of the pineal recess

The PR of 15 ovariectomized ewes with E2 implants was surgically sealed. After general anesthesia of the ewes (halothane 4%, Pitman-Moore France, Meaux, France), the scalp was stripped from midline on the left side of the head and a 50-mm-diameter hole was made in the skull behind the three-way junction of the parietal and two frontal bones. The dura mater was opened carefully in half moon on the left side of the midsagittal sinus. The left hemisphere was retracted gradually, and adjacent blood vessels were correctly photocoagulated with diode laser 980 nm (CERALAS D25, CeramOptec, Bonn, Germany) to expose the pineal gland. The roof of the IIIV was opened 1–2 mm in front of the pineal gland, above the PR, using the diode laser. Two drops of biological glue (Histoacryl, B. Braun Surgical GmbH, Melsungen, Germany) were placed, sealing the PR and closing the ventricular system. The dura mater was sutured and the hole in the skull was filled with acrylic cement. Six sham ewes underwent the same surgical approach including the pineal exposure but without opening of the IIIV. As a control for the presence of biological glue in the ventricular system, a 20-μl piece of prepolymerized glue was introduced in the lateral ventricle.

Cannula implantation and assessment of sealing-off effectiveness

About 2 months after the surgical sealing off of the recess, a guide-cannula was implanted in IIIV, using a lateral x-ray picture. This picture enabled to see whether the radio opaque fluid penetrates the PR. Optical density level in the PR and in the area dorsal to the Sylvius duct used as a black standard for the amount of x-rays crossing head tissue, was measured on x-ray picture via image analysis framework (Biocom Histo 500, Les Ulis, France). The absence of radio opaque in the PR was interpreted as complete sealing off of the PR. In contrast, the presence of radio opaque in this structure (*i.e.*, the same distribution as in sham (S) animals) was interpreted as a failed sealing off (see Fig. 5). Experimental animals were distributed accordingly into two groups: sealed off (SO) and failed sealed off (F). At the end of the procedure, a 50-mm guide-cannula was implanted in the IIIV, allowing the CSF withdrawal, as described above.

Blood and CSF collections

The effect of surgery on pineal activity was assessed by measuring blood melatonin levels on average 13 d before and 30, 58, and 124 d after the sealing off (min, max: d −21 to d −4; d +26 to d +35; d +50 to d +67, and d +116 to d +133, respectively). On each occasion, four blood

samples were obtained by jugular venipuncture at 2330, 000, 0030, and 0100 h.

On d +119, a polyethylene catheter was inserted into the jugular vein and secured to the skin of the 18 animals (8 SO, 4 F, and 6 S animals) to collect 3-ml blood samples, and CSF was collected continuously via a peristaltic pump. We obtained CSF fractions and blood samples every 30 min, except for one SO animal and one S in which CSF collection was not successful. The collection period started 1 h before the beginning of an 8-h night and finished 1 h after its end.

Sample processing and melatonin assay

Plasma and CSF fractions were stored at −20 C until assay. Plasma melatonin concentrations were determined in 100-μl aliquots, using a well-validated RIA (19). This assay system was also used to measure melatonin concentrations in 1- to 50-μl aliquots of CSF samples, but the standard curve was made in assay buffer instead of plasma (11). Assay sensitivity averaged 4 pg/ml for 100-μl plasma sample and 40 pg/ml for 10-μl CSF sample. Intra- and interassay CVs were 7.4% and 11.4%, respectively, for plasma, and 12.4% and 10.9%, respectively, for CSF.

Analysis

In Exp 1, log-transformed CSF concentrations were analyzed by repeated-measure ANOVA (day of sampling [low *vs.* fast flow rate]), period [experimental *vs.* control], and sample number as within factors). In Exp 2, blood concentrations on d −13, +30, +58, and +124 were analyzed by a repeated-measure ANOVA (group as between factor and day as within factor) after log transformation. On d +119, blood and CSF concentrations were analyzed by repeated-measure ANOVA (group and compartment as between factors, time of sampling as within factor) after log transformation.

Results

Exp 1: does melatonin reach the CSF in the third ventricle directly from the pineal gland?

Melatonin concentrations were much higher in the PR than in the vIIIV during simultaneous collection at a flow rate of 30 μl/min (178 ± 70 *vs.* 19,934 ± 6,388 pg/ml, $P < 0.001$, ratio PR/IIIV of 182 ± 86, Figs. 2, 3, and 4, mean ± SEM). Melatonin concentrations were very variable among animals (PR from 2,200 to 34,700 pg/ml; IIIV from 74 to 417 pg/ml). During h 3 and 4 of the experimental period, vIIIV concentrations increased 63 ± 46-fold when CSF collection stopped in the PR (from 178 ± 70 to 8,400 ± 3,775 pg/ml, $P < 0.05$, Figs. 2 and 3), but they did not in control period. However, those last vIIIV melatonin concentrations (control period: h 3 and 4) were not significantly different from those measured at the beginning of the experimental period (h 1 and 2) (296 ± 132 *vs.* 403 ± 238 pg/ml, experimental *vs.* control, Figs. 2 and 3). At the lower collection flow rate, concentrations were also much higher in the PR than in the vIIIV (18,106 ± 5,484 *vs.* 1,682 ± 585, $P < 0.05$, Figs. 2 through 4). However, this difference was smaller than that observed at the higher flow rate because, with the reduction in flow rate, levels in the vIIIV increased (178 ± 70 *vs.* 1,682 ± 585, 30 μl/min *vs.* 6 μl/min, respectively, $P < 0.05$, Figs. 2 through 4) and were not different in the PR (19,934 ± 6,388 *vs.* 18,106 ± 5,484 pg/ml; 30 *vs.* 6 μl/min, respectively, Figs. 2 through 4). The examination of the x-ray picture revealed a relationship between precise placement of the cannulae and observed melatonin concentrations at the flow rate of 30 μl/min: the ratio PR/IIIV is positively correlated with the distance between the thalamic mass and the tip of guide-cannulae located at the base of the PR ($r^2 = 0.53$; $P < 0.05$) and negatively correlated with the distance between the two cannulae ($r^2 = 0.63$; $P < 0.05$). These correlations are not significant at a lower collection flow rate.

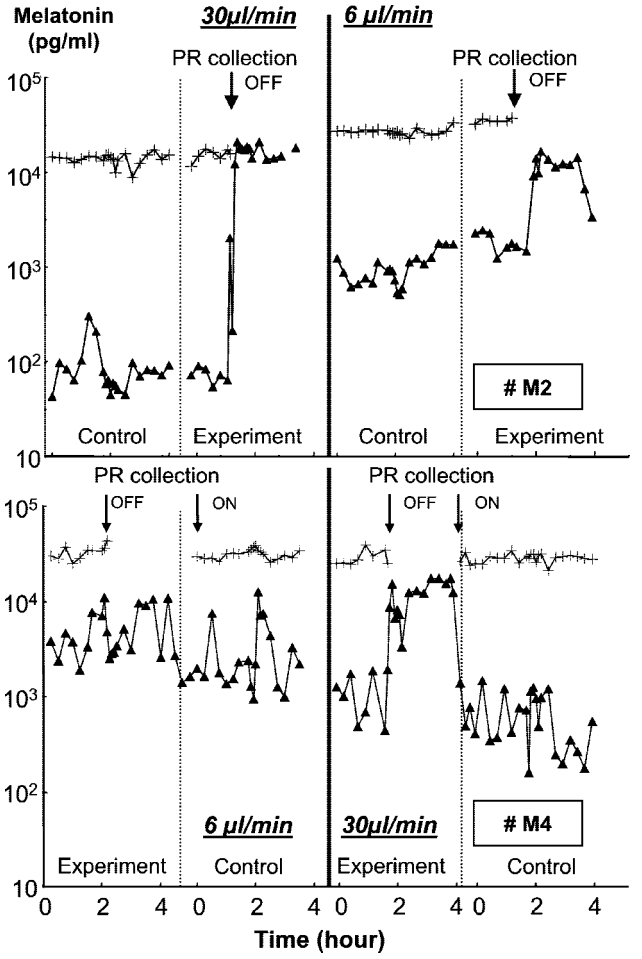

FIG. 2. Melatonin concentrations in the CSF collected simultaneously in the pineal recess (+) and in the ventral part of the third ventricle (▲) of two individuals *(top and bottom),* at fast flow rate (30 μl/min) and slow flow rate (6 μl/min).

Exp 2: does surgical sealing off of pineal recess prevent melatonin release in the third ventricle?

The analysis of eight x-ray pictures fit the criteria of complete sealing off (absence of radio opaque in PR: optical density similar to the black standard), and four did not (presence of radio opaque in PR: optical density similar to that in S and significantly higher than that of completely SO animals; $P < 0.005$; Fig. 5). This observation led to groups of SO (n = 8) and F animals (n = 4) in addition to S animals (n = 6).

On d +119, a nighttime increase in blood melatonin concentrations was observed in all animals regardless of their group. In addition, nocturnal melatonin levels were not different among groups (338 ± 56, 459 ± 101, 374 ± 69 pg/ml for S, F, and SO animals, respectively, Figs. 5 and 6). In the CSF, a nighttime increase in melatonin concentration was also observed in all animals, but, in contrast to blood, its

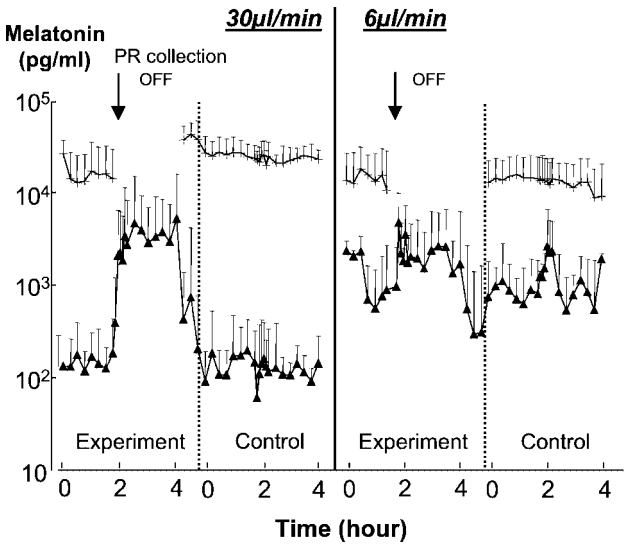

FIG. 3. Mean ± SEM melatonin levels in the CSF collected simultaneously in the pineal recess (+) and in the ventral part of the third ventricle (▲) of four sheep. Because the combination of phases (experiment *vs.* control) and flow rates (30 *vs.* 6 µl/min) were randomized according to a cross-over design, the phase order of presentation does not apply to all animals.

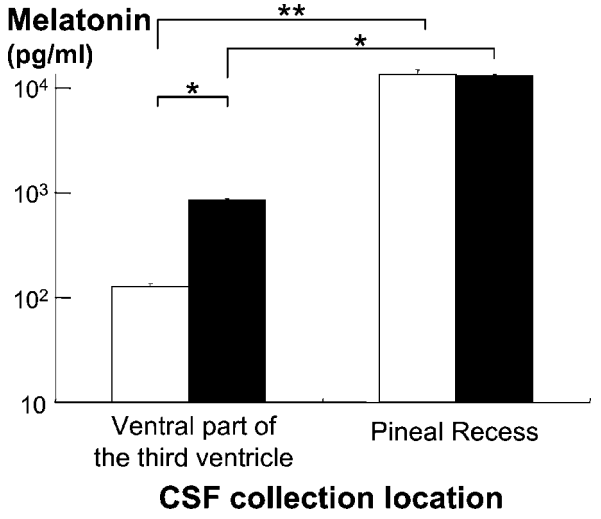

FIG. 4. Effect of the fast (□) or low (■) flow rate collection of CSF on melatonin levels (mean ± SEM) in the ventral part of the IIIV and in the PR. *, $P < 0.05$; **, $P < 0.001$.

amplitude varied greatly among groups. Nocturnal CSF melatonin concentrations were greatly lower in SO, compared with S and F animals, (ratio SO/S: 15%, $P < 0.05$ and SO/F: 17%, $P < 0.01$; 1020 ± 305, 5984 ± 1706, and 6917 ± 1601 pg/ml in SO, S, and F, respectively; Figs. 5 and 6).

The changes in melatonin concentrations in the jugular blood, used as an index of the secretory ability of the pineal gland, were not different among the three groups of animals following the surgery (no significant group × time interaction). An effect of time was found ($P < 0.05$) with d +30 being different from d +58 ($P < 0.05$) and no other difference between time points (d −13: 457 ± 50; d +30: 495 ± 66; d +58: 389 ± 49; d +124: 409 ± 47 pg/ml).

Discussion

In the literature, anatomical observations have suggested several hypotheses to explain how melatonin could be released in the CSF [*i.e.,* active uptake of melatonin from peripheral blood or release from choroid plexus after retrograde transport from the Galen vein (15–18)]. Our study performed in sheep, in which the localization of the pineal gland relative to the IIIV is similar to that in humans, is the first study aimed at testing one of these hypotheses. It demonstrates that the major part of CSF melatonin enters the IIIV through the PR, and from there diffuses to the whole IIIV. This conclusion is based on three pieces of evidence: 1) CSF nocturnal melatonin levels are much higher in the PR than in the vIIIV (*i.e.,* at a distance of less 10 mm); 2) CSF withdrawal in the PR causes a flow rate-dependent decrease in melatonin concentrations in the vIIIV; and 3) the surgical sealing off of the PR causes a dramatic decrease in nocturnal melatonin levels in the CSF.

The previous observation that melatonin levels were lower in the lateral ventricle than in the IIIV suggested that melatonin enters the CSF in the IIIV (15). The present demonstration of higher melatonin levels in the PR than in the vIIIV extends this conclusion and suggests strongly that the major access of melatonin to the CSF is near the PR. The most striking observation in favor of a PR origin is that the collection of CSF near this site causes a concentration drop in the vIIIV, and it is dependent on collection flow rate (high at fast rate, low at low rate). The most obvious interpretation is that collecting CSF at the source of melatonin deprived the whole compartment of the hormone; an increase in flow rate leads to a higher removal of melatonin and therefore a larger drop in its concentrations downstream, especially in the vIIIV. Alternatively, the high flow rate of collection (30 µl/min) in the PR is a significant proportion of CSF production (about 100 µl/min in sheep) (22) and causes CSF to circulate faster from the rostral to the caudal end of the IIIV. Therefore, melatonin could not have a chance to progress rostrally against this stream from the PR to the vIIIV. Regardless of the explanation, this observation strongly indicates that, in physiological conditions, CSF melatonin originates from the PR and diffuses rostrally toward the rest of the ventricle. This implies that melatonin diffuses from the PR to the vIIIV against the dominant rostrocaudal flow.

The difference in concentrations between the two sites observed during simultaneous collection must be taken with great caution in terms of physiological comparison because it was artificially overestimated by the collection procedure, particularly at a flow rate of 30 µl/min. Indeed, concentrations in the PR are not dependent on flow rate and are likely to reflect closely physiological concentrations in this site. However, concentrations in the vIIIV are greatly underestimated at a flow rate of 30 µl/min and, to a lesser extent, at 6 µl/min. In this latter situation, they agree with previous observations with a single site measurement at flow rates of 30 or 10 µl/min (15) and the increase following cessation of PR collection is much reduced, compared with the other flow rate. An additional difficulty for a precise assessment of concentrations lies in the interanimal variability. This variability originates in part in the well-described differences in

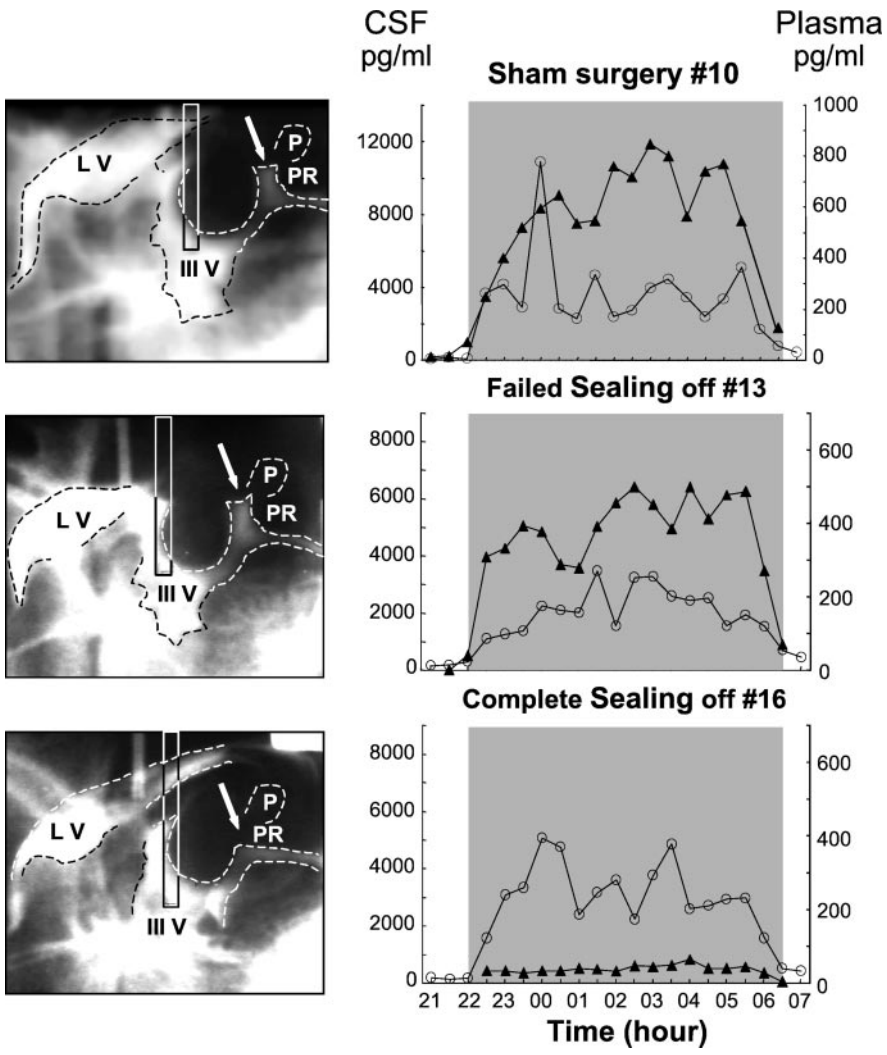

FIG. 5. *Left*, X-rays of ventricular system used to determine the completeness of PR sealing off (radio opaque fills or not the PR). LV, Lateral ventricle; P, pineal gland. *Arrow* points to PR. *Right*, Melatonin concentrations in the IIIV CSF (▲) and plasma (○) during an 8-h night, in an S, an F, and an SO animal.

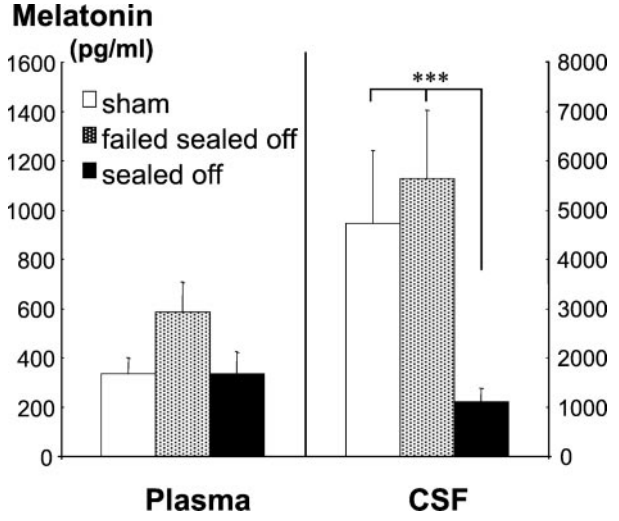

FIG. 6. Effect of the PR sealing off on the melatonin levels in jugular plasma and in the IIIV CSF. ***, $P < 0.005$.

melatonin production among animals (23). This biological variability could also be increased by the influence of the position of the cannula on the measured levels. Indeed, a gradient of melatonin concentrations exists between the PR and the base of the ventricle and a small difference in placement may cause substantial differences in measured concentrations. Regardless of this difficulty in quantifying precisely the difference between sites, the main conclusion that concentrations are higher in the PR than in the rest of the IIIV remains. Indeed, the concentrations measured in the PR (from 3,000 to 30,000 pg/ml) have never been found in the vIIIV in previous studies or during the single-site collection of this study (on average 1,700 pg/ml in this study and 2,000 pg/ml in Ref. 15).

In Exp 2, the obliteration of the PR allowed to avoid the penetration of CSF inside the PR as evidenced by the disappearance of this cavity on the x-ray pictures obtained after injection of radio opaque fluid in the ventricular system. This obliteration was obtained without preventing the flow of CSF toward the fourth ventricle, as evidenced by the presence of radio opaque fluid in the Sylvius duct and the fourth ventricle. This enabled distinguishing the part of CSF melatonin originating from the PR and that coming from other parts of the ventricular system. The dramatic reduction in melatonin concentrations in the IIIV in SO animals, compared with shams, demonstrates that at least 80% of IIIV CSF melatonin

enter the ventricle through the PR. One difficulty linked to the surgery was to spare the NPY nerve fibers in the pineal stalk that have been described in the rat (24) and that could be involved in the regulation of melatonin synthesis (25). The sham-operated animals allowed to remove partly this drawback because their pineal gland was exposed but not to the stage of opening the IIIV and inserting biological glue in the ventricular system. However, the F animals are much better controls because they underwent the same surgery and glue injection as the completely SO ones with the exception that biological glue fixed near the PR failed to prevent CSF circulation within the PR. Their CSF melatonin concentration was as high as in S animals, which indicates that the reduction of CSF melatonin levels in SO animals was the consequence of the obliteration and not a nonspecific consequence of the surgery. Moreover, the changes over time in blood melatonin concentrations, an index of the synthetic activity of the pineal gland, did not differ among groups, suggesting that pineal secretion of melatonin was not disrupted by the obliteration. Therefore, these data demonstrate that the major part of CSF melatonin comes from the PR.

A small amount of melatonin remains in IIIV CSF of SO animals, compared with S animals (15%). This remaining presence of melatonin is suggestive of the existence of another minor source of CSF melatonin. This hormone is small (232 D) and highly lipophilic, and it can cross easily the membranes and diffuse from surrounding tissue. It could also come from the choroid plexus that is possibly connected to the Galen vein by retrograde blood flow (16). An alternative explanation for the residual presence of melatonin is that the obliteration of the recess is not full, despite the absence of evidence for radio opaque in the recess. Considering the huge concentrations in the PR, a small communication between the pineal gland and the CSF will increase melatonin levels in the IIIV. In favor of this latter possibility, in some animals CSF melatonin levels were down to 70 pg/ml after obliteration. Regardless of its explanation, the presence of residual melatonin prevents the use of this model of SO animals to test the relative importance of the CSF and the blood signals to produce the biological effects of melatonin because, despite an 85% reduction, CSF concentrations remain about three times as high as blood concentrations in sealed off and therefore melatonin is not selectively suppressed in the CSF compartment.

The present data indicate that the major part of CSF melatonin originates from the PR in sheep, but how likely is this conclusion to apply to other species? Although the pineal bodies of different mammals possess a common developmental origin from the posterodorsal region of the diencephalic roof, there are variations with respect to pineal position. The sheep pineal gland is a pea-like organ, belonging to the proximal type: The bulk of the pineal tissue lies closely related to the IIIV and is supported by the stalk attaching the pineal body, which is separated by the PR (19). The human pineal gland is a piriform organ and presents the same anatomical organization as in sheep, suggesting that our data on CSF melatonin origin are likely to apply to humans. In contrast, the pineal gland of rodents extends from the IIIV ("deep" pineal) to immediately beneath the skull ("superficial" pineal) (19). Electron microscopy investigations in Mongolian gerbil and in the vole (20) demonstrated that the deep pineal gland-CSF interface is not covered by a typical ependyma usually protecting the ventricular walls and that some pinealocytes are protruded in the PR, being in direct contact with the CSF. These bulging pinealocytes of the deep pineal gland could therefore release melatonin directly into the PR, and the superficial pineal could release melatonin preferentially in blood. It will be of interest to determine whether species with a proximal type pineal, like humans and sheep, also display bulging pinealocytes, which would constitute an anatomical support for the melatonin release in the PR described in this study.

In conclusion, these results provide, for the first time, the localization of melatonin entry in the CSF. At least for its major part, melatonin is released into the PR and then is distributed throughout the ventricular system to reach possibly periventricular-binding sites. Further investigation is required to first determine the anatomical support for a direct secretion in the PR and then the functional role of CSF melatonin.

Acknowledgments

We wish to thank J.C. Thiéry for valuable comments on the manuscript; D. Skinner for helpful discussion; A. Daveau, O. Léon, and Y. Locatelli for technical assistance; F. Paulmier, F. Dupont, and their technical staff for animal management; and M. Charker for correcting the English manuscript. We are grateful to CeramOptec (Bonn, Germany) for generously providing the diode laser equipment (CERALAS D25).

Received August 15, 2001. Accepted September 19, 2001.

Address all correspondence and requests for reprints to: Benoît Malpaux, Physiologie de la Reproduction et des Comportements, UMR 6073 Inra-Cnrs-Université de Tours, 37380 Nouzilly, France. E-mail: malpaux@tours.inra.fr.

H.T. was supported by a Ph.D. grant from "Région Center."

References

1. **Nicholson C** 1999 Signals that go with the flow. Trends Neurosci 22:143–145
2. **Evans CA, Reynolds JM, Reynolds ML, Saunders NR, Segal MB** 1974 The development of a blood-brain barrier mechanism in foetal sheep. J Physiol 238:371–386
3. **Nilsson C, Lindvall-Axelsson M, Owman C** 1992 Neuroendocrine regulatory mechanisms in the choroid plexus-cerebrospinal fluid system. Brain Res Brain Res Rev 17:109–138
4. **Wood JH** 1982 Neuroendocrinology of cerebrospinal fluid: peptides, steroids, and other hormones. Neurosurgery 11:293–305
5. **Silver R, LeSauter J, Tresco PA, Lehman MN** 1996 A diffusible coupling signal from the transplanted suprachiasmatic nucleus controlling circadian locomotor rhythms. Nature 382:810–813
6. **Fang J, Wang Y, Krueger JM** 1998 Effects of interleukin-1 beta on sleep are mediated by the type I receptor. Am J Physiol 274(3 Pt 2):R655–R660
7. **Daniels WM, van Rensburg SJ, van Zyl JM, Taljaard JJ** 1998 Melatonin prevents beta-amyloid-induced lipid peroxidation. J Pineal Res 24:78–82
8. **Arendt J, Skene DJ, Middleton B, Lockley S, Deacon S** 1997 Efficacy of melatonin treatment in jet lag, shift work and blindness. J Biol Rhythms 12:604–617
9. **Liebmann PM, Wolfler A, Felsner P, Hofer D, Schauenstein K** 1997 Melatonin and the immune system. Int Arch Allergy Immunol 112:203–211
10. **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 secretion by *in situ* microimplant delivery. Endocrinology 139:1508–1516
11. **Rollag MD, Morgan RJ, Niswender GD** 1978 Route of melatonin secretion in sheep. Endocrinology 102:1–7
12. **Shaw PF, Kennaway DJ, Seamark RF** 1989 Evidence of high concentrations of melatonin in lateral ventricular cerebrospinal fluid of sheep. J Pineal Res 6:201–208
13. **Kanematsu N, Mori Y, Hayashi S, Hoshino K** 1989 Presence of a distinct

24-hour melatonin rhythm in the ventricular cerebrospinal fluid of the goat. J Pineal Res 7:143–152
14. **Reppert SM, Perlow MJ, Tamarkin L, Klein DC** 1979 A diurnal melatonin rhythm in primate cerebrospinal fluid. Endocrinology 104:295–301
15. **Skinner DC, Malpaux B** 1999 High melatonin concentrations in third ventricular cerebrospinal fluid are not due to Galen vein blood recirculating through the choroid plexus. Endocrinology 140:4399–4405
16. **Smulders AP, Wright EM** 1980 Role of choroid plexus in transport of melatonin between blood and brain. Brain Res 191:555–558
17. **Maurizi CP** 1991 Recirculation of cerebrospinal fluid through the tela choroideae is why high levels of melatonin can be found in the lateral ventricles. Med Hypotheses 35:154–158
18. **Quay WB** 1973 Retrograde perfusions of the pineal region and the question of pineal vascular routes to brain and choroid plexuses. Am J Anat 137:387–401
19. **Vollrath L** 1981 The pineal organ. In: Oksche A, Vollrath L, eds. Handbuch der mikroskopischen anatomie des menchen. Berlin: Springer-Verlag; VI/7
20. **Hewing M** 1982 Pinealocytes contacting the cerebrospinal fluid of the subpraipineal recess in the Mongolian gerbil (*Meriones unguiculatus*). Cell Tissue Res 222:177–185
21. **Skinner DC, Malpaux B, Delaleu B, Caraty A** 1995 Luteinizing hormone (LH)-releasing hormone in third ventricular cerebrospinal fluid of the ewe: correlation with LH pulses and the LH surge. Endocrinology 136:3230–3237
22. **Chodobski A, Szmydynger-Chodobska J, Cooper E, McKinley MJ** 1992 Atrial natriuretic peptide does not alter cerebrospinal fluid formation in sheep. Am J Physiol 262(5 Pt 2):R860–R864
23. **Zarazaga LA, Malpaux B, Guillaume D, Bodin L, Chemineau P** 1998 Genetic variability in melatonin concentrations in ewes originates in its synthesis, not in its catabolism. Am J Physiol 274(6 Pt 1):E1086–E1090
24. **Mikkelsen JD, Moller M** 1999 Neuropeptide Y in the mammalian pineal gland. Microsc Res Tech 46:239–256
25. **Ribelayga C, Pevet P, Simonneaux V** 1998 Possible involvement of neuropeptide Y in the seasonal control of hydroxyindole-*O*-methyltransferase activity in the pineal gland of the European hamster *(Cricetus cricetus).* Brain Res 801:137–142