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Photoperiod affects the cerebrospinal fluid proteome: a comparison between short day– and long day–treated ewes

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ABSTRACT

Photoperiod is the main physical synchronizer of seasonal functions and a key factor in the modulation of molecule access to cerebrospinal fluid (CSF) in animals. Previous work has shown that photoperiod affects the transfer rate of steroids and protein hormones from blood to CSF and modulates choroid plexus tight junction protein content. We hypothesized that the CSF proteome would also be modified by photoperiod. We tested this hypothesis by comparing CSF obtained from the third ventricle of mature, ovariectomized, estradiol-replaced ewes exposed to long day length (LD) or short day length (SD). Variations in CSF protein expression between SD- or LD-treated ewes were studied in pools of CSF collected for 48 h. Proteins were precipitated, concentrated, and included in a polyacrylamide gel without protein fractionation. After in-gel tryptic digestion of total protein samples, we analyzed the resulting peptides by nanoliquid chromatography coupled with high-resolution tandem mass spectrometry (GeLC-MS/MS). Quantitative analysis was performed using 2 methods based on spectral counting and extracted ion chromatograms. Among 103 identified proteins, 41 were differentially expressed between LD and SD ewes (with $P < 0.05$ and at least a 1.5-fold difference). Of the 41 differentially expressed proteins, 22 were identified by both methods and 19 using extracted ion chromatograms only. Eighteen proteins were more abundant in LD ewes and 23 were more abundant in SD ewes. These proteins are involved in numerous functions including hormone transport, immune system activity, metabolism, and angiogenesis. To confirm proteomic results, 2 proteins, pigment epithelium-derived factor (PEDF) and gelsolin, for each individual sample of CSF collected under SD or LD were analyzed with Western blots. These results suggest an important photoperiod-dependent change in CSF proteome composition. Nevertheless, additional studies are required to assess the role of each protein in seasonal functions.

1. Introduction

Seasonal functions are synchronized by several parameters, among which duration of night is the most important. This parameter is translated into a chemical signal by release of melatonin from the pineal gland. In sheep, an experimental model widely used for studies of seasonal biological

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rhythms, melatonin acts on several target sites in the brain including the pars tuberalis, the mediobasal hypothalamus, and the choroid plexus (CP) [1]. These latter, together with the cerebral endothelium, are part of the blood–brain barrier (BBB) system, and both are involved in blood filtration and in the synthesis of cerebrospinal fluid (CSF).

Proteins in the CSF originate from blood filtration by the CP, from local CP synthesis, and from drainage of the brain-borne compounds [2]. We have previously demonstrated that CSF renewal rate is modified by photoperiod and that long days (LD; 16L:8D) promote a smaller CSF production rate than short days (SD; 8L:16D) [3]. The passage of molecules through the BBB and therefore through the CP also differs with photoperiod in sheep [4,5]. For example, peripheral estradiol (E2) and progesterone passage from blood to brain is greater in LD than SD animals; this phenomenon is dependent on melatonin because pinealectomized animals do not show specific photoperiodic regulation. In rams, a protein hormone, leptin, has a higher transfer rate from blood to CSF under LD conditions [5]. More recently, we attempted to explain the cellular mechanisms involved in the regulation of blood–CSF transfer and found downregulation of specific tight junction proteins (afadin-6, zona occludens 1 and 2, and cadherin) of the CP under LD conditions [6]. This suggests that the permeability of the CP is decreased during SD periods.

We hypothesized that proteins passing through the CP, secreted by the CP, or drained from the brain may vary according to photoperiod. To test our hypothesis, we did a quantitative proteomic analysis on the CSF of ovariectomized, estradiol-replaced ewes kept under SD or LD conditions. We used an approach combining sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and trypsin digestion followed by nanoflow liquid chromatography tandem mass spectrometry (GeLC-MS/MS), associated with 2 label-free quantitative methods based on spectral counting (SC) and extracted ion chromatograms (XICs). Reliability of quantitative proteomic data obtained from pooled CSF was then checked for 2 proteins using Western blots.

2. Materials and methods

2.1. Animals, light treatment, and surgery

All animal experiments were conducted in accordance with French Authorization No. 37801 for Animal Experimentation and Surgery and approved by the Val de Loire Local Ethics Committee. Adult Ile-de-France ewes ($n = 10$) of similar age (2.5-yr old) and weight (52.5 ± 3.0 kg) obtained from UEPAO (Unité expérimentale PAO n°1297 [EU0028], INRA Centre Val de Loire, Nouzilly, France) were maintained indoors in separate pens under artificial lighting conditions and fed a constant maintenance diet of hay, straw, and commercial concentrates, with water and mineral licks available ad libitum. Ewes were submitted to a routine light treatment that induced a neuroendocrine status corresponding to an LD (16L:8D; $n = 5$) or SD (8L:16D; $n = 5$) photoperiod (ie, to an inhibited or stimulated pulsatile luteinizing hormone (LH) secretion, respectively [4]).

About 5 wk before starting CSF collection, all ewes were implanted with a stainless steel guide cannula (1.5 mm outer diameter and 40 mm length) in the third ventricle of the brain under general isoflurane anesthesia, according to the method described by Thiery et al [7]. During the surgery, they were also ovariectomized and subcutaneously implanted with E2 to maintain constant plasma E2 concentrations of 5 to 8 pg/mL [8], with an additional subcutaneous injection of morphine chlorhydrate (20 mg, CMD Lavoisier, 75,017, Paris, France) before the end of anesthesia to prevent postsurgical pain. During the sampling session, ewes were kept in comfortable, individual pens where they could lie down and had access to hay. They had visual contact with other sheep to prevent the stress of isolation.

2.2. CSF collection

Before CSF collection started, the endocrine status of each ewe was checked by assessing LH level in the blood. CSF was collected into 3 mL fraction tubes from the third ventricle for 48 h at a rate of 20 μL/min (Minipuls 3 peristaltic pump, Gilson, Villiers le Bel, France) in 5 ewes under SD conditions (LH levels >1 ng/mL) and in 5 ewes under LD conditions (LH levels <1 ng/mL). Fraction tubes were immediately put in ice to prevent protein degradation, and all fractions were subsequently pooled and stored at −20°C until further processing.

2.3. CSF preparation

The protein concentration in the CSF from 5 SD ewes and 5 LD ewes was determined using the Protein DC Assay (Bio-Rad, Marnes-la-Coquette, France) before concentration with a Vivaspin 500 with 10,000 molecular weight cutoff filters (Sartorius, Goettingen, Germany) according to the manufacturer's specifications. The protein concentration in the resulting samples was then determined once again using the Protein DC Assay. For quantitative mass spectrometry (MS) analysis, the CSF was pooled into 2 samples, one from SD ewes and the other from LD ewes, whereas for Western blots, the CSF of each individual was treated separately.

2.4. SDS-PAGE

SDS-PAGE electrophoresis was carried out according to the method of Laemmli [9]. For quantitative MS-based analysis, 50 μg of proteins from each pool was included in 10% SDS-PAGE (30 min at 50 V) without fractionation (1 protein band). Precision Plus Protein All Blue prestained standard (Bio-Rad) was used as molecular weight standard. Gel was stained with Coomassie Blue R-350 (PhastGel Blue R, GE Healthcare, Thermo Scientific, Illkirch, France), and the protein band was dissected out. For Western blotting, 20 μg of proteins from each CSF sample was separated on an 8% to 16% gradient SDS-PAGE. The gel was then run in a mini gel electrophoretic unit (Bio-Rad) at 180 V until the dye front was near the end of the gel.

2.5. Proteomic analysis

For quantitative MS-based analysis, proteins were digested in-gel with trypsin as previously described [10]. Peptide mixtures were analyzed (5 replicates) by nanoflow