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Original Research Article

Causal link of total locomotor activity, melatonin and rectal temperature daily rhythm in small ruminants

Claudia Giannetto [a], Vincenzo Carcangiu [b], Sebastiano Luridiana [b], Francesco Fazio [a], Maria Consuelo Mura [b], Albamaria Parmeggiani [c], Giuseppe Piccione [a,*]

[a] Department of Veterinary Science, University of Messina, Polo Universitario dell'Annunziata, 98168 Messina, Italy
[b] Department of Veterinary Science, University of Sassari, Via Vienna 2, 07100 Sassari, Italy
[c] Department of Veterinary Science, University of Bologna, Via Tolara di Sopra 50, 40064 Ozzano Emilia, Bologna, Italy

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ABSTRACT

To improve the knowledge in chronophysiology we investigated the causal link between the most important physiological variable studied until now; ten Sarda ewes and ten Sarda goats, pluriparus not pregnant and no lactating, were used. Animals were housed under natural environmental conditions in a common stall, alfalfa hay and water were available ad libitum. Each animal was equipped with an Actiwatch-Mini® for recording total activity. Blood samples were collected every 4 h over a 48 h period for the assessment of melatonin concentration. Rectal temperature was recorded with a digital thermometer immediately before the blood sampling at each data point. Single cosinor method showed a daily rhythm of studied variables. Higher MESOR and amplitude values of melatonin and rectal temperature were observed in sheep than in goats. The diurnal acrophase of locomotor activity was statistically different from the nocturnal acrophase of melatonin and rectal temperature, with no differences between the two species. Robustness was statistically lower in total locomotor activity in comparison with the others two variables, with a differences due to species in melatonin daily rhythm. In conclusion, in small ruminants, melatonin and rectal temperature daily rhythms are strictly correlated, and are not associated with the locomotor activity rhythm.

Introduction

In mammals, information about the environmental photoperiod is relayed from the retina to the suprachiasmatic nuclei (SCN) in the anterior hypothalamus. This is a basic structure where many physiological functions are controlled. Environmental conditions, especially light information, are mediate from the eye to hypothalamic structure. The mechanism of adaptation to these variations is seen to have an influence on

* *Corresponding author*. Tel.: +39 0903503584.
E-mail address: giuseppe.piccione@unime.it (G. Piccione).

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behavior such as locomotor activity and feeding, and in physiological functions, such as body temperature and hormonal secretion (Alila-Johansson et al., 2004). The light information received by the SCN is transduced into neural and hormonal output signals that affect various rhythms of the animal. Via the sympathetic nervous system the SCN sends impulse to the pineal gland where it influences the secretion of melatonin. The hormone melatonin is a chemical messenger that is synthesized during darkness; it is itself a messenger of the light/dark alternation in environment and is thought to serve as synchronizer for circadian rhythms. Its circadian periodicity may be understood as a coordinating signal for other biological rhythmicity, or as an endogenous synchronizer (Corbalan-Tutau et al., 2014). Daily oscillation in blood levels of melatonin has been documented in various species of birds and mammals (Hasegawa and Ebihara, 1992; Brandstätter et al., 2000; Zawilska et al., 2006). In sheep and goats, melatonin daily rhythm has been widely studied; it is endogenously generated and entrained by the light/dark cycle. In the study of biological rhythm the simultaneous investigation of many physiological variables plays an important role in consideration that there is a reciprocal relationship between the robustness of the endogenous circadian timing system and its dependency on regularly timed synchronizers (Berger, 2008). Although, daily rhythmicity is more robust in some physiological variables than in others, and more robust in some organisms than in other (Refinetti, 2006). As indicator of the rhythmicity of the biological clock the rhythmicity of body temperature has been widely used; because of the relative ease of monitoring, and because of the robustness of its rhythm (Piccione and Refinetti, 2003). Similarly it believed that locomotor activity ensures an optimal functioning of the biological system (Piccione et al., 2010); monitoring behavioral changes in farm animals can improve welfare by providing information on an individual health (Muller and Schrader, 2003). Previous research has shown that melatonin plays an important role in the modulation of the circadian rhythms of activity and body temperature (Aguzzi et al., 2006).

In the last years many studies have been conducted on the morpho-fucntional characteristics of the circadian system. All physiological variables that display a prominent circadian rhythm should be measured as a marker rhythm with an application in chronotherapy protocols and in the monitoring of welfare. Daily synchronization of physiological process contributes to the wellness of the organism; in order to investigate the potential causal link between activity, melatonin and body temperature we simultaneously investigated the rhythm of locomotor activity, melatonin serum levels and rectal temperature values in sheep and goats housed under environmental conditions.

Material and methods

Animals and housing

Ten Sarda breed pluriparus ewes, four years old, with a mean body weight 40.5 ± 1.8 kg, and ten Sarda breed pluriparus female goats, two years old, with a mean body weight 41.3 ± 2.8 kg, were enrolled in our study. All animals were clinically healthy, free from internal and external parasites, not pregnant and in dry period. All animals were kept in a common stall and had free access to water and to good-quality alfalfa hay. They were subjected to a natural photoperiod (Sunrise 07:00; Sunset 17:30). The environmental temperature and relative humidity ranges were 13–20 °C and 54–100%, respectively. All treatments, housing and care were carried out in accordance with the standards recommended by the EU Directive 2010/63/EU for animal experiments.

Data collection

Each animal was equipped with an Actiwatch-Mini® (Cambridge Neurotechnology Ltd., UK), actigraphy-based data loggers that record a digitally integrated measure of motor activity by means of collars that was accepted without any obvious disturbance. Total activity of each animal was recorded as the result of all movements, which includes different behaviors such as feeding, drinking, walking, grooming, and small movements during sleep, independent of the animal's position such as lying or standing, for 48 h with a sampling interval of 5 min, the values recorded every 5 min were the mean values of the sum of 32 recordings per second.

Also, all animals were cannulated the day before the start of the study and the cannula remained patent for the duration of sample collection. Blood samples were collected in heparinized tubes though jugular intravenous catheters (FEP G18 × 45 mm) secured in place with suture (Vicryl, Ethicon, Somerville, NJ) every 4 h over a 48 h period starting from 00:00 of day 1 and ending at 00:00 on day 3.

Rectal temperature was recorded with a digital thermometer (HI-92740, Hanna Instruments, Bedfordshire, UK) whose probe was insert 8 cm into the rectum immediately before the blood sampling at each data point. All animals tolerate rectal probes very well and show no sign of stress-induced hyperthermia (Piccione et al., 2002).

During the night, all data recording were performed using a dim-red light (<3 lux, 15 W Safelight lamp filter 1A, Kodak Spa) avoiding any direct lighting of the eyes in order not to influence melatonin secretion.

Melatonin assessment

Blood sample tubes were centrifuge at 2500 × *g* per 15 min and the obtained plasma were used in order to assess melatonin concentration. Plasma melatonin concentrations were determined with the aid of direct radioimmunoassay, adapted from that described by Fraser et al. (1983). The plasma sample (200 μl) was incubated with a specific antiserum to melatonin raised in the sheep (against *N*-acetyl-5-methoxy tryptophan/bovine thyroglobulin; Guildhay Antisera Ltd.) with a final dilution of 1:6000 and trace amounts of 3*H*-melatonin (*o*-methyl-3Hmelatonin, Amersham) were then added. The standard curve (Sigma melatonin) was constructed using charcoal-stripped pooled plasma from at least two ewes which had been kept in natural daylight for at least 4 h before sampling. The free and antibodybound fractions of melatonin were then separated using a dextran-coated charcoal solution. The free melatonin fraction was precipitated with charcoal by centrifugation and supernatant liquid counted in a scintillation-counter. The major