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Short communication

Diurnal and seasonal changes in endogenous melatonin levels in the blood plasma in dogs

R.S. Zań¹, Z. Roliński¹, C.J. Kowalski¹, A. Bojarska-Junak², J. Madany³

¹ Department of Pharmacology, Faculty of Veterinary Medicine, University of Life Sciences in Lublin, Akademicka 12, 20-033 Lublin, Poland

² Department of Clinical Immunology, Medical University in Lublin, Chodźki 4a, 20-093 Lublin, Poland

³ Department and Clinic of Internal Diseases, Faculty of Veterinary Medicine, University of Life Sciences in Lublin, Głęboka 30, 20-612 Lublin, Poland

Abstract

This paper describes research on the levels of endogenous melatonin (MLT) in the blood serum in dogs in different seasons (March, June, September, December) and at different times of day (11:00, 12:00, 23:00, 24:00 and 1:00), using immunoassay method. Blood samples were collected in the diurnal cycle, in consecutive seasons. The conducted studies show that MLT levels undergo clear changes in both the diurnal cycle, as well as in seasonal one in this species.

Key words: dogs, endogenous melatonin, immunoassay, plasma concentration

Introduction

Melatonin (MLT), a low molecular weight indoleamine (MW 232.27u) secreted primarily at night, coordinates the work of the master biological clock regulating circadian rhythms, including sleep and wakefulness. MLT levels are variable and are subject to fluctuations in the diurnal cycle as well as in different seasons. This comes from the fact that its synthesis and secretion is inhibited by light. In the literature there is a number of works describing both diurnal and seasonal changes in MLT levels in the blood plasma of animals (Andersson 2001, Diekman et al. 2002). The most common methods for determination of melatonin in blood are radio-immunoassay (RIA) and enzyme-linked immune-sorbent assay (ELISA) (Middleton 2006, De Almeida et al. 2011). In this study, we de-

cidated to use ELISA method because of its high sensitivity and selectivity.

Materials and Methods

Studies on the changes in endogenous MLT concentrations in diurnal cycle depending on seasons were conducted on always the same 6 healthy, crossbreed dogs. Blood samples were collected in the diurnal cycle, during the solstice at equal times: 11:00, 12:00, 23:00, 24:00 and 1:00 in consecutive seasons (March, June, September, December). Venous blood was collected in amount of 3 ml into tubes with heparin. Blood samples were collected under a point dim red light. Plasma was immediately separated by centrifugation for 15 min at 1500 rpm and stored in frozen tubes at

Correspondence to: R.S. Zań, e-mail: rafal.zan@up.lublin.pl

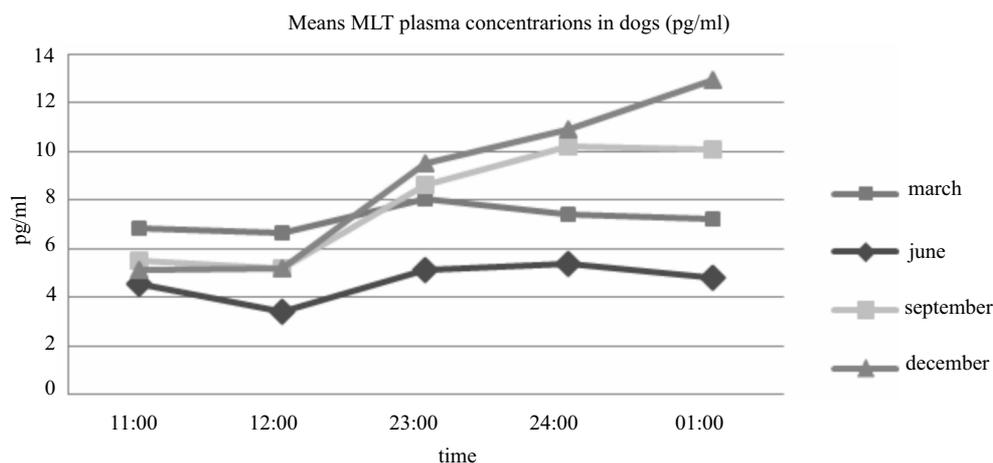


Fig. 1. Changes in circadian MLT concentrations in the blood plasma in dogs in various seasons.

– 20°C until assay. ELISA Kit for Canine Melatonin (Laboratory: Uscn Life Science Inc. Wuhan, China) test was used for the quantitative determination of MLT concentrations. MLT concentration in the samples was determined by comparing the optical density in comparison to the standard curve, using a reader: 1420 Multilabel Counter Victor³ (Perkin Elmer) and the computer program Work Out 2.0. Color change was measured spectrophotometrically at a wavelength of 450 ± 2 nm. The investigation revealed that the test used has a high sensitivity and specificity for canine MLT. The sensitivity of this assay was defined as the lowest MLT concentration that could be differentiated from zero. Student's t-test was used to compare differences between seasonal and daily MLT levels. $P \leq 0.05$ was considered statistically significant.

Results

The determinations showed that in winter (December) the average concentrations of MLT were contained within the limits of $5.11 \pm 4.21 - 5.20 \pm 3.08$ pg/ml during the daylight hours and $9.51 \pm 5.31 - 12.93 \pm 8.27$ pg/ml during the night, with a peak secretion of the hormone occurring at 01:00 a.m.. But, in the spring season (March) the daytime average concentrations of MLT in dogs ranged between $6.67 \pm 1.56 - 6.83 \pm 2.58$ pg/ml. During night hours, this level ranged from 7.20 ± 2.06 to 8.06 ± 2.11 pg/ml, with a peak secretion at 11:00 p.m.. For the summer season (June), these values were in the range $3.40 \pm 1.19 - 4.56 \pm 2.40$ pg/ml in the daytime and in the night-time average values ranged from 4.82 ± 2.06 to 5.39 ± 3.53 pg/ml. The peak of secretion occurred in the summer time at 12:00 a.m.. Average plasma levels of MLT in the autumn season (September) at night ranged between 8.56 ± 2.56 pg/ml – 10.21 ± 3.45 pg/ml,

whereby a peak secretion of MLT in this time of year occurred at 12:00 a.m. However, in the daylight hours the concentrations of this hormone were equal to $5.19 \pm 2.14 - 5.51 \pm 2.35$ pg/ml (Fig. 1.). Statistically significant differences in the levels of MLT determined during day and night ($p \leq 0.05$) were found in autumn and winter. Statistically significant differences ($p \leq 0.05$) were also found when comparing the levels of MLT during day and night in the summer and winter.

Discussion

In the light of our study, the MLT synthesis is always highest at night, decreases at dawn and remains at low levels throughout the day. In nearly all mammalian species tested so far, the MLT level is from a few to several times higher at night than during the day. Research on seasonal and daily changes in MLT concentrations in dogs were carried out on working Alaskan husky sledge dogs living in circumpolar zone, using a radioimmunoassay method. Blood samples were collected only in winter and summer at four time points (05:00 p.m., 02:00 a.m., 08:00 a.m. and 10:30 a.m.). These studies on husky confirmed the influence of the seasons and the light changes throughout the day on endogenous levels of MLT. The lowest levels of the hormone were equal to 1.14-1.91 pg/ml and were recorded in summer in the morning. Highest concentrations of MLT were given at night in winter and were above 15 pg/ml (Dunlap et al. 2007). Our research on the levels of endogenous MLT in dogs were carried out in contrast to the previous ones (Dunlap et al. 2007) during the four seasons (March, June, September, December), in dogs of a mixed race, and above all different method, enzyme immunoassay test, was used to study the concentrations of the hormone.

Results of our research were comparable with those obtained by RIA method, although in the dogs used in the experiment there were no such low levels of MLT in the summer (less than 2 pg/ml), nor as high ones in winter (above 15 pg/ml). The reason for these differences may be different time points of blood collection for test, differences due to race of the animals and different geographical latitude where these two experiments were conducted. The study also confirmed the dependence of MLT secretion on time of day in dogs. The lowest average concentrations found in plasma during the day was found in the summer and autumn season, they were respectively at 12:00 3.40 ± 1.19 pg/ml and 5.19 ± 2.14 pg/ml, and highest during the night at 1:00 in the winter season 12.93 ± 8.27 pg/ml. The study confirmed the usefulness of ELISA method for the determination of endogenous MLT concentrations in dogs and an appropriate limit of quantification of the commercial test „ELISA Kit for Canine Melatonin”.

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