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CIRCADIAN CHANGES OF THE MELATONIN CONCENTRATION IN THE BLOOD OF PREGNANT COWS AND CALVES

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Abstract

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It has been shown in numerous studies on animals and man that melatonin production is rhythmic and light-dependent. Since relevant literature concerning cattle is missing, and having in mind scientific and practical importance of such knowledge, we decided to investigate circadian changes of the melatonin level in the blood of cows and calves.

The experiment was carried out on pregnant cows and 2.5-month-old calves. Before and during the experiment animals were kept under the same light - dark regime (LD 10:14). The blood samples were drawn ninefold from the external jugular vein over a period of 24 hours, at 15:00, 18:00, 21:00, 24:00, 3:00, 6:00, 9:00, 12:00 and 15:00 h. Melatonin was determined by the radioimmunoassay (RIA) method. The findings were analysed using the Duncan's D test.

Highly significant differences ($P < 0.01$) were found between the level of melatonin in the light and the dark phase, both in cows and calves. The blood concentration of this hormone in calves in both phases was significantly lower ($P < 0.01$) when compared to cows. Also the overnight profile of the melatonin production was different in cows and calves; this is an evidence of changes in the melatonin synthesis and release in the dark phase with age.

Pregnant cows, calves, melatonin, circadian changes

From among all known biological rhythms the circadian rhythms play a major role in adaptation of organism to the conditions of surroundings. The pineal gland constitutes an important part of the mechanism that is capable of measuring out time by an organism. It acts in a synchronizing manner on the rhythmicity of different physiological processes by producing and regularly bringing about the release of melatonin. It is generally supposed that the main centre controlling the activity of the pineal gland and maintaining the rhythm of the secretion of melatonin is the nucleus suprachiasmaticus situated in the anterior part of the hypothalamus (Cymborowski et al. 1969; Deguchi 1979; Hasegawa et al. 1980; Klein 1979; Reiter 1991; Wurtman 1985).

Melatonin is synthesized by parenchymal cells of the pineal body that are capable of catching tryptophan, a precursor for indolic compounds, out from the circulation system. It shows a short biological half–life in blood (10-40 minutes), high systemic clearance and relatively low volume of distribution (Binkley 1979; Klein et al. 1996; Mallo et al. 1990; Reiter 1991; Waldhauser et al. 1984).

It has been shown in numerous studies on animals and humans that biosynthesis of melatonin takes a course according to the circadian rhythm that is light-dependent and generated by endogenic biological clock. The production of this hormone does not depend

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on the mode of life of a given species (nocturnal, diurnal, mixed) and is always highest at night or in the dark phase of the artificial light cycle. A very distinct circadian rhythm of the melatonin level was found in many animal species, with a low level during a day and higher one at night (Berthelot et al. 1990; Hedlund et al. 1977; Hougton et al. 1993; Klein et al. 1996; Nowak et al. 1990; Reiter 1991; Waldhauser et al. 1983; Waldhauser et al. 1984; Waldhauser et al. 1985; Wurtman 1985).

Melatonin shows variation in the secretion synthesis during the course of ontogeny. In the postnatal period, as the nervous system matures in respect of its functions, an organism starts to form its own circadian rhythm of producing and releasing melatonin; this rhythm is not always consistent with the rhythm occurring in adult individuals (Attanasio et al. 1986; Horton et al. 1992; Kivela 1991; McMillen et al. 1989; Waldhauser et al. 1984).

Many problems concerning the biology of the pineal gland and the physiological role of melatonin have not yet been explained. In particular, the contribution of melatonin in regulatory processes of the biological rhythms is crucial both from the scientific and practical point of view. The above have prompted us to undertake an investigation aimed at determining the circadian changes in the melatonin level in the blood plasma of cows and calves and to describe character of these rhythms.

Materials and Methods

The experiment was performed using five, clinically healthy, Black and White bull calves, 2.5-month-old, and five Black and White cows in the seventh month of pregnancy. During the studies cows were kept in a litter barn with tying system of stalls and calves in the separated part of the same barn, under uniform conditions of environment. Cows were fed rye and grass silage and concentrated feed mix. The feed ration was supplemented with hay and straw. The animals were allowed access to salt-lick and water. Calves were fed milk substituting preparation (7 l per day). They were given free access to concentrated fodder, hay and water.

Before the experiment and during the whole study period were the animals maintained under LD 10:14 (light phase – 5.30 to 15.30 h, dark phase – 15.30 to 5.30 h).

The blood samples were drawn from the external jugular vein, nine times over a period of 24, with 3-hour intervals, i.e., at 15:00, 18:00, 21:00, 24:00, 3:00, 6:00, 9:00, 12:00 and 15:00 h. The collected blood was heparinized (Heparinum Polfa) and centrifuged immediately. The blood plasma was stored at -20 °C until analysed. When drawing blood samples in the dark phase, the red spot-lighting was used (with wavelength > 600 nm).

Melatonin was determined by the radioimmunoassay (RIA) method (Arendt et al. 1977) with the use of the following reagents:

1. Melatonin, chromatographically pure (Sigma),
2. Melatonin, tritium-labelled (Amershman),
3. Norit A 0.4% activated carbon suspension with a 0.1% dextran addition,
4. Tricine 0.1% phosphate buffered solution, pH 7.7,
5. Anti-melatonin antibodies received from CSIRO (Australia).

Determinations were made in 500 µl of blood plasma. The calibration curve was prepared using the blood plasma of sheep, where endogenic melatonin was previously removed with the help of 4% activated carbon. The working dilution of antibodies was 300 000 ×, and the calibration curve range from 15.6 to 1 000 pg/ ml.

The findings were statistically evaluated with the method of analysis of variance using the Duncan's D test (Statgraphics 5.0).

Results and Discussion

The results obtained in the experiment are presented in Tables 1 and 2. In all animals studied differences were observed between the melatonin level in the blood plasma in the light and the dark phase.

The mean hormone concentration in the dark phase in the blood plasma of cows was at the level of 218.75 pg/ml. The highest concentration was obsereved at 18:00 and 3:00 h. It amounted to 234.8 pg/ml and 253.6 pg/ml, respectively. The lowest mean values of the melatonin level in the dark phase was observed at 24:00 h. The mean concentration of melatonin in the light phase was 47.08 pg/ ml. The lowest hormone concentration in the blood was found by the end of this phase (15:00 h). The amplitude of changes in the melatonin level in the blood between the light and the dark phase was 214.2 pg/ml (differences highly significant at $P < 0.01$). Similar differences in the melatonin concentration in the blood were found in cows by Newbold et al. (1991) and Berthelot et al. (1990; 1993).

Table 1

Melatonin levels in the blood plasma of cows (n=5) in pg/ml

Cow No	Time of sampling								
	1500	1800	2100	2400	300	600	900	1200	1500
1	67	269	166	196	271	47	52	41	47
2	40	254	302	133	348	47	70	33	30
3	49	253	227	293	300	57	42	53	40
4	40	128	114	118	179	109	41	50	43
5	31	270	170	214	170	40	29	42	37
mean	45.4	234.8*	195.8*	190.8*	253.6*	60.0	46.8	43.8	39.4
SD	12.2	53.9	64.0	62.7	69.2	25.1	13.7	7.1	5.7

Explanations: ☐ dark phase

*significant differences between phases ($P < 0.01$)

Table 2

Melatonin levels in the blood plasma of calves (n=5) in pg/ml

Calf No	Time of sampling								
	1500	1800	2100	2400	300	600	900	1200	1500
1	23	80	157	49	36	25	19	20	31
2	16	308	297	195	309	36	40	24	27
3	23	59	151	98	62	38	30	31	29
4	24	29	99	41	44	36	29	41	27
5	26	79	94	99	75	28	27	39	21
mean	22.4	111.0*	159.6*	96.4*	105.2*	32.6	29.0	31.0	27.0
SD	3.8	112.0	82.1	61.3	114.9	5.7	7.5	9.1	3.7

Explanations: ☐ dark phase

*significant differences between phases ($P < 0.01$)

The findings presented here point out that cows have a type of the night production of melatonin that is characterized by an increased secretion of the hormone shortly after the beginning of the dark phase, its high and not always stable level during this phase, and a rapid decrease of its level prior to the light phase.

Nowak et al. (1990) found a similar profile of changes of the melatonin level in the blood plasma of sheep. Significant differences in the daytime and overnight melatonin levels were found in adult humans. The concentration of melatonin ranged from 0 to 40 pg/ml in the light phase, and from 50 to 200 pg/ml in the dark phase (Waldhauser et al. 1983). Similar findings are reported by Grof et al. (1985). However, the night type of the melatonin production in humans is different.

In the presented experiment an individual variation of the melatonin level in the blood of cows was shown. The concentration of melatonin in the light phase ranged from 31 to 109 pg/ ml, while in the dark phase from 114 to 348 pg/ml.

The mean hormone concentration in the blood plasma of calves in the dark phase was 118.5 pg/ ml. In four calves studied the melatonin level increased slowly after the beginning of the dark phase, with its highest level occurring between 21:00 and 24:00 h. One calf displayed the same profile of changes of the melatonin concentration in the blood plasma as was found in adult cows. The results obtained point to significant individual differences in the melatonin concentration in the blood plasma of calves, in particular in the dark phase of the circadian cycle.

The mean level of melatonin in calves in the light phase was 28.4 pg/ml. The lowest concentration in the blood was found by the end of the light phase (15:00 h) similar to that found in cows. The amplitude of changes in the melatonin level in the blood between the light and dark phase was 89.65 pg/ml (differences highly significant at $P < 0.01$).

These findings point out that the overnight production of melatonin in calves represents a different profile than that of adult individuals characterized by a smooth increase in the melatonin secretion after the beginning of the dark phase and an equally smooth decrease of its level in the second half of this phase.

Hedlund et al. (1977), in their studies on 9-month-old calves, showed a similar profile of the night production of melatonin, too. In calves, according to them, there is only one night peak in the melatonin production at 3:00 h (220 pg/ml). A similar profile occurs in many other species, among others in rat, squirrel, hen and man.

It was also shown in the present studies that the mean level of melatonin in the blood plasma of calves was nearly two times lower than that of cows, both in the light phase (28.4 and 47.08 pg/ ml, respectively) and in the dark one (118.05 and 218.75 pg/ml, respectively).

As it appears from the results presented here, the production of melatonin in the pineal gland and its blood level are subject not only to the circadian changes but show variation in the course of ontogeny. An organism in the early postnatal period starts, in all probability in the connection with maturation of the nervous system being intermediary in synchronizing biological rhythms with changes in the environmental conditions, especially light and temperature, to form its own circadian rhythm of the melatonin secretion which is not alwyas the same as in adults in respect of its cycle and amplitude.

Waldhauser et al. (1984; 1985) found, studying the melatonin level in humans, that the concentration of this hormone in the blood plasma in children during daytime was low (below 20 pg/ml) and did not change with age. The overnight concentration of melatonin in children 1-5 years old is several times higher (about 200 pg/ml) than in juveniles and adults (about 50 pg/ml). Similar findings are given by Wurtman (1985).

When interpreting the results obtained in the studies one should consider the effect of pregnancy on the level and profile of the melatonin production. It is known from the literature that in the late pregnancy in humans (Kivela 1991; Maggioni et al. 1992), also in cows (Newbold et al. 1991), sheep (Hougton et al. 1993; Lee et al. 1993) and rats (Velasquez et al. 1978), the melatonin level in the blood is higher than in nonpregnant individuals. Most authors report, however, that the circadian profile of secretion of this hormone is constant over the whole pregnancy period. Kivela (1991) is of the opinion that an increase in the melatonin concentration during pregnancy does not result from the increased secreting activity of the pineal gland of foetus. On the other hand, it was confirmed (McMillen et al. 1989) in the studies on pregnant sheep and their foetuses that melatonin being present in the blood of foetuses does not come from their own pineal body but passes with the blood through placenta from their mothers.

In conclusion, the melatonin concentration in the blood of pregnant cows and calves showed highly significant differences ($P < 0.01$) during the light and the dark phase. The profile of the melatonin production in the dark phase varied in the course of ontogeny. The melatonin level in the blood of pregnant cows and calves showed high individual variations during the circadian cycle. The melatonin level in the blood of calves was significantly lower ($P < 0.01$) than in the blood of pregnant cows, both in the light and in the dark phase.

Cirkadiánní změny koncentrace melatoninu v krvi březích krav a telat

Cirkadiánní změny koncentrace melatoninu v krvi březích krav a telat byly sledovány u skupiny březích krav a u 2,5 měsíčních telat. Před pokusem a během pokusu byla zvířata ustájena v prostředí se stejným režimem střídání světla a tmy (LD 10:14). Krevní vzorky byly odebrány z v. jugularis externa 9krát během 24 h, v 15,00; 18,00; 21,00; 24,00; 3,00; 6,00; 9,00; 12,00; a v 15,00 h. Melatonin byl stanoven radioimunoenzymatickou metodou (RIA). Výsledky byly analyzovány pomocí Duncanova D testu.

Vysoce významné rozdíly ($P < 0{,}01$) koncentrace melatoninu byly zaznamenány při střídání světla a tmy u krav i u telat. U telat byla koncentrace tohoto hormonu v krvi v obou fázích významně nižší ($P < 0{,}01$) než u krav. Rovněž se lišila produkce hormonu u krav a telat v noci. Prokázali jsme tak, že syntéza melatoninu a jeho uvolňování ve tmě je ovlivněno věkem.

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