ACTA VET. BRNO 2000, 69: 177–182

MELATONIN CONCENTRATIONS IN THE GASTROINTESTINAL TISSUES OF BOVINE FETUSES

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Received May 2, 2000
Accepted July 27, 2000

Abstract

Bubenik G. A., G.M. Brown, R.R. Hacker, L. Bartoš: *Melatonin Concentrations in the Gastrointestinal Tissues of Bovine Fetuses.* Acta Vet. Brno 2000, 69: 177–182.

Day-time tissue levels of melatonin were determined in the rumen, reticulum, omasum, abomasum, jejunum, ileum and colon of cow fetuses of both sexes. The total body length of the fetuses was 14 to 53 cm which corresponds to the first half of pregnancy; the approximate gestation age was 85-150 days. The data were divided into five equal groups according to fetal length. Average total melatonin concentrations (34.0 to 49.8 pg/g) did not differ greatly between the developmental stages but nevertheless a positive correlation ($r_s = 0.35$, $P < 0.001$) was established between melatonin levels and fetal length. Significantly higher concentrations of melatonin ($P < 0.001$) were found in the male GIT. Finally, colon tissues exhibited higher concentrations of melatonin than any other GIT segment. As the average day-time values in fetal GIT (30.2 pg/g) were substantially lower than GIT levels found in adult cows (50-270 pg/g), it could be hypothesized that melatonin levels in the fetal GIT are derived from the maternal circulation. However, higher melatonin levels in older fetuses, the detection of higher levels in males and substantially higher levels of melatonin in the fetal GIT as compared to blood levels in adult cows, may indicate either an independent fetal production of GIT melatonin or the capacity of fetal GIT to concentrate melatonin derived from the maternal circulation. In view of these discrepancies it can be only concluded that the origin of melatonin in the bovine fetal GIT is at present unknown. The significantly higher concentration of melatonin found in the colon ($P < 0.05$) may be related to the presence of meconium detected in most fetuses in that GIT segment. This finding may indicate that melatonin has a physiological function in the fetal digestive system.

Stomachs, gut, ruminant, indolealkylamine, sex, development, embryo

Melatonin was detected in pineal glands of fetal bovids and a circadian rhythm of melatonin was observed in their plasma (Kennaway et al. 1977; Yellon and Longo 1988; Zemdegs et al. 1988). Because the fetal rhythm follows closely the maternal rhythm and pinealectomy abolished the rhythm and decreased plasma levels to or below the detection limit, it was speculated that melatonin in the fetus is derived entirely from the mother via transplacental transfer (Kennaway et al. 1977; Yallon and Longo 1987: Zemdegs et al. 1988; Okatani et al. 1998). However, because melatonin was also detected in the gastrointestinal tract (GIT) of chicken embryos (Herichová and Zeman 1996), where there is no connection to the maternal circulation, it could be hypothesized, that at least some melatonin found in the mammalian fetal GIT is produced by the fetus. Because of the widespread presence of melatonin binding in fetal tissues (Drew et al. 1997; Williams et al. 1997), it was speculated that the influence of melatonin in the embryo might not be limited to the entrainment of circadian activity (Thomas et al. 1998). As concentrations of melatonin were not yet measured in the fetal digestive system we decided to close this information gap and to determine melatonin levels in all segments of GIT of fetal calves.

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Materials and Methods

Animal tissues

Bovine fetuses were obtained from clinically healthy Canadian Holstein cows slaughtered between 8:00 and 9:00 hr in a local abattoir. The fetuses were donated to us by our colleagues from the Ontario Veterinary College, who investigated the function of corpus luteum in the pregnancy. The ovaries and uteri with fetuses of 35 animals were removed immediately after slaughter, cooled and transported on ice to the University of Guelph. Sex determination revealed that 16 fetuses were males and 16 females. In the 3 smallest fetuses we were unable to determine the sex of the animal. As no differences between melatonin levels of males and females were previously detected (Claypool et al. 1989; Bubenik et al. 1996; Williams et al. 1997), fetuses of both sexes were used in our study. The entire GIT of fetuses was removed within 2 hours of the slaughter, separated into seven segments (rumen, reticulum, omasum, abomasum, jejunum, ileum and colon) and frozen at -20 ºC. The length of fetuses ranged from 14 to 52 cm, which corresponds to days 85-150 of pregnancy. (The full gestation period in cows is approximately 280 days).

Radioimmunoassay

Melatonin levels were determined in segments of the entire digestive tube after washing out any debris accumulated in the lumen. A specific RIA was used after extraction; the assay was validated for monogastric and polygastric ungulates and the details of RIA were described in our previous publications (Brown et al. 1985; Bubenik and Smith 1987; Bubenik et al. 1996).

Statistics

All statistical analyses were performed with the aid of the Statistical Analysis System (SAS). The data were subjected to the General Linear Models Procedure (GLM). The classes were "GIT" (rumen, reticulum, omasum, abomasum, jejunum, ileum and colon), "Sex" (female and male) and "Group" (division based on the length of fetuses in cm: 14-20, 21-27, 28-31, 34-40 and 43-53; the cohort was divided into 5 equal groups of seven animals each). Least square means (LSMEANS) were computed for each class and differences between classes were tested by *t*-test.

Results

Animals

Melatonin was found in all segments of fetal GIT but the earliest trace of meconium was found in the ileum of the fetus at 42 cm of length and in the colon at 38 cm.

Statistic

The GLM model was significant ($F_{(12,244)} = 6.70$, $P < 0.001$). No interactions between classes were found. All three classes were significantly influential: GIT ($F_{(6,244)} = 2.16$, $P < 0.05$), Sex ($F_{(2,44)} = 17.97$, $P < 0.001$), Group ($F_{(4,24)} = 10.80$, $P < 0.001$). Of the GIT segments, the highest melatonin levels were found in the colon (at least $P < 0.05$) (Fig.1), but no other parts of the GIT differed significantly from each other. Male fetuses exhibited substantially higher concentration of melatonin than the female fetuses (Fig. 2). The two

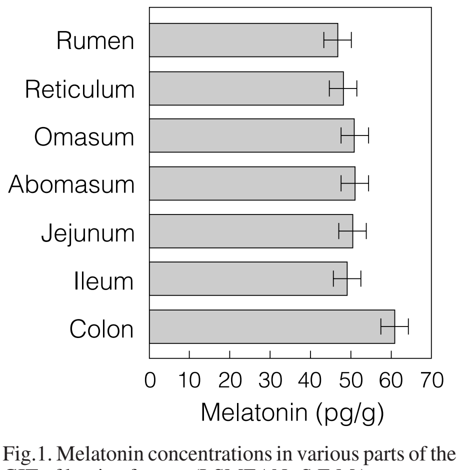

Fig.1. Melatonin concentrations in various parts of the GIT of bovine fetuses (LSMEANs S.E.M.)

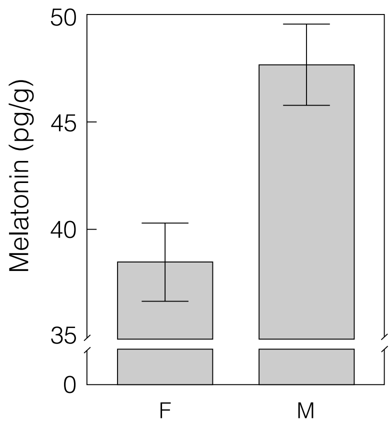

Fig.2. Melatonin concentrations in male and female bovine fetuses of all lengths (LSMEANs S.E.M.).

groups of smallest fetuses (14-20 and 21-27 cm) had also the lowest concentration (differing from all other groups by at least $P > 0.01$). The highest melatonin concentrations were found in fetuses of 28-31 cm in length but this difference did not reach the level of significance (Fig. 3). As we saw the trend to higher melatonin concentrations in larger fetuses, we constructed another GLM model, from which the class Group was removed and another variable "Length" (i.e. original measurement of length of fetuses) was applied. The GLM model was then significant; for melatonin $F_{(8,244)} = 3.99$, $P > 0.001$, for length $F_{(8,244)} = 7.32$, $P > 0.001$).

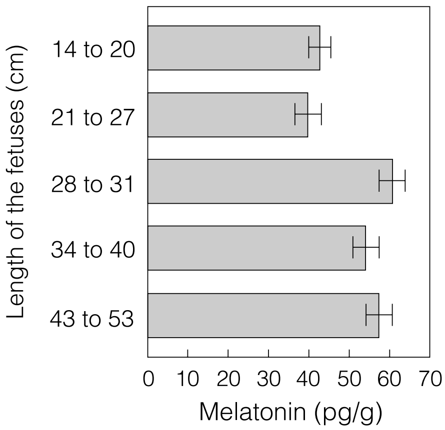

Fig.3. Comparison of melatonin concentrations in five groups of bovine fetuses based on their body length (LSMEANs S.E.M.). (n = 7 in each group)

Since the residual data did not show a normal distribution, we applied a non-parametric test. Residual Spearman correlation showed a significant positive relationship between melatonin levels and the fetal length ($r_s = 0.35$, n = 245, $P > 0.001$).

Discussion

Melatonin was detected in plasma of sheep fetuses (Kennaway et al. 1977; Yellon and Longo 1987; Zemdegs et al. 1988) and melatonin binding was also reported in deer and human fetal tissues (Drew et al. 1997; Williams et al. 1997). In addition, a substantial melatonin binding was found in the mucosa and muscular stroma of the gastrointestinal tract of red deer fetuses. It is significant, that melatonin binding in deer GIT appeared already in fetuses as young as 32 days of gestation (Williams et al. 1997), which was much earlier than the age of our bovine fetuses. (The gestation period in red deer is approximately 232 days, Guinness et al. 1978).

The range of melatonin levels in the fetal GIT tissues established in the present experiment (27-63 pg/g) was higher than blood levels in most bovids reported previously. An average baseline daytime melatonin levels in plasma of prepubertal heifers were around 20 pg/ml (Buchanan et al. 1992) and daytime levels of melatonin in plasma of adult cows were between 5-20 pg/ml (Berthelot et al. 1990; Bubenik et al 1999). Whereas average daytime plasma levels in pregnant sheep were 20-25 pg/ml, plasma levels in fetal lambs were almost twice as high, around 40 pg/ml (Yellon and Longo 1987). In addition our experiment confirmed that generally, daytime melatonin levels are higher in the GIT than corresponding values in plasma (Bubenik et al. 1992; Huether 1994; Bubenik and Brown 1997; Herichová et al. 1998).

Unexplained remains the finding of higher GIT concentrations in male fetuses. As mentioned previously, others studies found no sex differences in adult melatonin levels (Claypool et al.1989; Bubenik et al. 1996; Williams et al. 1997). It is well established that embryonic testes are producing testosterone in early stages of embryogenesis (Lincoln et al. 1973), which may lead to a generally higher weight of male fetuses. Whether this fact was responsible for the sexual difference cannot be answered, as in our study only length but not weight of fetuses was established.

The function of melatonin in fetuses has not yet been clearly elucidated but it was speculated that beside being involved in the regulation of circadian activity, melatonin might be important for the growth and early development and maturation of fetal tissues, perhaps via stimulation of growth hormone secretion (Williams et al. 1997; Beyer et al. 1998).

Similarly to its function in the postnatal period, melatonin may be involved in the early peristalsis of the fetal gut by modulating the intestinal tonus (Bubenik 1986; Bubenik et al. 1992). The significantly higher melatonin concentrations found in the colon may be physiologically important as this part of the GIT was the segment which contained most of the fetal feces, the meconium which moves slowly throughout the fetal GIT. Therefore, it can be hypothesized that melatonin may be utilized in fetal life to prepare the gut for its postnatal function (for a review, see Bubenik 1999). An alternative or supplementary explanation would be that melatonin functions as a scavenger of free radicals (Reiter et al. 1997), which accumulate in the GIT during embryogenesis.

Although the mean levels of melatonin in various segments of the fetal GIT did not differ substantially, nevertheless a significant correlation between fetal length and overall GIT melatonin concentrations was detected. That fact and the findings of significantly higher levels in male fetuses indicate that fetal GIT is capable of an independent melatonin synthesis. An alternative explanation for the detected differences would be a higher binding of melatonin in the colon and in male GIT. In that case, melatonin would be picked up either from the maternal or from the fetal circulation and then concentrated in the GIT. A similar explanation has been also postulated for the circadian variation of melatonin observed in some parts of the adult GIT (Bubenik 1980). However, it has to be pointed out that day-time levels of melatonin in blood of adult cows (5-20 pg/ml) were still substantially lower (Berthelot at al. 1990; Bubenik et al. 1999) than day time levels of GIT melatonin (34-50 pg/g) found in bovine fetuses. On contrary, daytime plasma levels in sheep fetuses were twice as high as melatonin levels in their mothers (Yellon and Longo 1988). These findings may indicate that fetal GIT either produces melatonin in their own tissues or that it concentrates melatonin found in the fetal circulation.

It can be concluded that melatonin was detected in all segments of the fetal GIT of calves sampled during the first half of gestation. In addition more melatonin was found in male fetuses, colon exhibited higher melatonin levels than other GIT segments and a correlation was determined between fetal length and GIT melatonin. These findings may indicate that fetal GIT either produces melatonin in their own tissues or that it concentrates melatonin found in the fetal circulation.

Koncentrace melatoninu v gastrointestinálních tkáních bovinních fétů

Denní koncentrace melatoninu byly stanoveny v rumenu, reticulu, omasu a abomasu, jejunu, ileu a kolonu u fétů skotu obojího pohlaví. Těla fétů měřila 14 až 53 cm, což odpovídá první polovině fetálního vývoje. Přibližný věk fétů byl 85 až 150 dní. Féty byly rozděleny podle délky do pěti stejně velkých skupin. Průměrné celkové koncentrace melatoninu (34,0 až 49,8 pg/g) se mezi vývojovými stádii příliš nelišily, avšak byla prokázána positivní korelace (P < 0.001) mezi délkou fétu a koncentrací melatoninu. Signifikantně vyšší koncentrace melatoninu (P < 0.001) byly zjištěny v gastrointestinálním traktu (GIT) samčích fétů. Ve tkáni kolonu byly zjištěny vyšší koncentrace melatoninu, než ve všech ostatních částech GIT. Protože průměrné denní koncentrace melatoninu v GIT fétů (30,2 pg/g) byly podstatně nižší, než hladiny v GIT u dospělých krav (50 - 270 pg/g), byla vyslovena hypotéza, že melatonin zjištěný u fétů pochází z krevního oběhu matky. Na druhé straně skutečnost, že ve starších fétech byly zjištěny vyšší koncentrace melatoninu, než v mladších, že u samčích fétů byly naměřeny vyšší koncentrace melatoninu než u samičích, a především, že v GIT fétu byly vyšší koncentrace melatoninu, než v krevním oběhu dospělých krav, naznačuje buď nezávislou produkci melatoninu v GIT fétů, nebo schopnost fetálního GIT shromažďovat melatonin získaný z krevního oběhu matky. S ohledem na tyto

rozporné nálezy nemůžeme s určitostí říci, jaký má melatonin v GIT fétů původ. Signifikantně vyšší koncentrace melatoninu stanovené v kolonu ($P < 0,05$) mohou být důsledkem přítomnosti mekonia zjištěného u většiny fétů v příslušné části GIT. Toto zjištění naznačuje, že melatonin má fysiologickou funkci ve fetálním travicím traktu.

Acknowledgements

We would like to acknowledge the exemplary laboratory help of Ali Choudhari and Erika Johannson, the technical help of Greg Humphreys and the clerical help of Yoko Imai.

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