GENETIC VARIABILITY OF THE PATTERN OF NIGHT MELATONIN BLOOD LEVELS IN RELATION TO COAT CHANGES DEVELOPMENT IN RABBITS

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INTRODUCTION

The pineal gland, via melatonin secretion is a key element in the neuro-endocrine control of pelage changes by photoperiodism in various mammalian species including mink, sheep, goat and rabbits (Allain *et al*., 1994) as it is for many other functions including reproduction, regulation of body weight and more generally the control of circadian rhythms (Arendt, 1995). The photoperiodic information received by the retina is transduced into a neuroendocrine signal through the nocturnal duration of the increase in the pineal hormone, melatonin (Malpaux *et al*., 1996). It is well established that blood melatonin levels are highly variable in both duration and amplitude between individuals. Recently, it has been shown in sheep that variability in nightime melatonin plasma levels is under a strong genetic control (Zarazaga *et al*., 1998a) but no similar information is available about the duration of the nocturnal increase of melatonin levels. Furthermore, it is well known that time of coat changes in response to changes in photoperiod are variable among individuals and could be modified by a selection programme in some mammals (Lynch *et al*., 1989). The present study was conduct to assess the degree of genetic contributions of both variability in the nocturnal pattern of melatonin plasma concentrations and photoresponsiveness in coat changes in rex rabbit bred for fur production.

MATERIAL AND METHODS

Animals. This experiment was carried out at the Rex rabbit farm of the Institut National de la Recherche Agronomique, Le Magneraud, BP 52, Surgères, France. Rex rabbits were born between January and mid–September 1998. Animals were maintained under long photoperiods (LD 16:8, light from 4 to 20 h) from birth to 8 weeks of age and then moved to short photoperiods (LD 8:16, light from 8 to 16 h) until the blood sampling procedure which occurred at 12 weeks of age. The Rex rabbit strain is managed in 10 sire reproduction groups composed of 1 male and 10 females. A total of 422 animals of both sexes born from 23 males issued from the 10 sire groups (50 to 55 animals / sire group; 1 to 3 males / sire group; 3 to 40 animals / sire) were used and their genealogy was known over several generations. Animals were housed individually from 8 weeks of age in closed buildings with a natural ventilation and where temperature varied between 15 °C in winter and 25 °C in summer. Animals were fed ad libitum with a complete pelleted commercial food and had free access to water.

Blood sampling and melatonin radioimmunoassay. Animals were distributed into 8 groups of about 50 animals (bleeding group) and blood sampled at 12 weeks of age according to 4

different periods over the year 1998 : mid-March, mid-June, mid-September or mid-December. At each period 2 bleeding groups were sampled at weekly interval. Blood sampling occurred 6 times during the dark phase at 5th (D5), 6th (D6), 7th (D7), 8th (D8), 13th (D13) and 15th (D15) hour after light switch off and one time during the light phase, 1 hour after light on (L1). Blood samples were obtained by venipuncture of the ear artery and were collected under dim red light (<1 lux at 20 cm), avoiding any direct illumination of eyes. Melatonin was assessed by RIA (Fraser *et al*., 1983 ; Tillet *et al*., 1986). The sensitivity of the assay was 4 pg/ml.

Measurement of fur priming on dry pelts. At pelting (18 weeks of age) skins were dried and evaluated for fur priming by determining the ratio of the area of skin blue pigmented to total skin area.

Statistical analysis. A mean mid-night melatonin plasma concentration was assessed for each rabbit from the four samples taken during mid night at hourly interval from 5th to 8th hours after light off. Normalization of the variable rate of fur priming area, ranging from 0 to 1, was performed by using an arcsine transformation. Statistical analysis was performed on these average mid-night concentration as well as on the other blood sample and fur priming ratio. Genetic parameters for each variable (Y) were estimated by using REML VCE, a multivariate restricted maximum likelihood variance component estimation program (Groeneveld, 1997) with an animal model according to the following mixed linear model :

$$\boldsymbol{Y}_{ijklm} = \mu + \boldsymbol{S}_i + \boldsymbol{M}_j + \boldsymbol{BG}_{kj} + \boldsymbol{a}_l + \boldsymbol{c}_l + \boldsymbol{e}_{ijklm}$$

with $\boldsymbol{\mu}$ the overall mean, $\boldsymbol{S}_i$ is the sex effect (2 levels: male or female), $\boldsymbol{M}_j$ the month of the year effect (4 levels: March, June, September or December), $\boldsymbol{BG}_{kj}$ the bleeding group within the month of the year effect (8 levels : 2 bleeding groups per period), $\boldsymbol{a}_l$ a random vector of direct additive genetic effects of animals, $\boldsymbol{c}_l$ a random vector of common environmental or litter effects of animals, and $\boldsymbol{e}_{ijklm}$ a random vector of residuals. Random effects a, c and e were assumed to be normally distributed, $a \sim N(0, A\sigma^2 a)$, $c \sim N(0, I\sigma^2 c)$, $e \sim N(0, I\sigma^2 e)$ where A is the relationship matrix among animals, I is the identity matrix, and $\sigma^2 a$, $\sigma^2 c$ and $\sigma^2 e$ are the additive genetic, common environment and error variance, respectively.

RESULTS

There is a large variability between animals in both mean mid-nigthtime and late dark phase melatonin concentrations (table 1). High concentrations (between 174 to 154 pg/ ml) with a large individual variability were observed around mid-night (from D5 to D8) and until D13 (144 pg / ml). In the late part of the dark phase, a sharp decrease of melatonin concentration was observed with a very large variation between animals. One hour before light switch-on, some animals exhibited high levels similar to those observed at mid-night, while very low levels close to zero and similar to diurnal levels were observed in others.

According to genetic parameter estimates, this variability in the pattern of plasma melatonin concentrations over the dark phase in both amplitude at mid-nigthtime and duration is under a strong to moderate genetic control (table 2). Heritability estimates of plasma melatonin concentrations are moderate to high during the night (from 0.42 at mid-night to 0.11 for D15) and low during the daylight period (0.05). Genetic correlations between melatonin levels at consecutive times are positive high (0.83 to 0.54) but decrease as the interval between

sampling time increases. There were high negative genetic correlations between the rate of fur priming and melatonin levels in the late part of the dark phase or 1 hour after light was on (-0.64 and -0.50 respectively), and they were low or close to zero with melatonin levels at mid-night.

Table 1. Basic statistics of plasma melatonin concentrations at different times over the night and rate of fur priming area at pelting

Variables	N	Mean	Standard deviation
Melatonin concentrations			
D5 (5h after light off)	421	174.7	75.4
D6 (6h after light off	419	171.3	76.9
D7 (7h after light off)	420	161.4	72.2
D8 (8h after light off)	421	154.3	70.7
Mid-nightime (mean D5 to D8)	421	165.5	63.9
D13 (13h after light off)	421	143.9	68.6
D15 (15h after light off)	421	36.1	51.2
L1 (1h after light on)	412	7.3	4.6
Rate of fur priming area (%)	255	0.82	0.16

Table 2. Estimates of heritability (h^2 on bold on diagonal) and genetic correlations (above diagonal) of plasma melatonin levels at different times over the night and rate of fur priming at pelting in Rex rabbits

	Midnight	D13	D15	L1	% fur priming area at pelting
Mid-night level	**0.42**	0.83	0.29	0.04	0.09
D13 level (13 H after light off)		**0.17**	0.70	0.07	-0.26
D15 level (15 H after light off)			**0.13**	0.54	-0.64
L1 level (1h after light on)				**0.05**	-0.50
% fur priming area at pelting					**0.11**

DISCUSSION

Heritability estimate of mid-nightime plasma melatonin concentration is high and very similar to that observed in ewes (Zarazaga *et al*., 1998a), and thus it demonstrated that the large variability in mean melatonin plasma concentrations at mid-nightime is under a strong genetic control in rabbits as it is in sheep (Chemineau *et al*., 1998). It has been also shown in ewes that genetic variability in melatonin concentrations originates in its synthesis, and not in its catabolism (Zarazaga *et al*., 1998b).

More interesting are the moderate heritability estimates of plasma melatonin concentrations in the late part of the night (0.17 and 0.11 at D13 and D15 respectively). Such parameters are indicators of the duration of the nocturnal increase of melatonin as it is well established in most species including rabbit (Young Lai *et al*., 1986) that it begins as soon as light is switched off. Thus the very large variability in melatonin concentrations in the late part of the dark phase observed between animals, indicating that some animals are able to anticipate the end of the dark phase, is under genetic control. To our knowledge, no other report about genetic control of

the duration of plasma melatonin levels during the night in any mammalian species has been published.

Measurement of the rate of fur priming area at pelting is an indicator of the photoresponsiveness of coat changes as animals have been raised under a well defined lighting pattern known to induce coat development changes in rabbits (Vrillon *et al*., 1988). There are no significant genetic relationship between rate of fur priming ratio and melatonin levels at mid-nightime. But strong and negative genetic correlation estimates were observed with melatonin concentrations in the later part of the dark phase or 1 hour after light was switched on. The lower the melatonin levels are, the higher the rate of fur priming area is. Our result first indicated that the main cue of coat changes photoresponsiveness is rather the duration of high nocturnal melatonin levels than levels at mid-nightime and confirmed that photoperiodic information is transduced by the duration of nocturnal increase of plasma melatonin levels (Malpaux *et al*., 1996). Secondly, it appears that under a short photoperiod animals exhibiting a shorter duration of nocturnal high melatonin levels, or able to anticipate the end of the dark phase, would respond better to the photoperiod.

In conclusion, we have demonstrated that i) the high variability of mid-nightime concentration and duration of the nocturnal increase of melatonin blood levels are both under a strong genetic control in rabbits and ii) the duration of the nocturnal plasma melatonin increase seems to be an interesting genetic component of photoresponsiveness in coat changes.

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