Brief report

Melatonin, a candidate signalling molecule for energy sparing

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Homeotherms, including birds living at high latitudes, often encounter simultaneously extremes of cold ambient temperature (Ta), darkness and food scarcity that cause competition for energy resources between the systems for thermoregulation and energy balance. As an adaptation to food scarcity some homeotherms enter into torpor or hibernate for prolonged periods time. Virtually similar adjustments to limited energy resources appear during the circadian cycle. Diurnal animals display a circadian rhythm of body temperature (Tb) exhibiting high Tb during the day and low at night. The magnitude of Tb changes is related to the animal's energy reserves. Typically, diurnal birds exhibit energy sparing at night. Fasted quails, for example, increase the magnitude of nocturnal hypothermia 1°C per day of fasting. After every bout of nocturnal hypothermia, Tb is adjusted to the original high day level of 41–42°C (Hohtola et al. 1991).

Apparently the need for energy sparing follows from inadequacy of food ingestion, since the adjustment of metabolism and body temperature to the proper level is dependent on the amount of food ingested during the day (Rashotte et al. 1993, 1999). Thus, it seems likely that lowering of Tb during inactive phase in diurnal species is a result of controlled integration of photic, thermal and energy balance information leading to optimal adjustments of multiple effectors. The question arises as to how animals are sensing and signalling their energy state to the thermoregulatory system. One suggestion is that animals are able to measure the energy content of food. Another theory is that the digestive load is sensed by gastrointestinal stretch receptors. Also one candidate for such a signal is a hormone or a transmitter secreted from gastrointestinal cells.

Recent studies on the mechanisms involved in sensing the physical information about energy reserves in fasted pigeons indicate that there is an inverse relationship between the volume of the digestive load and the drop of nocturnal Tb (Reinertsen & Bech 1994; Geran & Rashotte 1997). The more non-nutritive cellulose pellets pigeons consume in the immediate preceding light phase the less Tb drops at night. Thus it appears that the stretch of the digestive system, rather than food energy content, provides feedback signal to the thermoregulatory center. However, it still is unclear whether the signal is mediated via the stretch receptors of digestive organs located for example in the crop of the pigeon. Respectively, the firing rates of sensory nerves should turn down when the crop is emptying. Full understanding of neural and functional connections related to gastrointestinal filling and emptying needs further studies.

Our objective in this study is to consider the role of chemical signals as a transducer mediat-

ing information about energy state. For several reasons, the pineal gland and its hormone melatonin is viewed as a candidate signalling molecule for energy sparing (Saarela and Reiter 1994). Firstly, melatonin drives circadian and seasonal rhythms both in birds and mammals. Secondly, plasma melatonin rhythm exhibits a mirror profile for Tb of diurnal animals including a man (Badia et al. 1993). For example timing of rewarming in the morning seems to be coupled to an abrupt decrease of high melatonin levels at this time. Among many other physiological functions of melatonin, a role in gastrointestinal processes has also been suggested after high concentrations of melatonin were found in the pigeon (Vakkuri et al. 1985) and rat (Bubenik et al. 1977) intestine and it was localised immunohistochemically in enterochromaffin cells.

Evidence for melatonin synthesis and a possible existence of enzymatic machinery in enterochromaffin cells is provided by the experiments conducted by Huether et al. (1992) with rats. Melatonin precursor, tryptophan, increased blood melatonin concentration after being given either in food or via i.v. injection. The response was similar with pinealectomized rats. On the other hand, the response to tryptophan was absent in animals whose liver portal vein were dissected. Moreover, melatonin increase was higher after a tryptophan load in the gut (1458% vs. controls) than in the pineal gland (598%) or blood (533%) suggesting that there is a melatonin synthesis machinery in enterochromaffin cells. It has been calculated that rat intestine contains 400 times more melatonin than the rat pineal gland (Huether 1993). Thus it appears that changes in gastrointestinal melatonin are not derived only from the pineal gland. Since about 90% of melatonin is metabolised during primary circulation of liver and secreted by kidneys (Pardridge & Mietus 1980), only a minor amount of melatonin can leak from gastrointestinal tract to systemic circulation.

The function of melatonin as a signalling molecule for energy reserves became more plausible since an increase in the pineal and serum concentrations, and also the pineal N-acetyltransferase (NAT) activity in response to fasting was found in rats (Stokkan et al. 1991). Furthermore, food restriction appears to increase brain, stomach, small intestine and also colon melatonin concentration in the mouse (Bubenik et al. 1992).

We found that Japanese Quails (*Coturnix coturnix japonica*) bred under 12:12 LD exhibit the normal, significantly higher melatonin concentrations both in plasma and gastrointestinal tissues at midnight as compared with those at noon (Table 1). Melatonin concentration in the digestive system was higher in 72 h fasted quails than in controls both at noon and at night (Fig. 1). The small intestine day values were 338 ± 68 pg/g *vs.* 137 ± 7.6 pg/g, and night values $6\ 747 \pm 643$ pg/g *vs.* $1\ 816 \pm 389$ pg/g. Similarly, melatonin night-time concentrations of colon and stomach were significantly higher in fasted birds than in controls, $5\ 157 \pm 422$ pg/g *vs.* $1\ 525 \pm 66$ pg/g and 2001 ± 239 pg/g *vs.* 860 ± 120 pg/g, respectively. Fig. 2 shows that plasma melatonin concentration of control quails was 76 times higher at night than at noon ($P < 0.001$). Night-time melatonin concentration was significantly higher ($P < 0.001$) in fasted quails. Plasma daytime melatonin concentration of fasted quails was 1.7 times higher ($P < 0.05$) than in controls. However, the difference between fasted and control quails was not significant in plasma at night.

Our results with quails confirmed recent findings in the duodenum, ileum and colon of chicks (Herichova et al. 1998) and an earlier report in pigeons (Vakkuri et al. 1985). It appears that the melatonin daily rhythm in the gastrointestinal tract of quails parallels that seen in plasma. Our experiment shows that melatonin is released from

Table 1. Means ± S.E. of melatonin concentration in gastrointestinal tissues and plasma of Japanese Quails.

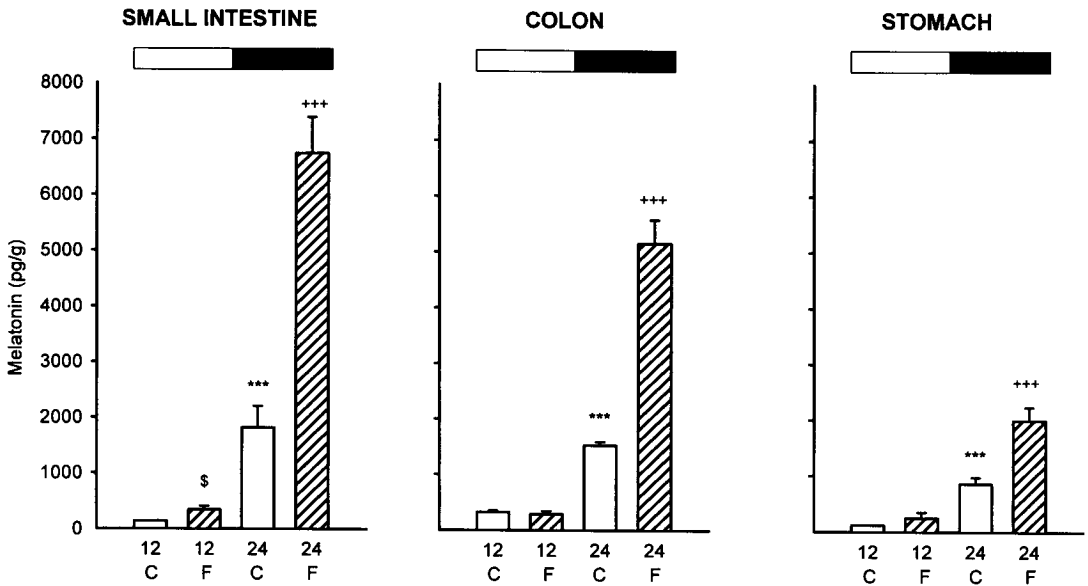|	Small intestine (pg/g)	Large intestine (pg/g)	Stomach (pg/g)	Plasma (ng/l)
Day	137 ± 7.6	324 ± 31	121 ± 11	10 ± 0.6
Night	1816 ± 389	1525 ± 66	860 ± 120	765 ± 99
Ratio	13.3	4.7	7.1	76.5
P <	0.001	0.001	0.001	0.001

SMALL INTESTINE **COLON** **STOMACH**

Fig. 1. Mean (± S.E.) values of melatonin concentration in gastrointestinal tissues of Japanese Quails at midday (12 hours) and at midnight (24 hours). Open columns refer to control quails (C) and shaded to 72 h fasted quails. Day and night are indicated visually by the white and black vertical bars at the top of panels. $ = $p < 0.05$ difference between fasted and control groups at noon 12 hours, +++ = $p < 0.001$ between fasted and control groups at night 24 hours. Statistical differences between day and night in control groups are indicated by *** ($P < 0.001$) and respectively between day and night in fasted groups by £££ ($P < 0.001$). The number of animals in each group was eight (n = 8).

the gastrointestinal tract in rather substantial amounts; under some circumstances the quantity of melatonin released from the gut may exceed that discharged by the pineal gland. For example, small intestine and colon night-time melatonin concentrations were 3.7 and 3.4 times higher in fasted quails (Fig. 1) than in controls but fasting resulted only 1.35 times higher concentration in plasma (Fig. 2). A correlation analysis showed that changes in plasma melatonin concentrations due to pineal and retinal melatonin syntheses and secretion explain by 62% the difference observed in gut and stomach melatonin concentrations between controls and fasted quails. While 32% of changes remained unexplained, melatonin is likely synthesised also in gastrointestinal cells. The opinion is supported by the finding that pinealectomy reduces melatonin levels in the serum but not in the gastrointestinal tract of rats and pigeons (see Bubenik & Brown 1997). Recently, a daily rhythm of melatonin forming enzyme NAT activity was reported also in the duodenum of quails (Hong & Pang 1995).

Caloric restriction is a common feature in animals in the wild particularly in winter at high latitudes. Only the animals that are adapted to reduced caloric intake or endure food shortage survive. Recent findings indicate that food can act as a pacemaker in birds (Rashotte & Stephan 1996). Obviously interactions of multiregulatory systems such as the circadian clock and thermoregulatory center are necessary. We believe that melatonin may act as a transducer and provide a hormonal message from the gastrointestinal tract to the SCN (suprachiasmatic nuclei) and the PoAH (the preoptic area of anterior hypothalamus) to adjust the set point of Tb consistent with the metabolic rate of animal.

Selostus: **Toimiiko pimeähormoni melatoniini energiatasapainosta tiedottavana signaalimolekyylinä?**

Pohjoisilla leveyspiireillä ympärivuotisesti elävien tasalämpöisten eläinten hengissäpysymisen edel-
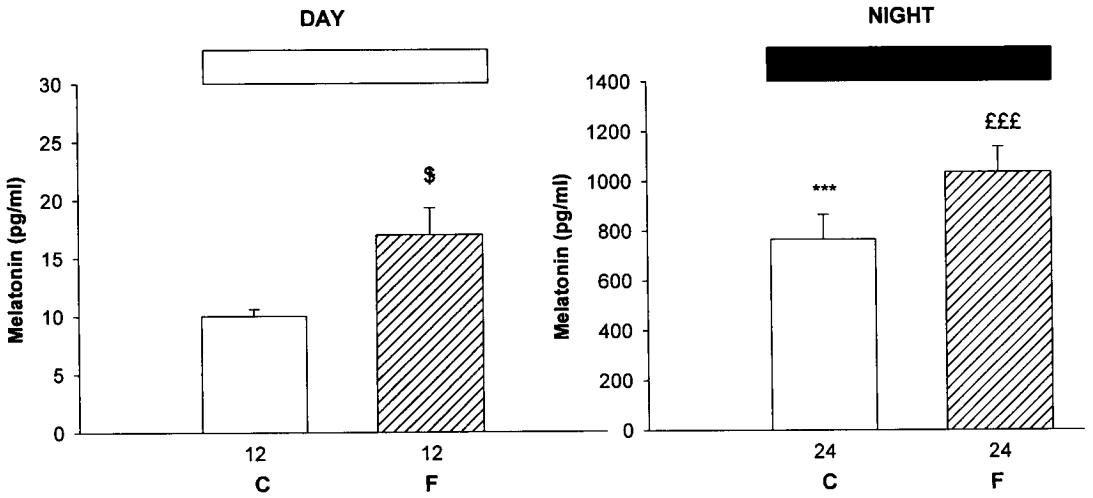
Fig. 2. Mean (S.E.) values of plasma melatonin concentrations of quails at midday (12 hours) and at midnight (24 hours). Open columns refer to control quails (C) and shaded to 72 h fasted quails (F). Statistical difference between day values is indicated by $ (P < 0.05). Significant difference between day and night values of controls is indicated by *** (P < 0.001) and by £££ (P < 0.001) in fasted groups. The number of animals in each group was eight (n = 8). Note that the day and night scales are different.

lytyksenä on, että ne sopeutuvat ravinnon niukkuuteen. Jos ravintoa ei ole saatavilla, on sillä hetkellä elimistössä olevaa energiaa ryhdyttävä säästämään. Kirjoituksessa tarkastellaan, miten valaistus-, lämpötila- ja energiainformaatiota ajatellaan kontrolloitavan lintujen ja nisäkkäiden keskushermostossa ja effektoritasolla. Erityisesti pohditaan sitä, miten energiatason mittaaminen voisi tapahtua elimistössä. Aiemmin esitetyn ruuansulatuskanavan venytysreseptorien välittämän signaalin lisäksi tuodaan esiin toisena vaihtoehtona pimeähormoni melatoniini, joka voisi toimia kemiallisena viestimenä. Kirjoittajat tutkivat valaistuksen ja ravinnon määrän vaikutusta viiriäisen (Coturnix coturnix japonica) plasman ja ruuansulatuskanavan eri osien melatoniinipitoisuuksiin. Viiriäiset jaettiin koe- ja kontrolliryhmiin. Kussakin ryhmässä oli kahdeksan viiriäistä. Viiriäiset lopetettiin keskipäivällä ja keskiyöllä, pimeäjakson keskivaiheilla. Linnuista kerättiin veri talteen ja ruuansulatuskanavasta preparoitiin ohutsuoli, paksusuoli ja lihasmaha talteen melatoniinimäärityksiä varten. Kuva 1 osoittaa, että ruuansulatuskanavan melatoniinitasot olivat keskiyöllä merkitsevästi (P < 0.001) korkeammalla kuin keskipäivällä. Paasto (72 h) nosti jo muutoinkin yöllä koholla olevan ohutsuolen (small intestine), paksu

suolen (colon) ja lihasmahan (stomach) melatoniinipitoisuuksia erittäin merkitsevästi (P < 0.001) verrattuna kontrolliviiriäisiin. Myös plasman melatoniinitaso oli paastonneilla viiriäisillä korkeampi (P < 0.05) kuin kontrolleilla. Korrelaatioanalyysi osoitti, että plasman melatoniinipitoisuuden muutokset, jotka ovat peräisin käpyrauhasen ja retinan hormonierityksestä, selittävät 62% viiriäisen ruuansulatuskanavassa havaituista melatoniinin muutoksista paaston jälkeen. Siis 32% veriplasman pitoisuusvaihtelusta saattaa olla peräisin juuri ruuansulatuskanavan omasta melatoniinierityksestä. Tätä hypoteesia tukee aiempi havainto, että kyyhkyillä ja rotilla käpyrauhasen poisto laskee seerumin mutta ei ruuansulatuskanavan melatoniinin konsentraatiota (Bubenik & Brown 1997) ja että viiriäisen ohutsuolen etuosassa on havaittu melatoniinin synteesiä säätelevän entsyymin aktiivisuudessa vuorokausirytmi (Hong & Pang 1995). Kirjoittajat uskovat, että melatoniini toimii hormonaalisena viestinä ruuansulatuskanavan ja suprakiasmaattisessa tumakkeessa sijaitsevan biologisen kellon välillä, josta on hermostollinen yhteys lämmönsäätelykeskuksen preoptiseen tumakkeeseen. Näiden yhteyksien kautta aineenvaihdunta ja ruumiinlämpötila säätyvät elimistön energiavarantojen mukaiselle tasolle.

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