

Absence of a nocturnal rise in either norepinephrine, *N*-acetyltransferase, hydroxyindole-*O*-methyltransferase or melatonin in the pineal gland of the domestic pig kept under natural environment photoperiods

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Castrated male and intact female pigs were kept under natural photoperiodic and temperature conditions and were killed over a 24 h period in either May (under long days) or in December (under short days). Neither the pineal norepinephrine (NE) concentration nor the melatonin content rose at night: likewise, neither the activities of *N*-acetyltransferase (NAT) nor hydroxyindole-*O*-methyltransferase (HIOMT) increased during darkness. In May pineal melatonin content actually decreased ($P < 0.05$) at night while in December NAT activity fell ($P < 0.05$) at night. Daytime levels of each of these variables were equivalent to those measured in other species. The absence of a nocturnal increase in pineal melatonin production in either May or December raises the possibility that pineal melatonin may be involved in regulating seasonal breeding in the pig in a manner different from other mammals. Alternatively, pineal melatonin production may be unrelated to seasonal reproduction in the pig.

Few reports have examined levels of melatonin in the blood of the wild boar and domestic pig. Kennaway et al. [8] measured melatonin in the plasma of one boar and found that the midnight level was about 3 times higher than the midday value. The photoperiodic conditions under which the boar was kept were not specified. According to McConnell and Ellendorff [10], the plasma melatonin profile in domestic adult German Landrace sows is determined by the photoperiod environment to which the animals are exposed. Thus, under photoperiodic conditions of LD 12:12 they found that 3 of 4 sows exhibited a nighttime rise in plasma melatonin (the increase ranged from 2 to 5-fold). However, when the animals were shifted to a summer photoperiod,

i.e. LD 16:8, the nighttime melatonin surge was abolished in these animals and, furthermore, it could not be reinstated by subsequent exposure of the animals to a winter photoperiod, i.e. LD 8:16. Indeed, in the latter photoperiod regimen the mean nighttime melatonin values (31 pg/ml) were lower than the daytime values (55 pg/ml), although the means did not differ significantly. A similar absence of a nocturnal rise in serum melatonin in prepubertal gilts has been noted by Brandt et al. [3]. When they kept young pigs under artificially regulated short photoperiods (light–dark cycles of 10:14), they found that daytime and nighttime melatonin values were equivalent.

Although pineal melatonin contents have not been reported for the pig, the implication of the study of McConnell and Ellendorff [10] and Brandt et al. [3] is that under photoperiodic regimens other than LD 12:12, the pineal does not produce or secrete melatonin in a rhythmic manner under a light–dark environment.

For the present study, pineal glands were collected from pigs killed over a 24-h period either in May (long days) or December (short days). The pineals of these animals were used to measure the norepinephrine (NE) and melatonin contents as well as the activities of hydroxyindole-*O*-methyltransferase (HIOMT) and *N*-acetyltransferase (NAT) to determine if any of these variables changed as a function of the LD cycle. NE is the neurotransmitter mediating the nocturnal rise in pineal melatonin production in most species [21] while HIOMT and NAT are the enzymes involved in converting serotonin to melatonin [6], a hormone of the pineal gland.

The pigs (*Sus scrofa*) used in this study were crossbreeds (Yorkshire × Landrace); the animals had been weaned at 28 days of age and were killed between 90 and 100 days. The animals had been maintained under natural photoperiods at 35° 45'N latitude (Raleigh, NC) from birth until the experiment was conducted. Because of varying amounts of cloud cover, etc., the intensity of light to which the pigs were exposed during the day varied widely. Minimal environmental temperatures were decreased gradually from about 25°C during the first month after birth to about 20°C at autopsy. Maximal temperatures were allowed to vary with the daily ambient temperatures through ventilators in the building. For other reasons, the males (26 each) had been castrated while the females (20 each) were intact. Each group of animals contained both intact females and castrated males. The pigs were killed by electrocution, without exposure to light during the dark period. As quickly as possible (within 4–5 min) the skull was opened and the pineal gland was dissected and frozen in liquid N₂. Thereafter the glands were transported frozen to San Antonio, TX, for analysis. NE levels were measured by HPLC, NAT and HIOMT activities by radioenzyme assays, and melatonin by radioimmunoassay using the methods of Champney et al. [4]. Protein estimates were made using the technique of Lowry et al. [9]. Pineal levels of the variables measured are expressed relative to protein values.

The first experiment was conducted on May 14–15. Daylength at this time was approximately 14 h; 2 groups of 6 animals each were killed at 10.00 h and 18.00 h (daytime samples), respectively, while 2 other groups (5 each) were killed at 24.00 and 04.00 h (nighttime samples), respectively.

The second experiment was conducted on December 16–17. Daylength at this time

was approximately 10 h; 1 group of 8 pigs was killed at 14.00 h (daytime samples) while 2 other groups (8 each) were killed at 24.00 h and 04.00 h (nighttime samples), respectively. Data were statistically analyzed using an ANOVA and Student–Newman–Keuls test.

In animals killed in May, pineal NE levels exhibited no 24-h rhythm (Fig. 1). Also, neither of the enzymes involved in the conversion of serotonin to melatonin, i.e. NAT and HIOMT, changed as a function of the LD cycle. Finally, pineal melatonin concentrations exhibited a slight, but statistically significant, decrease at night when compared to daytime levels of the indoleamine (Fig. 1).

The results of experiment 2 were similar to those of the initial study. In December when day lengths were short (night lengths were long), pineal NAT values at 24.00 h were significantly depressed compared to values measured at either 14.00 h or 04.00 h (Fig. 2). Pineal melatonin concentrations were equivalent at all time points.

These results clearly show that under either naturally long or short days, none of the constituents normally associated with pineal melatonin production exhibit a nighttime increase. Quite the contrary, nighttime melatonin concentrations (May experiment) and NAT activity (December experiment) were actually diminished

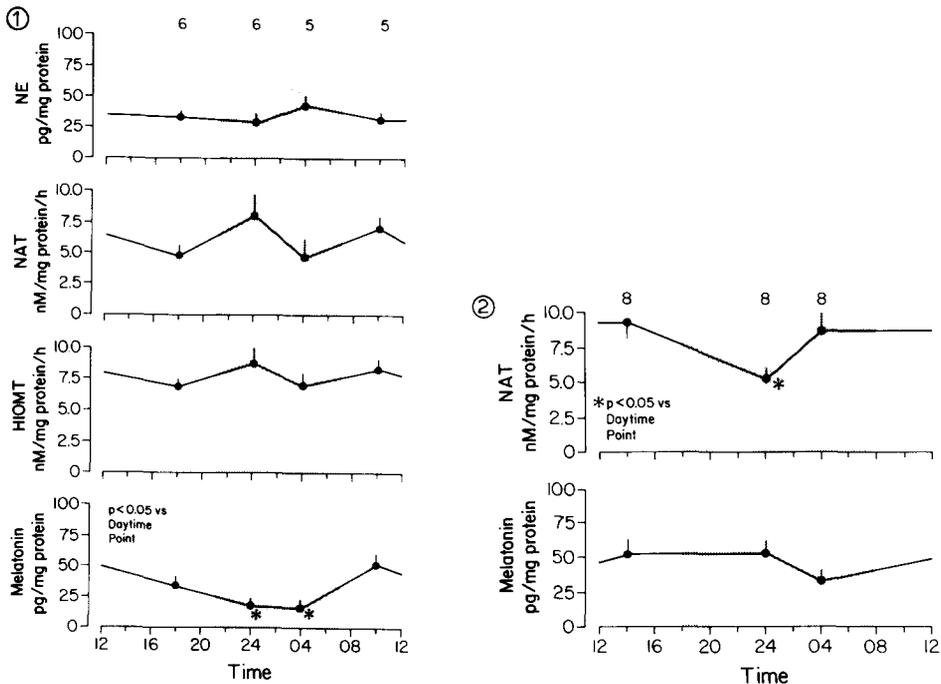


Fig. 1. Pineal NE, NAT, HIOMT and melatonin in the pineal gland of pigs killed throughout a 24-h period in mid-May. Vertical lines associated with the points represent the S.E.M. Group size is indicated by the numbers in the top panel. Shaded area represents the period of darkness.

Fig. 2. Pineal NAT and melatonin in the pineal gland of pigs killed throughout a 24-h period in December. Vertical lines associated with the points represent the S.E.M. Group size is indicated by the numbers in the top panel. Shaded area represents the period of darkness.

slightly. These findings are consistent with the reported lack of a nighttime rise in blood melatonin levels in prepubertal [3] and adult [10] pigs kept under either artificially long or short days and strongly suggest that the failure of plasma melatonin concentration to rise at these times is a direct consequence to the failure of the pineal gland to produce and secrete high nighttime levels of melatonin under these photoperiodic regimens. Interestingly, McConnell and Ellendorff [10] also reported that under LD cycles of either 8:16 or 16:8 plasma melatonin titers were in fact depressed (not statistically significantly so) compared to daytime levels. Likewise, in the present study were observed a statistically verified reduction ($P < 0.05$) in nocturnal pineal melatonin concentrations (May experiment) and NAT activity (December experiment). NAT is usually considered to rate limit melatonin production. Thus, at photoperiods where the ratio of light:dark varies somewhat from 1:1 the pig may actually reduce its pineal melatonin production at night. This is a marked exception to the known increase in pineal melatonin synthesis in many other mammals [16].

The failure of pig pineal NE concentrations to exhibit a 24-h rhythm is not novel. While in the rat [20] pineal NE values rise at night, this is not the case in either the Syrian hamster [12] or the Mongolian gerbil [11] pineal gland. Also, the fact that HIOMT values in the pig pineal did not exhibit a 24-h cycle is not unique inasmuch as the activity of this enzyme typically does not exhibit a rhythm in the pineal gland [6]. NAT activity, however, shows a nocturnal increase in all animals where it has been examined although the magnitude of the 24-h rhythm varies widely [18].

That the male pigs in these experiments had been castrated probably had little effect on the pineal constituents that were measured. In neither the rat [17] nor in the rabbit [2] does castration markedly change the 24-h pattern of melatonin production. Also, each group of pigs in the present study contained both intact females and castrated males; all these animals had similar melatonin levels during the day and at night.

Ebihara et al. [7] have shown that certain inbred strains of mice, e.g. C57BL/J6, lack a 24-h rhythm of pineal melatonin. Furthermore, they found that this lack of rhythmicity was due to a genetic absence of the two enzymes which are involved in converting serotonin to melatonin, i.e. NAT and HIOMT. The situation in the pig pineal gland is quite different, however. NAT and HIOMT are not lacking in the pig pineal gland. Indeed, the daytime activities of these two enzymes in the pineal gland of the pig are equivalent to those in other species; however, NAT simply does not increase during darkness as is the usual case [18].

It should be noted that even though the pig pineal did not exhibit a nighttime rise in melatonin production either in May or December, it is possible that at other times of the year the rhythm is present. Thus, McConnell and Ellendorff [10] found that under artificially regulated LD cycles of 12:12, plasma melatonin levels rose at night. Had the present studies been conducted in either late March or late September (when LD cycles are near 12:12) perhaps an increased production of melatonin at night would have been measured.

Melatonin rhythms have frequently been related to seasonal cycles of reproduction in photoperiodic mammals [14, 15, 19]. The domestic pig is generally found to exhibit a biphasic pattern of seasonal reproduction with reproductive performance being

better during either spring or fall than during the summer and winter [1, 5, 13]. During summer or winter there is a higher rate of anestrus after weaning in sows and puberty is transiently delayed in gilts. Thus, reproductive ability would seem to be greatest at the time when pigs have been reported to exhibit a nighttime pineal and plasma melatonin rise and lowest at the times when a nocturnal melatonin increase is absent. Although much of the early evidence [14, 19] suggested that melatonin was primarily inhibitory to successful reproduction it is now obvious that this hormone is a mediator of the effects of seasonal changes in photoperiod on reproduction and, as a consequence, it is not solely inhibitory or stimulatory to reproduction. Hence, in the pig pineal, melatonin production may be essential for seasonal changes in reproductive capability despite that at certain times of the year there is no nighttime rise in pineal melatonin production and secretion. The pig may be another variation on the theme which links photoperiod, the pineal and seasonal reproduction in mammals. Conversely, it is possible that seasonal reproductive performance in the pig is unrelated to pineal melatonin production.

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