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N-ACETYLTRANSFERASE ACTIVITY, HYDROXYINDOLE-O-METHYLTRANSFERASE ACTIVITY, AND MELATONIN LEVELS IN THE HARDERIAN GLANDS OF THE FEMALE SYRIAN HAMSTER: CHANGES DURING THE LIGHT:DARK CYCLE AND THE EFFECT OF 6-PARACHLOROPHENYLALANINE ADMINISTRATION

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SUMMARY: The activities of NAT and HIOMT and the melatonin content of the Harderian glands of female Syrian hamsters were studied. When hamsters were kept under a light:dark cycle of 14:10 (lights on at 06.00 h), NAT activity exhibited a sharp, short term rise at one hour after lights on. Simultaneously, the activity of HIOMT, which forms melatonin, exhibited a rapid decline. Melatonin levels, like HIOMT activity, also showed a precipitous drop at one hour after light onset. After the respective changes, both NAT and HIOMT activity reverted back to night time levels. Melatonin levels remained depressed for several hours but by 1400 h (8 hours after lights on), nighttime melatonin values were re-established. Treatment of female hamsters with PCPA, a trytophan hydroxylase inhibitor, led to depressed levels of Harderian melatonin without affecting the activities of either NAT or HIOMT.

In rodents, the Harderian gland is a large tubuloalveolar gland which is located in the posteromedial aspect of the orbital cavity (1). Initially, it was presumed to function in lubricating the cornea and the nictitating membrane (2, 3). Recently, several other functions have been proposed for the rodent Harderian gland including the transfer of photic information to the pineal gland of neonatal rats (4), the modulation of reproductive functions (5,6), the production of pheromones affecting aggression and sexual behavior (7,8), and the regulation of the body temperature (9).

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Abbreviations used are: HIOMT, hydroxyindole-O-methyltransferase; NAT, N-acetyltransferase; PCPA, parachlorophenylalamine.

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In Syrian hamsters, the Harderian glands show a remarkable sexual dimorphism. Anatomically, female glands are smaller, pigmented and possess one glandular cell type (5). Male glands, however, are pale in comparison and have two glandular cell types. Biochemically, female glands possess large concentrations of porphyrins as well as high levels of the pineal hormone, melatonin (10). On the other hand, the gland of males contains small amounts of intraluminal porphyrins and low levels of melatonin.

It has been well documented that castration of males converts the glands, both anatomically and biochemically, to the female type (5,6,11,12). The administration of testosterone prevents these changes (5,11). Moreover, androgen receptors have recently been described in the male hamster Harderian gland (13). This fact suggests that the gland may be androgenically regulated (11,14). It has also been proposed that some pineal product(s) also may be directly involved in the conversion of male to female type gland (6,14).

In addition to its presence in the Harderian gland of the Syrian hamster (10,15), melatonin has been reported in the Harderian glands of the rat (16,17) and the Richardson's ground squirrel (18). In the female hamster, Hoffman et al (10) reported a 24 h rhythm in the melatonin content of the Harderian gland with high levels being maintained for most of the 24 h period with a rapid and dramatic drop in the melatonin content of the gland occurring shortly after lights on.

The activities of the enzymes which convert serotonin to melatonin, NAT and HIOMT have never been examined in the Syrian hamster Harderian gland. Thus, studies were undertaken to determine whether changes in the activities of these enzymes in the hamster Harderian gland correlate with the amount of melatonin present.

METHODS

Female Syrian hamsters (75-150g) were purchased from Sasco (Omaha,NE) and housed under a light:dark cycle of 14:10 (lights on daily from 06.00 to 20.00). Animals were housed 4-5 per cage and food and water were provided ad libitum.

Experiment 1. The purpose of the initial study was to verify the marked drop in Harderian gland melatonin in female hamsters shortly after lights as observed by Hoffman et al (10) and, furthermore, to determine the activities of the two enzymes which convert serotonin to melatonin,i.e., NAT and HIOMT, during

the time when pineal melatonin levels supposedly decreased. For this, Harderian glands were collected from female hamsters in the late dark period (03.00 and 06.00h) and at several times during the light phase, begining shortly after lights on (07.00, 08.00, 10.00 and 14.00h). At each collection point, 8 animals were utilized; the animals were killed by decapitation. During the dark phase tissue was collected with the aid of a dim red light (25W tungsten bulb behind a 1A safe light Kodak filter). Harderian glands were removed and immediately frozen in solid CO2. Within 72 hours after collection, tissues were analyzed using radioenzymatic methods for NAT, HIOMT and immunoreactive melatonin according to techniques previously described (19,20). Protein content of the samples were obtained by the method of Lowry et al (21). The enzymatic activities and the immunoreactive melatonin were expressed as pM/mg (HIOMT) or nM/mg of protein (NAT) and pg/mg of protein, respectively.

Experiment 2. This experiment was designed to provide evidence that melatonin may be synthesized within the Harderian glands rather than being taken up from circulation. PCPA is a drug which acts to inhibit the synthesis of serotonin (melatonin precursor) by acting on the enzyme tryptophan hydroxylase. Treatment with PCPA thereby also blocks melatonin production (22). PCPA was prepared and administered according to the method of King et al (23). At 03.00 h late in the dark phase each of 16 hamsters received an intraperitoneal injection of PCPA (125mg/kg body weight). Harderian glands were subsequently collected at 7 hours (10.00h) and 11 hours (14.00h) after the PCPA injection; the glands were processed for their melatonin content and enzyme activities as in experiment 1.

Statistical analysis. Data are expressed as means ± SE and were analyzed using a ANOVA followed by a t-test.

RESULTS

Experiment 1. NAT activity in the Harderian gland was equivalent at both dark time points (03.00 and 06.00h) (Fig.1, top panel). Within 1 hour after lights on (07.00h), NAT activity more than doubled; however, by two hours after lights on (08.00h) NAT activity had returned to basal levels and remained there during the subsequent 2 time points (at 10.00 and 14.00h).

The changes in HIOMT activity followed a pattern inverse to that of NAT activity (Fig. 1, middle panel) . During darkness at 03.00 and 06.00h HIOMT activity was high; however, within an hour after lights on it had plummeted to almost undetectable levels. Shortly thereafter, HIOMT activity rose so that within 2 hours after lights on it had again achieved high activity which was maintained at both 10.00 and 14.00h.

The Harderian gland melatonin pattern was similar to that for HIOMT activity (Fig. 1, botton panel). Again, at night (03.00 and 06.00h) the melatonin content of the Harderian gland was high, averaging 50-55 pg/mg protein. However, the onset of light was associated with a marked drop in the melatonin concentration of the Harderian gland. Thereafter, values began to

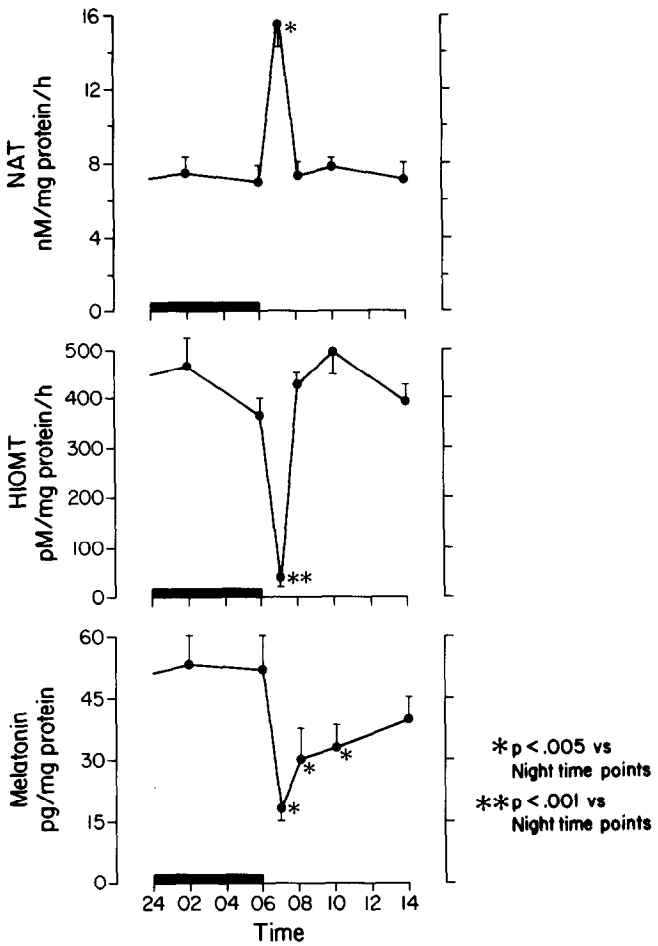

Fig. 1. NAT and HIOMT activities and melatonin concentrations in the Harderian gland of female Syrian hamsters during the night (horizontal black bar) and in the day. Each of the constituents changed dramatically shortly after lights on.

increase and by 14.00h they had again achieved concentrations equivalent to those measured at night.

Experiment 2. Inhibition of tryptophan hydroxylase with PCPA resulted in reduced melatonin levels in the Harderian gland both 7 and 11 hours later (Fig. 2); the drug, however, had no effect on either NAT or HIOMT activities.

DISCUSSION

The present findings confirm the marked drop in melatonin concentration in the female hamster Harderian gland shortly after light onset in the morning as first reported by Hoffman and colleagues(10). The present results extend these findings by showing for the first time that the drop in Harderian gland

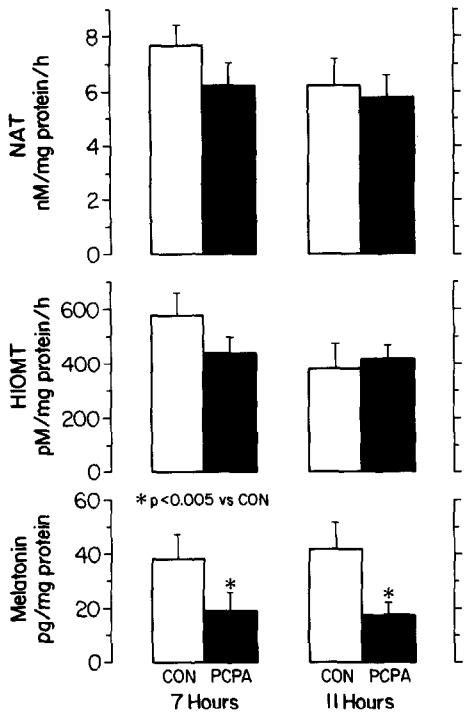

Fig. 2. NAT and HIOMT activities and melatonin concentrations in the Harderian gland of female Syrian hamsters with and without PCPA treatment. The animals were killed at either 7 or 11 hours after PCPA treatment.

melatonin is accompanied by an equally dramatic reduction in the activity of the melatonin forming enzyme, HIOMT, while NAT activity exhibits an unexpected increase at this same time. Clearly, the implications of these findings is that melatonin levels fall because of the drop in the synthesis of the indolamine due to a reduction in HIOMT activity.

HIOMT activity has previously been reported in the rat Harderian gland (24). Likewise, immunoreactive melatonin has been reported in the rat (16,17), Syrian hamster (10,15) and Richardson's ground squirrel (18) Harderians glands. Also in male hamster HIOMT activity has been observed (15) but herein are the first data concerned with NAT and HIOMT activities in the Harderian gland of the female hamster.

Melatonin is a well known pineal secretory product (25). In the pineal gland it is formed from serotonin using enzymes similar to those present in the Harderian gland, i.e., NAT and HIOMT. However, the patterns of NAT and HIOMT activities in the hamster pineal gland are very different than in the Harderian

glands. Hamster pineal NAT is controlled by a mechanism involving the noradrenergic innervation to the gland (26), β-adrenergic receptors on pinealocyte membranes (27), and an intracellular second messenger cyclic AMP (28). Hamster pineal NAT activity exhibits a marked nocturnal increase 6-8 hours after darkness onset (29) and returns to basal levels before lights on. Also, acute light exposure at night causes a precipitous decline in pineal NAT activity (30). In contrast, in the Harderian glands NAT activity exhibits a marked increase shortly after lights on but it remains elevated for only a brief interval. Since the noradrenergic innervation of the Harderian glands ends primarily on the blood vessels within the organ (31) (rather than on the secretory cells), it would seem that NAT activity in the Harderian gland is regulated very differently than in the pineal. Moreover, the number and density of β-receptors in the Harderian gland of the hamster is low (Gonzalez-Brito et al, personal communication) compared to the number in the hamster pineal gland (27).

Hamster pineal HIOMT activity is constant throughout the light:dark period (32). However, in the Harderian gland there is conspicuous reduction in the activity of the methylating enzyme shortly after lights on. As noted above, HIOMT activity has been described in the Harderian glands of rats (24) and male hamsters (15). Harderian HIOMT differs somewhat from that found in the pineal gland; the former has a much higher Km. Considering the parallel drop in HIOMT activity and the melatonin concentration in the female hamster Harderian gland, it seems likely that the methylating enzyme rate limits melatonin formation in this organ.

Our results with female hamster Harderian gland HIOMT differ somewhat from those of Pevet et al (15) in male hamsters. They reported that, in the male, HIOMT activity reached a peak near the end of the daily dark period while in the female hamster HIOMT activity clearly falls after lights on. A possible explanation for these results is the conspicuous morphological (5), and possibly physiological, sexual dimorphism in the Harderian gland of male and female hamsters. In a separate study, however, we have been unable to confirm an

HIOMT rhythm in the Harderian gland of the male hamster (Menendez-Pelaez et al, unpublished results) as observed by Pevet and collegues (15).

PCPA has been successfully used as a specific blocker of melatonin synthesis in hamster pineal gland (23). In the present experiment, a 60% reduction of Harderian gland melatonin in animals treated with the drug was observed while NAT and HIOMT activities were not affected. Melatonin values presumably fell after PCPA treatment because serotonin, the melatonin precursor, is depleted due to its reduced synthesis; thus, even though NAT and HIOMT activities were unaffected reduced amounts of melatonin were formed.

Female hamster Harderian glands possess high levels of both porphyrins (33) and melatonin (10) compared to males. It has been demonstrated that light exposure stimulates porphyrin formation in Harderian glands of castrated male hamsters (34). Also, blinding of castrated male hamsters normally results in the morphological conversion of the Harderian gland to the female type, a response that is prevented by pinealectomy (6). Moreover, removal of the Harderian glands reportedly diminishes the nocturnal rise in melatonin in the hamster pineal gland (26). In view of these findings, further investigations into the bilateral interactions between the pineal and the Harderian gland in the Syrian hamster may prove fruitful.

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