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INDOLE AND PORPHYRIN CONTENT OF THE SYRIAN HAMSTER HARDERIAN GLANDS DURING THE PROESTROUS AND ESTROUS PHASES OF THE ESTROUS CYCLE

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Summary—Porphyrin and indole metabolism was studied in the Harderian glands of Syrian hamsters during the proestrous and estrous stages of the estrous cycle. Porphyrins remained unaltered during these stages, but levels of different indoles (5-hydroxytryptophan, 5-hydroxytryptamine, *N*-acetyl-5-hydroxytryptamine, and 5-hydroxyindole acetic acid) exhibited pronounced changes during the dark:light period in both proestrous and estrous. There was a strong parallelism between 5-hyroxytryptamine, *N*-acetyl-5-hydroxytryptamine and 5-hydroxyindole acetic acid levels. Hydroxytryptophan rhythms appeared slightly shifted from those of the other indoles. Immunoreactive melatonin present in the Harderian glands did not show a significant day–night change during the stages studied.

INTRODUCTION

The Harderian glands have been used as a model for studying porphyrin [1–3] and indole synthesis [4, 5]. In the Syrian hamster, these particular orbital glands show a remarkable sexual dimorphism [6]. Contrary to situation in the glands of the females, the male-type gland contains very low porphyrin [1, 6], melatonin [4, 7] and somatostatin levels [8]. Androgens are probably responsible for these sexual differences since castration converts the male-type gland to the female type [1, 6]. This feminization of the gland can be prevented by the exogenous administration of testosterone [1, 7]. It has been proposed that the androgenic control of the Harderian glands is similar to that of accessory sex glands in which testosterone is converted to 5α-dihydrotestosterone [9, 10]. However, the role of ovarian steroids in the control of the Harderian gland indole metabolism has been poorly defined. Also, there are few studies of Harderian gland porphyrin content during the estrous cycle [11]. Recently, an effect of pituitary hormones on Harderian gland porphyrin synthesis was proposed [3]. The purpose of this work was to study the possible changes in porphyrin and indole metabolism in Harderian glands of female Syrian hamsters during proestrous and estrous phases of the estrous cycle when circulating steroids change markedly.

MATERIALS AND METHODS

Female Syrian hamsters (*Mesocricetus auratus*) were purchased from Sasco (Omaha, Neb.) and housed (4 or 5 per cage) under a light–dark cycle of 14:10 (lights on at 07.00 daily). Food and water was provided *ad libitum*. All animals were monitored for estrous cyclicity according to the method of Orsini [12].

Harderian glands were collected at several times during the proestrous (01.00, 07.00, 09.00, 13.00, 15.00, 16.00, 17.00, 18.00, 19.00 and 21.00 h) and estrous stages (01.00, 07.00, 13.00, 19.00 h). Each group comprised 7–9 animals. During the dark phase, tissues were collected with the aid of a dim red light (25 W Tungsten bulb behind a 1 A safe light Kodak filter). Harderian glands were rapidly removed, weighed, divided into small pieces and immediately frozen in solid CO_2. Total porphyrin concentration was measured by fluorescence spectroscopy according to the procedure described by Buzzell *et al.* [3]. Other pieces were

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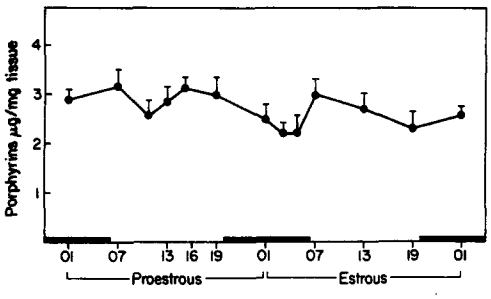

Fig. 1. Porphyrin content in Harderian glands of Syrian hamsters during proestrous and estrous. There are no statistically significant differences between any two points. The dark bars on the horizontal axis indicate the daily dark periods.

processed for HPLC in order to determine the concentrations of 5-hydroxytryptamine (5-HT), *N*-acetyl-5-hydroxytryptamine (NAS), 5-hydroxyindole acetic acid (5-HIAA), and 5-hydroxytryptophan (5-HTP) [13]. Immunoreactive melatonin was determined according to Rollag and Niswender [14]. Protein content was measured using the method of Lowry *et al.* [15]. Results are expressed as μg/mg tissue (porphyrins) or ng/mg protein (indole).

Data are expressed as means $\pm$ SEM. Statistical significance among groups was determined using an analysis of variance followed by the Student–Newman–Kuels test.

RESULTS

The Harderian gland porphyrin content was constant during the proestrous and estrous phases of the estrous cycle (Fig. 1). However, the indole content exhibited substantial changes. A strong correlation between the content of 5-HT, NAS and 5-HIAA was observed (Fig. 2). During proestrous, the 5-HT content of Harderian glands exhibited fluctuations. The highest concentration was observed at 13.00 h, falling 2 h later. A significant increase in 5-HT was detected at 16.00 h. After this rise 5-HT levels dropped to basal levels (0.25 ng/mg protein). During the dark phase between proestrous and estrous, a significant increase in Harderian gland 5-HT content was detected. After a rapid decrease, 5-HT concentrations remained constant during estrus.

The NAS content of the glands ranged from undetectable quantities to 2 ng/mg protein; NAS was highly correlated with changes in its precursor 5-HT (Fig. 2). In some cases the NAS peak was slightly retarded (17.00 h). No differences were observed during the light phase of the day of estrous.

The 5-HIAA content of female Harderian glands was very low during early proestrous.

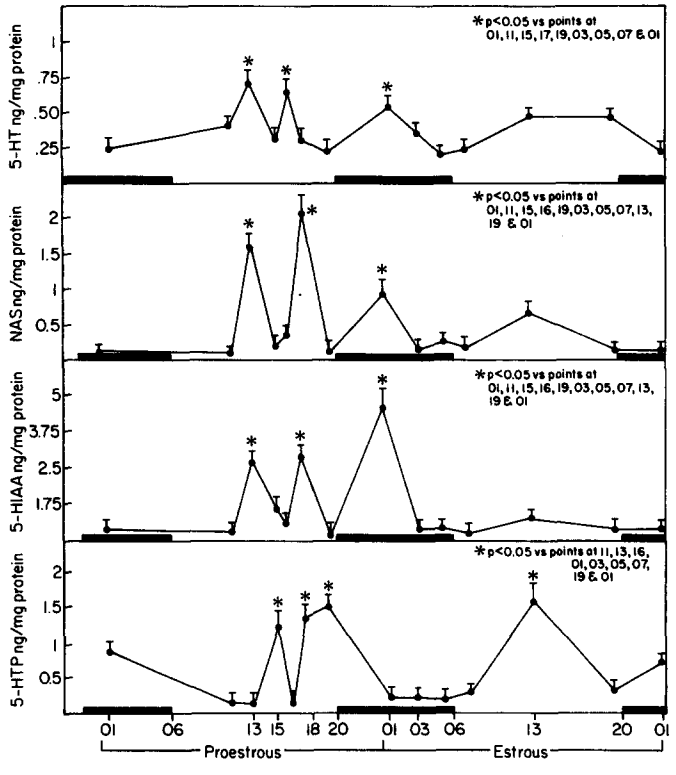

Fig. 2. Fluctuations in 5-hydroxytryptophan (5-HTP), 5-hydroxytryptamine (5-HT), *N*-acetyl-5-hydroxytryptamine (NAS), 5-hydroxyindole acetic acid (5-HIAA) in Harderian glands of Syrian hamsters during proestrous and estrous. The dark bars on the horizontal axis represent the daily dark period. $*P \leqslant 0.005$.

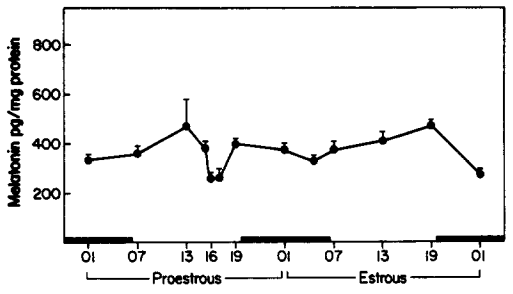

Fig. 3. Immunoreactive melanonin in Harderian glands of Syrian hamsters during proestrous and estrous. The dark bars on the horizontal axis represent the daily dark period. There are no statistically significant differences between any two points.

Early in the afternoon, coinciding with the 5-HT peaks, the 5-HIAA concentrations reached high values (3 ng/mg protein). The highest value was observed in the middle of the dark phase between proestrous and estrous (4.5 ng/mg protein). On the day of estrous, the 5-HIAA values were very low.

The 5-HTP content changed considerably during proestrous and estrous (Fig. 2). Interestingly, 5-HTP peaks appeared slightly shifted from that of 5-HT (15.00, 17.00 and 19.00 h). There was no 5-HTP peak during the dark phase of estrous; 5-HTP levels progressively increased during the light phase reaching the highest values (1.5 ng/ mg protein) when 5-HT and the other indoles analyzed were almost undetectable.

The immunoreactive melatonin content of the glands remained constant throughout the proestrous and estrous stages with concentrations ranging from 275 to 450 pg/mg protein (Fig. 3). Although not statistically significant and coinciding with the drop in the other indoles studied, a decrease in the melatonin content at 16.00 h on the proestrous day was observed.

DISCUSSION

In a previous study, van Jaarsveld *et al.* [16] demonstrated the lack of a dark:light associated rhythm in the porphyrin content of female Syrian hamster Harderian gland. However, in that study the ovarian cycle was not taken into account. Our results, which measured porphyrins during proestrous and estrous phases of estrous cycle, have shown that they do in fact remain unaltered during this 48 h period in spite of marked changes in ovarian steroid secretion during this time. Payne *et al.* [1] have reported differences in the total porphyrin concentration of female hamster Harderian glands during the estrous cycle. However, it is difficult to compare their results with ours. In contrast to our experiment, their animals were exposed to unusual photoperiodic conditions (12 h light, 12 h dark; some of the groups had a reversed lighting regimen). Under these conditions, female Syrian hamsters exhibit short-day induced gonadal regression [17]. They also used a different method for extraction of porphyrins [11]. These factors could be responsible for the enormous individual variations observed in their study. In our experiment, as shown in Fig. 1, the variation was small. There is other evidence suggesting that the porphyrin content of hamster Harderian glands is independent of ovarian steroids. Hence, it is well known that ovariectomy has no measurable effect on the porphyrin content of Harderian glands in this species [6, 9, 18]. Also, the activity of the enzyme aminolevulinate synthetase, which limits porphyrin synthesis, does not fluctuate over the estrous cycle [19].

This is the first report of the indole content of the Harderian glands during the proestrous and estrous stages of the estrous cycle. However, several papers have described day–night differences in the melatonin content of female hamster Harderian glands [4, 5]. In these latter studies the estrous cycle was not taken in account. In the present experiments we were unable to find significant variations in the melatonin content either over the light:dark period or as a function of the estrous cycle. A 5-HT rhythm has been found in the Harderian glands of male Syrian hamsters [20]. In that study, higher 5-HT concentrations were observed during the night than during the day. In the females studied in the present paper, the highest 5-HT levels were found during afternoon of proestrous; during estrous, however, the highest 5-HT concentrations were found during the dark period. The indoles in the Harderian glands of Syrian hamsters seem to be under pituitary hormonal control since, during the gonadotropin surge associated with ovulation (17.00 h, unpublished results), the concentration of all indoles studied fell dramatically. The formation of 5-HT is produced by the action of the enzyme 5-HTP decarboxylase. However, 5-HTP can be used as substrate by the HIOMT producing 5-methoxytryptophan. During estrous, 5-HT levels do not correlate with 5-HTP indicating that this second pathway might be predominant during this stage.

There is an obvious parallelism between 5-HT, NAS and 5-HIAA content. In the pineal gland, 5-HT is converted to NAS by the action of

N-acetyltransferase (NAT); 5-HT is also acted upon by monoamine oxidase (MAO) which converts it to 5-hydroxyindole acetaldehyde (the precursor of 5-HIAA). Both NAT and MAO are found in the Harderian glands of female hamsters [5, 21].

The rodent Harderian gland has been proposed to produce pheromones [22]. Ovarian steroids may play an important role regulating pheromone secretion from the gland. The possible participation of indoles in this process should be investigated. On the other hand a possible endocrine function of the Harderian glands cannot be excluded. Thus, NAS rhythms in the serum of Syrian hamsters are abolished after the surgical removal of the Harderian glands [23].

In conclusion, the indole metabolism of female hamster Harderian gland, contrary to the porphyrogenic activity, seems to be strongly affected by the steroid fluctuations during the ovarian cycle. Also, a possible direct influence of pituitary hormones cannot be excluded.

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