Cell Tissue Res (1983) 229:461–466

Cell and Tissue Research

Short Communication

Depressive effect of LHRH on the numbers of "synaptic" ribbons and spherules in the pineal gland of diestrous rats*

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Summary. Previous studies have shown that LHRH or LHRH-like substances are present in the pineal gland. In order to investigate whether exogenous LHRH may affect the pineal gland, in the present study the effects of a single dose of LHRH (1 μg, i.p.) on pineal "synaptic" ribbons and spherules as well as serum melatonin levels were examined in diestrous Wistar rats. One hour after the injection both ribbons and spherules exhibited a statistically significant decrease in number. Serum melatonin levels were not affected. It is concluded that humoral feedback mechanisms may exist between the hypothalamus and the pineal gland.

Key words: LHRH, effect of – Pineal gland – Synaptic ribbons – Synaptic spherules – Melatonin – Rat

There is evidence that the mammalian pineal gland contains LHRH or LHRH-like substances (White et al. 1974; Pelletier 1976; Wilber et al. 1976; Duraiswami et al. 1976; Millar et al. 1977; Wheaton 1980; Pévet et al. 1980; King and Millar 1981; Piekut and Knigge 1981, 1982; Piekut 1982); however, contradicting results have also been obtained (Gross 1976; Carson et al. 1977; Weindl and Sofroniew 1978). Since immunoreactive LHRH material in the rat pineal gland is preferably localized in profiles near blood vessels, but usually not in perikarya of pinealocytes, one train of thought has been that LHRH may not be synthesized in the pineal but is sequestered from the general blood circulation (Piekut and Knigge 1981, 1982). This assumption is plausible since radioactively labelled LHRH is taken up by the pineal gland (Dupont et al. 1974). In view of possible interrelationships between the pineal gland and the hypothalamus and vice versa, it is of

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* Supported by grant Vo 135/7 within the "Schwerpunktprogramm Neuroendokrinolgoie" of the Deutsche Forschungsgemeinschaft

interest to study whether administration of LHRH has an effect on the pineal gland. In the present investigation the "synaptic" ribbons and spherules of pinealocytes are examined; these elements have been shown to be useful morphological parameters for monitoring intrapineal events (cf. Vollrath 1981).

Materials and methods

In the present study a total of 30 female Wistar (Hannover) rats were kept for 5 weeks under routine laboratory conditions (5 animals per Macrolon® cage; LD 12:12, lights on from 7 a.m. – 7 p.m., 300 lux, 22±2 °C; 50–60% relative humidity; Altromin rat pellets and water ad libitum), during which time daily vaginal smears were taken to select twelve 4-day cyclic rats in the diestrous stage. Diestrous animals were used since in this stage LHRH concentrations are lowest during the estrous cycle. Two groups consisting of six animals each were formed. One group (194±3 SD g b.w.) served as controls and received 0.5 ml physiological saline intraperitoneally 1 h prior to sacrifice; the experimental group (195±6 g b.w.) was injected i.p. with 1 µg LHRH (Sigma) in 0.5 ml physiological saline and was killed 1 h later between 10 a.m. and noon. For technical reasons (i.e. perfusion fixation) the experiments were carried out on two consecutive days (December 8–9, 1981), the same number of control and experimental animals being killed on each day.

Under Nembutal anesthesia, the animals were killed by cardiac perfusion, after 3 to 5 ml of blood had been drawn from the abdominal aorta for melatonin radioimmunoassays (see below). Perfusion fixation was carried out using Karnovsky's (1965) fluid (0.2 M cacodylate buffer, pH 7.2, 4° C). Immediately after perfusion, the pineal gland was excised and postfixed in the same fixative for 1 h and bisected along the midsagittal and midcoronal planes into four pieces of equal sizes. The tissue blocks were postfixed for another hour in the above fixative, postfixed in OsO_4 for 1 h, dehydrated in graded series of acetone, stained in 1% uranyl acetate+1% phosphotungstic acid in 70% acetone overnight, embedded in Epon, and oriented in such a way that frontal sections were cut from the center of three of the four blocks, the left anterior piece being reserved for future studies.

Thin sections were mounted on uncoated copper grids (400 mesh), stained with uranyl acetate and lead citrate and examined in a Zeiss EM 10. For quantitation of the "synaptic" ribbons and spherules (for definition, see Results) from each of the three blocks the section lying closest to the center of the grid was selected and the structures in question were counted in 42 grid holes measuring 45 µm × 45 µm, so that the total area scanned for each animal was 255,150 µm^2. The values obtained were converted to 20000 µm^2 and subjected to the Wilcoxon-Mann-Whitney two sample test (Steel and Torrie 1980) for statistical analysis. A pilot statistical analysis (ANOVA) did not reveal significant differences in the amount of "synaptic" structures between the three regions of the pineal gland. Hence, the figures were pooled.

Serum melatonin was measured by radioimmunoassay (Commentz and Willig 1981), after extraction as described by Arendt et al. (1977). The detection limit of the assay was 13 pg/ml; coefficients of variation were 12.1% (intraassay) and 12.2% (interassay). For the statistical analysis the Wilcoxon-Mann-Whitney two sample test was used (Steel and Torrie 1980).

Results

"Synaptic" ribbons and spherules have been previously described in detail (cf. Vollrath 1981; Karasek and Vollrath 1982). Briefly, "synaptic" ribbons appear as electron-dense rods of up to 1.5 µm in length and 30–40 nm in width, surrounded by mostly electron-lucent vesicles of 30 nm in diameter, whereas spherules are spherical structures measuring 120–180 nm, surrounded by electron-lucent vesicles of 30–40 nm. Both structures are confined to pinealocytes where they are found in both perikarya and processes.

Table 1. Effect of LHRH administration on "synaptic" ribbons and spherules in the rat pineal gland, expressed as mean ± S.E.M./20,000 μm^2

	Total number of "synaptic" structures	Ribbons		Spherules	
		Perikaryon	Processes	Perikaryon	Processes
Controls	21.88 ± 2.88	9.60 ± 1.25	11.90 ± 1.63	0.20 ± 0.05	0.19 ± 0.11
LHRH	12.62 ± 1.01 *	5.18 ± 0.59 **	6.97 ± 0.49 *	0.08 **	–

* $p < 0.05$; ** $p < 0.01$

The quantitative results are given in Table 1. It can be seen that in LHRH-treated rats both ribbons and spherules decrease significantly in number. The ribbons show the same degree of decrease in perikarya and pinealocyte processes.

Lipid droplets, which are a characteristic feature of rat pinealocytes (cf. Vollrath 1981), exhibited a distinct decrease in number and size in the LHRH-treated animals.

Serum melatonin levels were 54.9 ± 7.2 pg/ml in the control and 51.0 ± 3.4 pg/ml in LHRH-treated rats, the differences being non-significant ($p > 0.05$). Correlation studies revealed that the number of "synaptic" structures and serum melatonin levels were positively correlated ($r = 0.9$) in the controls, whereas in the experimental animals there was a slight negative (-0.37) correlation.

Discussion

Pineal "synaptic" ribbons have been frequently investigated; they show characteristic numerical changes under various physiological and experimental conditions. Their numbers are low during the day and high at night (Vollrath 1973; Kurumado and Mori 1977; Theron et al. 1979; King and Dougherty 1980; McNulty 1981; Karasek and Vollrath 1982), paralleling melatonin synthesis in the pineal (Rudeen et al. 1975). The close parallelism between these structures and melatonin formation is corroborated by the findings in the present study showing that in the control animals the number of "synaptic" structures is positively correlated with serum melatonin levels. "Synaptic" ribbons can be easily manipulated and appear to be regulated by β-adrenergic mechanisms (Vollrath and Howe 1976; King and Dougherty 1982a, b). In the pineal, "synaptic" spherules show comparable day/night changes as the ribbons, but there are a number of features that make them appear functionally distinct from the ribbons (Karasek and Vollrath 1982).

The results of the present study show that the administration of LHRH at diestrus leads to a clear depression of the numbers of both "synaptic" ribbons and spherules. The exact mechanism of action of LHRH on the structures in question is not known. As ribbons and spherules are present in equal amounts in male and female rates and as they do not exhibit statistically significant numerical changes during the estrous cycle (Kosaras et al.

1982), it is not likely that female sex steroids play an important role in their regulation. Nevertheless, this aspect should be experimentally clarified.

There is some evidence that a number of proteohormones, glycoprotein-hormones and peptide hormones may have a direct effect on the pineal gland. FSH, LH or prolactin given to castrated rats increases pineal HIOMT activity (Cardinali et al. 1976). Adenohypophyseal tissue (Karasek 1974), HCG or PMSG (Karasek et al. 1978) added to cultured rat pineal glands results in ultrastructural changes suggestive of activation of pinealocytes. Interestingly, the "synaptic" ribbons increased also in amount, although actual counts were not carried out.

The effect of LHRH on the pineal has also been investigated. In vivo, LHRH administration was followed by an enhanced release of vasotocin, which was thought to stem from the pineal gland (Goldstein and Pavel 1977). However, it should be noted that the presence of vasotocin in the pineal gland has been disputed (Pévet et al. 1980; Reiter 1981). Castration followed by LHRH administration leads to a more marked stimulation of pinealocytes than castration alone (Karasek et al. 1976). In vitro LHRH did not lead to clear morphological changes (Karasek et al. 1978). LHRH has been shown to inhibit the noradrenaline-stimulated cAMP accumulation in the rat pineal gland (Tsang and Martin 1976), but was without effect on cAMP in homogenates of human pineal glands (Tsang et al. 1980). As the pineal gland lacks a distinct blood-brain (pineal)-barrier (cf. Vollrath 1981) and as radioactively labelled LHRH accumulates in the pineal (Dupont et al. 1974), it would appear that LHRH when given systemically may well affect the pinealocytes. Further studies are needed to clarify the functional significance of the apparent interrelationships between LHRH and the pineal gland. Perhaps, humoral feedback mechanisms exist between the hypothalamus and the pineal gland.

Acknowledgements: The authors are grateful for the expert technical assistance of Frau Karin Schulze and the secretarial services rendered by Frau Iris Liebthal and Frau Ursula Hulick.

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Accepted November 22, 1982