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The adenohypophysial hormone content of the rat pars tuberalis[1]

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Summary. The pars tuberalis of the hypophysis was shown to contain LH, which increases after castration, TSH and a very low amount of PRL. FSH was found after castration.

Although the adenohypophysial rat pars tuberalis (PT) has been thoroughly studied, its function is not clear. It is mainly composed of chromophobe cells but it also contains follicular cells and a much smaller number of cells similar to the gonadotrophs of the pars distalis (PD)[2-4]. No morphological changes in the chromophobe cells were found after adrenalectomy, castration, thyroidectomy, PTU treatment, osmotic changes or modification of the calcemia[2]. Immunocytochemical studies have only shown cells positive to LH[5] and that these hypertrophy after castration[6]. Besides, hypothalamic extracts that include the PT have shown thyrotrophic activity[7].

The aim of this study was to evaluate whether the rat PT contains immunoassayable LH, FSH, PRL and TSH and compare the content of these with their level in the PD and their concentration in serum, under normal and experimental conditions that modify these hormonal levels.

Male and female adult Holtzman rats, housed under controlled conditions, and weighing 250-350 g were used. The animals were beheaded and trunk blood was collected and allowed to clot at 4 °C. Serum was then separated and frozen at −20 °C until used for hormone determination. The PT and surrounding tissue, including part of the basal hypothalamus, was dissected, taking as limits the optic chiasm, the mammillary bodies and 2 lateral cuts, each at 1 mm from the median eminence. A dorsal cut was performed as proximal to the PT as possible. The weight of the dissected fragment was about 4 mg. In this way, the PT surrounding both the median eminence and the pituitary stalk was taken. The PD was dissected using a different set of surgical instruments to avoid any contamination of the PT with adenohypophysial hormones.

The PT and PD were immediately homogenized in all-glass homogenizers containing 1% BSA in PBS pH 7.6 (50-100 µl/PT; 1000 µl/PD), centrifuged at 3000 rpm for 10 min at 4 °C. The supernatants were removed and kept at −20 °C until assayed. LH, FSH, TSH and PRL were determined by radioimmunoassay according to the NIAMDD 2nd antibody technique using as reference hormones the rat -RP_1 preparations[1].

As in some conditions we found too many values concentrated in the lower tail of the distribution curves, some of which were below the limit of sensitivity of the RIA assay, we could not assume that we had normal distributions. Therefore, we used the non-parametric Mann Whitney U test to analyse the significance of differences between groups, and thus the hormonal levels are expressed as medians.

LH was the gonadotrophin found in the highest level both in the pars tuberalis and pars distalis of female rats. After 2 weeks of castration both values increased significantly. The FSH content in the PT of the normal rat was below the limit of sensitivity for the FSH-RIA in our hands. These values were not modified after 2 weeks of castration, while the content of the PD increased significantly. The serum concentrations of both LH and FSH were significantly increased (table 1). After 3 weeks and 4 months of castration the PT content of FSH was slightly increased, becoming assayable in most cases. The median values found were 19 (n = 8) and 37 (n = 10) ng FSH/PT respectively.

The median TSH content in adult male rats (n = 30) was 237 ng/PT and 1277 µg/PD, while the serum concentration was 1700 ng/ml.

Table 2 shows that the PT of rats separated from their litters for 4 h contains PRL and that its content increases

Table 1. Median gonadotrophin values in the pars tuberalis (PT), pars distalis (PD) and serum in the female rat

	LH			FSH		
	ng/PT	µg/PD	ng/ml serum	ng/PT	µg/PD	ng/ml serum
Control	37 (30)	527 (30)	134 (30)	< 15 (19)	25 (19)	330 (19)
2 weeks castration	91* (30)	1160* (30)	407** (30)	< 15 (20)	99** (10)	1725** (20)

< 15 = lower than the sensitivity of the method. Number of animals in parentheses. * $p < 0.01$; ** $p < 0.001$.

Table 2. Median prolactin values in the pars tuberalis (PT), pars distalis (PD) and serum of the lactating rat

	Prolactin		
	ng/PT	µg/PD	ng/ml serum
4 h pup separation	0.39 (10)	99.72 (10)	32 (10)
4 h pup separation + 30 min suckling	1.14* (10)	153.16 (10)	420** (10)

For symbols see Table 1.

significantly after 30 min of suckling. A significant increase was found in PRL serum concentrations, but not in the PD content.

These results show that the pars tuberalis of the adult female rat contains immunoassayable LH and that the amount increases after castration. Although the FSH content in intact rats was below the sensitivity limits of the method, after castration the level became assayable. Thyrotrophin activity has been described in the rat stalk-median eminence[7], and the activity found was 100 times lower than that in the PD. Our results show that the immunoassayable TSH of the PT is about 5000 times lower than that found in the PD. This small amount of TSH could be due to the relative scarcity of this type of cell in the PT and explains why it could not be detected in rats by immunocytochemical methods[5].

The amounts of LH and FSH found in the PT after castration, as well as the amount of TSH, speak against the possibility of contamination by blood. Although the low amount of PRL found could be due to the blood present in the sample, this is highly improbable. In the former case, the contribution of the blood levels to the hormonal content in the PT would be negligible, even if it were considered that the 4 mg of PT tissue was all blood. In the case of PRL, the hormonal blood levels would only account for the PRL content in the PT if 100% of the tissue were blood, which is far from reality.

In spite of the fact that our PT samples also contain adjacent basal hypothalamic tissue, according to previous immunocytochemical studies[5] we can assume that all the hormones studied are localized within the PT. The total amounts of these hormones were in all cases much lower in the PT than in the PD. Furthermore, to obtain the same concentrations of these hormones in both structures, the PT would have to weigh a maximum of 6 µg. Thus, we can consider that not only the hormonal content but also the concentration of the hormones tested is much lower in the PT than in the PD.

We can therefore conclude that the rat PT contains FSH, LH, PRL and TSH. However, the low hormonal levels found could imply that the PT plays no very important role in the hormonal control of the target organs, except perhaps after hypophysectomy. Nevertheless, other interpretations are at hand. The direction of blood flow in the portal circulation is not yet clearly established, and loops starting in the PT reach the median eminence and return to the PT[8,9]; consequently, we can assume that the PT secretes to the hypothalamus-median eminence (H-ME). Therefore, our hypothesis is that the hormones secreted by the PT reach the hypothalamus-median eminence, where they could modify the release of factors which regulate PT as well as adenohypophysial secretion. Thus, a circuit PT ⇄ H-ME → PD could be present to maintain a basal secretion. Long feedback, starting from the target organs to H-ME, PD or PT could modify this situation to release a greater or smaller amount of adenohypophysial hormones.

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Effect of removal of the Harderian glands on pineal melatonin concentrations in the Syrian hamster[1]

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Summary. Peak melatonin levels which are normally present in male Syrian hamsters at 8 h after the onset of darkness in animals maintained under a light:dark cycle of 14:10, were significantly decreased following the removal of the Harderian glands.

In rodents, the Harderian gland is a large, lobulated, compound tubuloalveolar gland situated within the orbital cavity directly behind the eyes[2]. Although its function remains largely undefined, it has been considered to provide a lipoidal secretion for lubrication of the nictitating membrane[2-4]. Likewise, several investigations suggest that in rats and hamsters the glands may function as a link in a retinal-pineal-gonadal system[5,6]. For example, the Harderian glands have been shown to have a regulatory effect on pineal serotonin and on an enzyme involved in the synthesis of melatonin, hydroxyindole-O-methyltransferase (HIOMT)[5,6]. Pineal synthesized melatonin may normally act as a hormone in mediating gonadal regression in hamsters exposed to restricted photoperiods (< 12.5 h of light/24-h period)[7]. In the following experiment, we tested whether the removal of Harderian glands would have any effect on peak pineal melatonin concentrations in the Syrian hamster.

Materials and methods. Adult male Syrian hamsters, *Mesocricetus auratus*, weighing 80–100 g (Lakeview Hamster Colony, Newfield, N.J.) were housed in polycarbonate cages (6 animals/cage) and were supplied food (Wayne Lab-Blox) and tap water ad libitum. Lights were turned on at 06.00 and off at 20.00 h daily (14 h light and 10 h darkness). 3 days after arrival, hamsters were divided into 2 groups. One group had their Harderian glands removed and the other served as unoperated control animals. 2 weeks after surgery, the hamsters were decapitated at 22.00, 04.00 and 07.00 h and their pineal glands were collected and stored on solid carbon dioxide. Animals sacrificed during the hours of darkness were exposed to a dim red light (25 W tungsten bulb behind a No. 1 A Safe