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EFFECT OF GONADAL STEROIDS ON HYPOTHALAMIC LUTEINIZING HORMONE RELEASING HORMONE (LH-RH) CONTENT IN GONADECTOMIZED RATS

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Summary

The amount of LH-RH in the hypothalamus and serum levels of gonadotrophins in rats under various experimental manipulations were determined by radioimmunoassay. There were no significant changes in hypothalamic LH-RH content during the period of the preovulatory gonadotrophin surge. Orchidectomy or ovariectomy decreased hypothalamic LH-RH content by 75-80% and simultaneously elevated serum levels of LH and FSH. Daily administration of testosterone (100 μg/100 g body weight) or estradiol benzoate (EB; 5 μg/100 g body weight) to orchidectomized rats for 7 days, increased hypothalamic LH-RH content and reduced gonadotrophin release. A single injection of EB (50 μg/rat) to ovariectomized rats increased hypothalamic LH-RH content three days later, while serum levels of LH and FSH fell. Testosterone and progesterone had no such effect. These results suggest a negative feedback action of testosterone and EB on gonadotrophin release via hypothalamic LH-RH release.

Introduction

Gonadal steroids are known to influence the secretion of gonadotrophins, follicle-stimulating hormone (FSH) and luteinizing hormone (LH). The effects of steroids on the secretion of gonadotrophins may be both inhibitory or stimulatory[1-3]. These effects are, although in part exerted on the anterior pituitary[4,5], mainly exerted via the hypothalamus through the release of gonadotrophin releasing hormone or luteinizing hormone releasing hormone (LH-RH)[2,3]. Negative and positive feedback mechanisms, at the level of the hypothalamus, have been shown to be exerted by changes in serum levels of the gonadal steroids[3]. On the other hand, gonadal steroids have been shown to affect the basal secretion of gonadotrophins from the anterior pituitary[4,5] and to moderate the responsiveness of this gland to LH-RH[6,7]. Estrogen and progesterone[8,9] as well as testosterone[10,11] lower serum gonadotrophin levels in ovariectomized rats. However, whether the action of these steroids exerted by effecting hypothalamic LH-RH is still obscure.

Using a specific and sensitive radioimmunoassay for LH-RH[12] the correlation between gonadotrophin release and the amount of LH-RH content in the hypothalamus of proestrous rats has been determined. Moreover the sites of action of various gonodal steroids, estrogen benzoate, testosterone or progesterone on gonadotrophin release in the gonadectomized male and female rats before and after administration of those steroids are demonstrated.

Materials and Methods

Animals and Hormones

Wistar-derived rats were housed in air-conditioned quarters illuminated between 5:00 hours and 19:00 hours. Pelleted food (Ralston Purina Co.) and water were offered without restriction. Vaginal smears were examined daily. Sixty four proestrous rats (three month-old, 180–200 g body weight) were used after completion of two normal four-day-cycles. Twenty one orchidectomized rats (five-month-old, 200–220 g body weight) and thirty five ovirectomized rats (four-month-old, 180–220 g body weight) were used 10 weeks and 8 weeks after operation respectively.

Estradiol benzoate (EB), progesterone or testosterone (Ikapharm, Ramat Gan, Israel) dissolved in peanut oil was subcutaneously administered to castrated animals; controlled rats received peanut oil as specified in Results section.

Orchidectomized rats were treated daily for 7 days with testosterone (100 μg/100 g) or with estradiol benzoate (5 μg/100 g). Animals were killed 6 h after the last injestion. The content of LH-RH in the median eminence (ME) and the preoptic area (PO), as well as serum levels of LH and FSH, were determined by radioimmunoassay.

Ovariectomized rats received a single dose of estradiol benzoate (50 μg), progesterone (25 mg), testosterone (2 mg) or a combination of estradiol benzoate (50 μg) and progesterone (25 mg). Hypothalamus and blood were collected 72 h after injection. The content of LH-RH in the median eminence and in the preoptic area, as well as serum levels of LH and FSH, were determined by radioimmunoassay.

Tissue preparation

Hypothalamus from decapitated rats were excised; the median eminence (medial basal hypothalamus) and the preoptic area of the brains were dissected according to De Groot[13] as shown in Fig. 1. The tissue was boiled for 3 min in 1 ml, 0.01 M phosphate-buffered saline (PBS) pH 6.9, and applied seven strokes in a Thomas teflon homogenizer and then boiled again for another 3 min. The supernatant fraction, collected after 30 min centrifugation at 17,000 × g at 4°C was taken for LH-RH radioimmunoassay.

Radiommunoassay

Blood samples were collected from decapitated rats. The blood was allowed to clot at 4°C for 24 hours. The serum was collected by centrifugation at 1,200 × g for 15 min at 4°C and stored at-20°C until assayed. Serum levels of LH and FSH were determined by radioimmunoassay as described by Daane and Parlow[14] using reagents supplied by the National Institute of Arthritis and Metabolic Diseases (NIAMD), through the courtesy of Dr. A.F. Parlow. The results were expressed in terms of the reference preparations NIAMD-Rat-LH-RP–1 and NIAMD-Rat-FSH-RP–1 respectively.

LH-RH amount was determined by radioimmunoassay described by Koch *et al.*[12]; LH-RH for standard and iodination was generous gift of Hoeschst A.G. (Frankfurt, Germany)

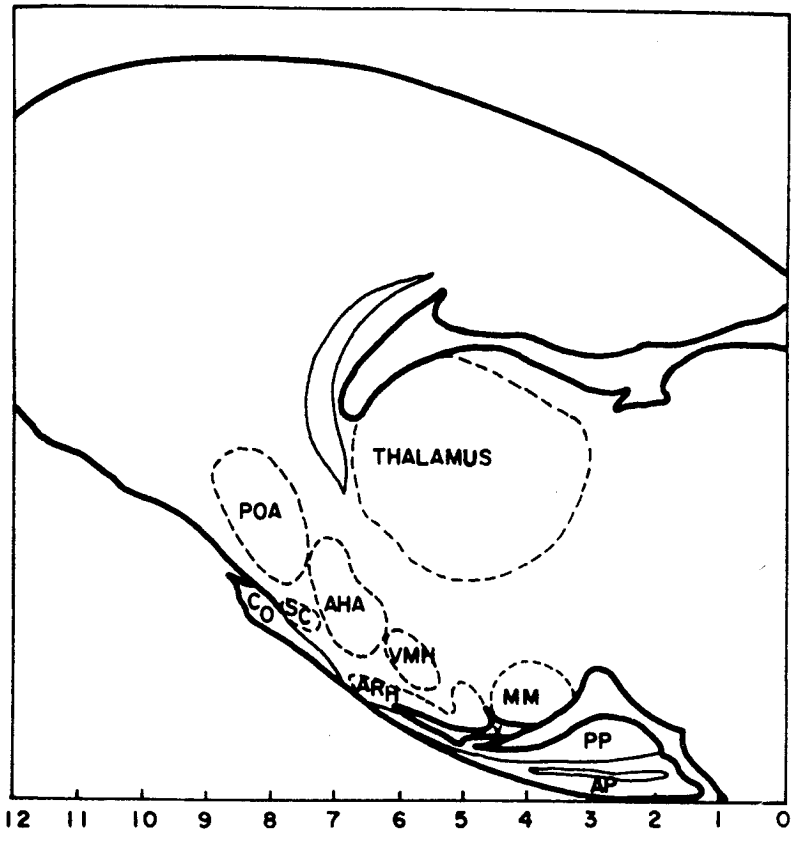

Fig. 1. Midsagittal diagram of the rat brain showing the location of nuclei in the basomedial hypothalamus. The median eminence projects from the basal surface of the brain between A 6.6 and A 4.7 (after De Groot[13]). Abbreviations: AHA, anterior hypothalamic area; AP, anterior lobe of pituitary; ARH, arcuate nucleus; CO, optic chiasma; MM, mammillary nucleus; POA, preoptic area; PP, posterior lobe of pituitary; SC, supraoptic nucleus; V, third ventricle; VMH, ventromedial nucleus.

Results

Hypothalami from proestrous rats were excised and assayed for LH-RH content. No significant changes of LH-RH content in the hypothalamus could be detected during different hours including the period of the preovulatory gonadotrophin surge (18:00 h) as shown in Table I.

Ten weeks after orchidectomy decreased hypothalamic LH-RH content and elevated serum levels of LH and of FSH were observed (Table II). Daily administration of testosterone (100 μg/100 g body weight) for 7 days, increased hypothalamic LH-RH content and reduced LH release. Estradiol benzoate (5 μg/100 g body weight) increased hypothalamic LH-RH content and reduced both LH and FSH release (Table II).

Two months after ovariectomy, the median eminence LH-RH content was decreased by 80%, while the LH-RH content in the preoptic area remained unaffected; concurrently serum levels of LH and of FSH were greatly elevated (Table III). Administration of EB (50 μg/rat) as well as a combination of EB (50 μg/ rat) and progesterone (25 mg/rat), brought about increased concentration of LH-RH both in the median eminence and in the preoptic area whereas serum LH and FSH levels were significantly reduced. Testosterone (2 mg/rat) or progesterone (25 mg/ rat) increased the preoptic LH-RH content only.

TABLE I: THE AMOUNT OF LH-RH IN HYPOTHALAMI OF PROESTROUS RATS.

Time	LH-RH (ng/hypothalamus)
10:00	4.50 ± 0.31^a
12:00	3.70 ± 1.20
14:00	5.18 ± 0.47
15:00	4.65 ± 1.18
16:00	4.41 ± 0.29
17:00	4.30 ± 0.60
18:00	4.88 ± 0.99
20:00	4.18 ± 0.49

[a]Mean value ± SEM, (N = 8).

TABLE II: EFFECT OF TESTOSTERONE OR ESTRADIOL BENZOATE ON HYPOTHALAMIC LH-RH CONTENT AND GONADOTROPHIN RELEASE IN ORCHIDECTOMIZED RATS.

Treatment	LH-RH		LH	FSH
	ng/ME	ng/PO	ng/ml serum	ng/ml serum
Control (0.5 ml peanut oil)	1.52 ± 0.38^a	0.145 ± 0.045	1689.5 ± 336	3155.8 ± 347
Testosterone (100 μg/100 g)	4.00 ± 0.029^b	0.598 ± 0.014^b	198.0 ± 34^b	3508.7 ± 394
EB (5 μg/100 g)	3.58 ± 0.66^b	0.320 ± 0.014^b	107.5 ± 7.2^b	1010.1 ± 95^b
Intact rats	6.13 ± 0.01	0.132 ± 0.026	59.1 ± 3.0	449.2 ± 87

[a]Mean value ± SEM, (N = 7).

[b]Significant difference from control ($p < 0.01$).

TABLE III: EFFECT OF EB, PROGESTERONE AND TESTOSTERONE ON HYPOTHALAMIC LH-RH CONTENT AND GONADOTROPHIN RELEASE IN OVARIECTOMIZED RATS.

Treatment	LH-RH		LH	FSH
	ng/ME	ng/PO	ng/ml serum	ng/ml serum
Control (0.5 ml peanut oil)	1.13 ± 0.22[a]	0.027 ± 0.03	516.0 ± 80	1352.3 ± 104
EB (50 μg)	2.46 ± 0.28[b]	0.709 ± 0.12[b]	154.3 ± 19[b]	772.6 ± 30[b]
EB (50 μg) + progesterone (25 mg)	2.54 ± 0.26[b]	0.413 ± 0.04[b]	169.5 ± 17[b]	784.6 ± 31[b]
Progesterone (25 mg)	1.28 ± 0.24	0.510 ± 0.02[b]	664.8 ± 138	1508.8 ± 149
Testosterone (2 mg)	1.66 ± 0.23	0.442 ± 0.06[b]	412.7 ± 6.2	1400.5 ± 108
Intact rats	5.24 ± 0.58	0.256 ± 0.01	55.3 ± 5.3	348.3 ± 21.2

[a]Mean value ± SEM, (N = 7).

[b]Significant difference from the control ($p < 0.01$).

Discussion

It is generally believed that changes in plasma gonadotrophin, observed during the estrous cycle in rats, are dependent on alteration in the secretory rate of gonadotrophin releasing hormone from the hypothalamus into the hypophysial portal system[15, 16]. It may well be that under condition of vigorous secretion of releasing hormone, its amount in the hypothalamus will be decreased due to insufficient rate of synthesis. Significant changes in the levels of releasing hormone in the hypothalamus, using bioassay, have been reported during the estrous cycle in rats[17, 18]. Kalra *et al.*[16] showed that a rise in LH-RH content preceded the preovulatory gonadotrophin surge in proestrous rats and was followed by a decrease, suggesting that enhanced synthesis of gonadatrophin releasing factors preceded their release during proestrous.

In this experiment, using a specific and sensitive radioimmunoassay system for LH-RH, there was no change in LH-RH content when measured in the whole hypothalamus (Table I), or the median eminence, where the bulk of LH-RH is stored prior to its release, or in preoptic area during the day of proestrous (data not shown). The results obtained can be explained by three different mechanisms: (i) any loss of LH-RH from the hypothalamus is quickly compensated for by an enhanced synthesis: (ii) the preovulatory surge of LH-RH is a relatively small portion (10-20%) of the total hypothalamic content and thus within the error of the assay: (iii) there is no preovulatory surge of LH-RH, but the sensitivity of the anterior pituitary to LH-RH, is enhanced by other factors, e.g. gonadal steroids[4, 6, 7].

The hypothalamic LH-RH content in castrated male rats, 10 weeks after operation, is lower by 75% than in normal intact male rats; concurrently, serum levels of LH and FSH are elevated (Table II). These results could be due to the removal of the negative steroid feedback on the hypothalamus, bringing about increased release of LH-RH from the hypothalamus. Daily administration of testosterone (100 μg/100 g body weight) or of EB (5 μg/100 g body weight) for 7 days increased LH-RH content in the hypothalamus and simultaneously reduced serum revel of LH (Table II).

Thus it seems that administration of testosterone or EB suppresses the release of LH from the anterior pituitary by decreasing hypothalamic LH-RH release. EB, but not testosterone, suppressed serum FSH levels; the reason for this difference is not understood. The higher dose of testosterone required to elicit the same effect as estradiol benzoate (100 μg vs. 5 μg) may be due to the need for this steroid to be converted into estradiol before it can act[19]. Shin *et al.*[20] also reported that hypothalamic LH-RH content was lowered in gonadectomized male rats as compared to intact male rats. However daily administration of 200 μg testosterone for 4 days increased hypothalamic LH-RH, but did not prevent LH release[8]. Nevertheless, it has been established that the elevated gonadotrophin levels following castration can be suppressed by administration of testosterone[10,11,16].

Similarly, ovariectomy elevated serum level of the gonadotrophins and decreased LH-RH content in the median eminence by 80% as compared with control intact rats. After removal of steroid feedback inhibition, the anterior pituitary exhibits enhanced release of LH and FSH, probably provoked by increased secretion of LH-RH from the hypothalamus. A single subcutaneous injection of EB (50 μg) or a combination of EB (50 μg) and progesterone (25 mg) lowered serum levels of LH and FSH while the amount of LH-RH in the hypothalamus was increased. Progesterone by itself had no such effect (Table III). The suppressing effect of estrogen on gonadotrophin release in ovariectomized rats has been also reported by Legan *et al*[8]. (50 μg EB/rat) and by Ramirez and Sawyer (5 μg/100 g)[9]. However, whether the effect of estrogen on LH and FSH release is exerted on the hypothalamic level or at the level of anterior pituitary is still a matter of controversy[3,5,21,22]. These results indicate that at least part of the EB effect is exerted at the level of the hypothalamus.

Several reports claimed to measure serum level of LH-RH in male[23] or in proestrous[24] rats. The serum LH-RH levels detected by these authors were 600 pg/ml and 900 pg/ml respectively. These serum levels of LH-RH seem to be too high in view of the total amount of LH-RH present in the hypothalamus (about 4 ng) and the rapid clearance of LH-RH from the blood (half life of about 2-7 min according to different authors[25,26]. It is not possible to detect LH-RH in blood circulation or even significant changes in hypothalamic LH-RH content during the preovulatory gonadotrophin surge period. Thus it seems that only a small portion of hypothalamic LH-RH need to be released, and due to the small blood flow in the hypothalamic hypophysial portal system, this hormone might reach the pituitary in high concentration.

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บทคัดย่อ

เพื่อศึกษากลไกการหลั่งฮอร์โมนส์โกนาโดโทรพีนส์จากต่อมใต้สมองโดยฮอร์โมนส์สเตอรอยด์จากอวัยวะเพศ ว่ามีผลที่ระดับสมองโดยมีผลต่อการหลั่งฮอร์โมนส์ที่ควบคุมการหลั่งฮอร์โมนส์โกนาโดโทรพีนส์ที่เรียกว่าโกนาโดโทรพีนส์รีลิสซิงฮอร์โมนส์ (LH-RH) หรือไม่ จึงหาความสัมพันธ์ระหว่างจำนวนฮอร์โมนส์ LH-RH ในสมอง ส่วนไฮโปธาลามัส และระดับฮอร์โมนส์โกนาโดโทรพีนส์ทั้ง FSH และ LH ในเลือดของหนูทดลองโดยวิธีเรดิโออิมมูโนแอสเสย์เมื่อหนูเหล่านั้นได้รับฮอร์โมนประเภทสเตอรอยด์ชนิดต่าง ๆ จำนวน LH-RH ในไฮโปธาลามัสของหนูตัวเมียปกติในระยะก่อนการตกไข่ไม่มีการเปลี่ยนแปลงในช่วง

ชั่วโมงที่มีการหลั่งโกนาโดโทรพีนส์มากกว่าปกติ จำนวน LH-RH ในหนูทั้งตัวผู้และตัวเมียที่ถูกตัดอวัยวะเพศออก จะลดลง 75-80% ขณะเดียวกันระดับ FSH และ LH ในเลือดจะสูงขึ้น เมื่อฉีดเทสโตสเตอโรนจำนวน 100 ไมโครกรัมต่อน้ำหนักหนู 100 กรัม หรือเอสตราไดออลเบนโซเอท จำนวน 10 ไมโครกรัมต่อน้ำหนักหนู 100 กรัม ให้กับหนูตัวผู้ที่ตัดอวัยวะเพศออกทุกวันเป็นเวลา 7 วันติดต่อกัน พบว่าจำนวน LH-RH ในไฮโปธาลามัสจะสูงขึ้น ขณะที่ทั้ง FSH และ LH ในเลือดลดลง เมื่อฉีดเอสตราไดออลเบนโซเอท จำนวน 50 ไมโครกรัมให้กับหนูตัวเมียที่ตัดรังไข่ออก จำนวน LH-RH เพิ่มขึ้นและระดับ FSH กับ LH ในเลือดลดลง แต่เมื่อให้เทสโตสเตอโรน หรือโปรเจสเตอโรนจะไม่มีผลต่อระดับ LH-RH, FSH และ LH แต่อย่างใด จากการทดลองครั้งนี้ พอที่จะสรุปได้ว่า ผลของสเตอรอยด์จากอวัยวะเพศต่อการหลั่งโกนาโดโทรพีนส์จากต่อมใต้สมอง มีผลที่ระดับสมองส่วนไฮโปธาลามัส โดยมีผลต่อการหลั่ง LH-RH อีกทีหนึ่ง