Luteinizing hormone-releasing hormone in human pituitary blood

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Luteinizing hormone (LH) and LH-releasing hormone (LHRH) were measured by radioimmunoassay in blood samples collected from the pituitary gland during transsphenoidal surgery in 19 patients. Detectable levels of LHRH were present in 12 patients. Wide fluctuations of LHRH were seen in sequential samples collected at 10-minute intervals, suggesting a pulsatile mode of release. This technique may yield useful data on hypothalamic control of pituitary secretion.

KEY WORDS • pituitary gland • luteinizing hormone-releasing hormone • luteinizing hormone • transsphenoidal surgery • pituitary tumor

HYPOTHALAMIC control of anterior pituitary function is dependent on a unique neurovascular connection, the hypophysial portal system. Hypothalamic releasing hormones, such as luteinizing hormone-releasing hormone (LHRH) have been localized in nerve terminals that abut the primary capillary plexus of the portal system and, through these vessels, reach the secretory cells of the pars distalis.

Recently, methods have been devised for collecting pituitary portal blood in the rat and monkey, and LHRH has indeed been shown to be present in portal blood. In the monkey, the techniques, while useful, have the disadvantages of interrupting the vascular link that controls pituitary secretion, or of removing the pituitary gland, and therefore do not permit the simultaneous study of hypothalamic releasing hormones and their target pituitary hormones. Blood within the pituitary should, however, contain both substances.

In this paper, we report on LHRH and luteinizing hormone (LH) levels in blood collected from the pituitary parenchyma during transsphenoidal surgery in humans.

Materials and Methods

Pituitary blood samples were obtained during transsphenoidal surgery in 19 patients, 11 women and eight men, whose ages ranged from 17 to 68 years (Table 1). All patients had histologically verified pituitary adenomas, except for Cases 10 and 11, and those patients underwent total hypophysectomies for metastatic breast carcinoma. Both of these patients had previously undergone bilateral oophorectomy. Four patients had amenorrhea and galactorrhea, and another four had clinical evidence of acromegaly. Four of the eight male patients had decreased sexual potency.

Radiological evaluation in these patients included angiography and/or pneumoencephalography. Cases 9 and 17 had large suprasellar extension of the tumor, and a small suprasellar component was also demonstrated in Cases 8, 15, 16, 18, and 19. With the exception of a single eosinophilic
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**TABLE 1**

Clinical features and hormonal levels in pituitary and peripheral blood

<table>
<thead>
<tr>
<th>Case No.</th>
<th>Sex, Age (yrs)</th>
<th>Clinical Features</th>
<th>Sample No. (10-min intervals)</th>
<th>Pituitary Blood</th>
<th>Peripheral Blood</th>
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</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>LHRH (pg/ml)</td>
<td>LH (mIU/ml)</td>
</tr>
<tr>
<td>1</td>
<td>F, 30</td>
<td>amenorrhea; galactorrhea</td>
<td>1</td>
<td>60</td>
<td>—</td>
</tr>
<tr>
<td>2</td>
<td>F, 29</td>
<td>amenorrhea; galactorrhea</td>
<td>1</td>
<td>&lt; 12.5</td>
<td>57</td>
</tr>
<tr>
<td>3</td>
<td>F, 25</td>
<td>amenorrhea; galactorrhea</td>
<td>1</td>
<td>1000</td>
<td>237</td>
</tr>
<tr>
<td>4</td>
<td>F, 35</td>
<td>amenorrhea</td>
<td>2</td>
<td>675</td>
<td>—</td>
</tr>
<tr>
<td>5</td>
<td>F, 32</td>
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<td>6</td>
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<td>2</td>
<td>190</td>
<td>&gt; 2000</td>
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<td>2</td>
<td>&lt; 12.5</td>
<td>1898</td>
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<tr>
<td>8</td>
<td>F, 68</td>
<td>postmenopause</td>
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<td>&lt; 12.5</td>
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<td>9</td>
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<td>F, 48</td>
<td>hypophysectomy for cancer; oophorectomy</td>
<td>1</td>
<td>12.5</td>
<td>23</td>
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<tr>
<td>11</td>
<td>F, 30</td>
<td>hypophysectomy for cancer; oophorectomy</td>
<td>1</td>
<td>&lt; 12.5</td>
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<tr>
<td>12</td>
<td>M, 17</td>
<td>—</td>
<td>2</td>
<td>&lt; 12.5</td>
<td>728</td>
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<tr>
<td>13</td>
<td>M, 28</td>
<td>decreased sexual potency; acromegaly</td>
<td>1</td>
<td>115</td>
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<tr>
<td>14</td>
<td>M, 42</td>
<td>acromegaly</td>
<td>2</td>
<td>12.5</td>
<td>13.8</td>
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<td>15</td>
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<td>16</td>
<td>M, 40</td>
<td>—</td>
<td>2</td>
<td>&gt; 2000</td>
<td>338</td>
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<tr>
<td>17</td>
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<tr>
<td>18</td>
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<td>2</td>
<td>&lt; 12.5</td>
<td>1000</td>
</tr>
<tr>
<td>19</td>
<td>M, 63</td>
<td>decreased sexual potency</td>
<td>1</td>
<td>184</td>
<td>98</td>
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<tr>
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<td>M, 63</td>
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<td>3</td>
<td>&lt; 12.5</td>
<td>384</td>
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<tr>
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<td>&lt; 12.5</td>
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<td>19</td>
<td>M, 63</td>
<td>decreased sexual potency</td>
<td>2</td>
<td>&gt; 2000</td>
<td>2340</td>
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</tbody>
</table>

*Normal value = 1–5 mIU/ml; postmenopause and castrate > 15 mIU/ml.
†Normal value = men, 0–20 ng/ml; women, 1–25 ng/ml.

In all, all the tumors proved to be chromophobe adenomas on routine stains.

A detailed endocrine work-up was obtained in all patients. Serum prolactin, growth hormone, luteinizing hormone (LH), follicle-stimulating hormone, thyroxine, and in some cases tri-iodothyronine and testosterone were measured by routine radioimmunoassay. Adrenal function was tested by measuring the serum levels of cortisol before and after adrenocorticotropic hormone (ACTH) stimulation. The urinary excretion of 17-ketogenic steroids and 17-ketosteroids was also measured.

Thyroid and adrenal functions were normal in all patients except for the two who had large suprasellar components (Cases 9 and 17). All four acromegalic patients had elevated basal levels of growth hormone. Prolactin was elevated in eight cases (Table 1). Testosterone was decreased in five of the male patients (Cases 12, 13, 15, 17, and 19).
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A decrease in serum gonadotropin levels was noted in Cases 8, 9, 12, 14, 17, and 18.

Patients were premedicated with Seconal (secobarbital, 100 mg) and atropine (0.5 mg) administered intramuscularly. Operations were performed under general anesthesia (N₂O and oxygen), using the rhinoseptal microsurgical approach. The microsuction apparatus was modified to trap blood into a small heparinized tube, and an attempt was made to keep the cannula within the normal pituitary parenchyma at all times. A second suction device was used to remove blood coming from extrapituitary sources, thus minimizing dilution by systemic blood. Samples of blood, 1 to 2.5 ml each, were collected over a 30-second period, at 10-minute intervals. In 13 patients, it was possible to collect more than one sample without prolonging the surgical procedure. In most instances peripheral blood samples were obtained at the same time. This protocol was approved by the Human Investigation Committee in our institution.

In unextracted serum samples, LHRH was measured by radioimmunoassay* using the method of Nett, et al., as modified by Araki, et al. The synthetic decapeptide was used as reference standard. Human LH† and human prolactin (F₃) were used as reference standards in their respective radioimmunoassays.

**Results**

Data on LHRH and LH levels in both pituitary and peripheral blood samples are shown in Table 1. We detected LHRH in pituitary blood in 12 patients and in 20 of 39 samples tested; the levels ranged from 40 to more than 2000 pg/ml. Curves of serial dilutions of pituitary blood from two patients were found to parallel the standard curve obtained with the synthetic decapetide. In the same patients, LHRH levels in serial pituitary blood samples taken at 10-minute intervals showed wide fluctuations, ranging from more than 2000 pg/ml to undetectable levels. The LHRH values were not measurable in any of the peripheral blood samples tested.

Concentrations of LH in pituitary blood were always higher than in peripheral blood samples obtained simultaneously. Pituitary LH levels also showed marked fluctuation, while in the same patient, the peripheral levels were quite uniform.

An interesting finding was the inverse relationship between simultaneous LHRH and LH levels in many pituitary blood samples, an example of which is illustrated in Fig. 1. Rapid falls in LHRH concentration (for instance, from > 1000 pg/ml to 190 pg/ml) were paralleled by rapid rises in LH (10.2 to > 2000 mIU). Conversely, rises in LHRH (for instance, from 40 to 184 pg/ml) were echoed by falls in LH (from > 2000 to 98 mIU).

**Discussion**

Detectable levels of LHRH were present in pituitary blood samples from 60% of the patients. It is generally accepted that in man most of the blood supply to the adenohypophysis derives from the hypophysial portal system, which in turn originates from the superior hypophysial arteries. Thus, it may be assumed that pituitary levels of LHRH reflect hypothalamic secretory patterns. Mixing with blood from the neurohypophysis may also occur to a variable degree. It is interesting that large concentrations of LHRH detectable by radioimmunoassay have been found in the neurohypophysis of the rhesus monkey.

*Anti-LHRH R-42 pool was kindly supplied by Drs. Nett and Niswender.
†LER-907 human luteinizing hormone is distributed by the Center for Population Research of the National Institute of Child Health and Human Development.

**Fig. 1.** Luteinizing hormone (LH) and LH-releasing hormone (LHRH) levels in pituitary blood samples obtained simultaneously at 10-minute intervals in Case 15.
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monkey, and using specific immunocytochemical methods, fibers containing LHRH have been traced to the pars nervosa.

In the remaining 40% of the patients, LHRH remained undetectable in the pituitary blood. This may be attributed to various factors. First, it is possible that there was dilution from extrapituitary sources. It is unlikely however, that this played a major role, for in all cases the high levels of LH in pituitary blood samples indicate that pituitary sinusoidal blood was indeed being collected. Second, the pituitary blood samples may have been collected in a phase of low secretory output. Indeed, we have previously shown that LHRH is released in a pulsatile fashion into the blood vessels of the pituitary stalk in the rhesus monkey. It is also known that in primates, as well as in other species, LH release is pulsatile. The frequency of the LH pulses varies with the hormonal milieu, and is lower in the absence of estrogens. In this respect, it is of interest to note that in none of the ovariectomized patients were there measurable LHRH levels.

The marked variations in LHRH levels in sequential 10-minute samples in the same patient suggest that in the human LHRH is also released in a pulsatile fashion. Similar oscillations were noted in the levels of LH measured in pituitary samples. An interesting observation is the apparent inverse relationship between LHRH and LH levels in many samples of pituitary blood. Although the number of observations is limited and precise quantitative relationships are difficult to draw under these conditions, the results would be consistent with the hypothesis that a short-loop feedback may intervene in the regulation of these hormones, as proposed by Motta, et al., in the rat. Additional support has come from recent experiments in rabbits and humans in which LH or human chorionic gonadotropin were shown to decrease circulating LH levels.

The collection of pituitary portal blood during surgery may yield useful information on the secretory capacity of the hypothalamus and on hypothalamo-pituitary relationships. Care must be taken however, in interpreting these data. Pituitary tumors may not maintain physiological blood flow or hormonal secretion and both may also be affected by the surgical and anesthesia procedures in progress during the collection.

Nevertheless, this approach allows the study of release patterns of hormones, such as LHRH, which are difficult to assay in peripheral blood samples, and permits the simultaneous measurement of both the pituitary hormones and their hypothalamic releasing factors. Thus, the technique may help to clarify the role of the hypothalamus in the pathogenesis of pituitary tumors.

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References

2. Blake CA: Temporal patterns in plasma luteinizing hormone concentration in ovariectomized rats during different times of the day. Endocrinology 95:813-817, 1974
8. Neill JD, Patton JM, Dailey RA, et al: On the regions of the brain regulating LH secretion in the rhesus monkey. Presented at the Meeting...
of the Endocrine Society, San Francisco, 1976, Abstract No. 59


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