Stimulatory Action of Ovine LH Releasing Factor on Synthesis of Luteinizing Hormone (LH) Demonstrated by the Rat Intratesticular Test System

YOUNG CHIN LIN, MICHIKO TAKAHASHI AND YOSHIHEI SUZUKI
Department of Veterinary Physiology, Faculty of Agriculture, University of Tokyo, Tokyo

Synopsis

Owing to the action of the intratesticular LH release reported in the previous paper (Suzuki et al., 1970), LH content of the rat anterior pituitary implanted into the rat testis rapidly decreased to about 1/100 of the original amount at 25 hr after the implantation. By this time, once highly stimulated testosterone output from the treated testis by the action of released LH, returned to almost the intact level again.

The anterior pituitary of extremely low LH content thus obtained was utilized to see whether LRF induces LH release after promoting de novo synthesis of LH, or not. An ovine LRF preparation was infused for 20 minutes into the testis bearing a pituitary implant from 28 hrs after the pituitary implantation. At the assessment of 2 hrs after the start of infusion, LH content of the implant elevated from nearly zero level to the amount surpassing the pre-implanting level by 40%. And a concomitant maximal increase of testosterone output from the testis bearing the implant was also observed.

At least in the preparation used, it is reasonably concluded that the LRF possesses the dual action of release and synthesis of LH as its primary action.

It is well established that the hypothalamic LH releasing factor (LRF) promotes a rapid release of LH from the anterior pituitary without involving either protein or RNA synthetic processes (see Geschwind, 1970). However, the stimulatory effect of LRF on the de novo synthesis of LH in the gland has also been suggested by several workers (Kobayashi et al., 1966, 1968; Jutisz et al., 1967; Mittler et al., 1970; Suzuki et al., 1970). As is already claimed (Wakabayashi and McCann, 1970), even if the reaccumulation of the hormone in the pituitary takes place after the LRF treatment, whether it is a direct consequence of LRF or the secondary effect to the depletion of LH in the gland, is a matter of unsettled problems.

Recently, the present authors (Suzuki et al., 1970) have found the intratesticular LH release (ITLR) action of rat testes, in which a large amount of LH is released promptly from the rat anterior pituitary implanted in the rat testis, associated with an increase of testosterone output from the testis bearing pituitary. Thus the LH content of the pituitary implant in the testis is expected to decrease to an extremely small amount in a relatively short period. The pituitary of low LH content of this kind seems one of adequate targets to see whether LRF can induce a release of LH after de novo synthesis, or can not induce because of the lack of LH reservoir.

Materials and Methods

Wistar strain rats bred in our laboratory were used throughout the experiments. They were fed on a commercial chow (Japan Clea Co.) and water ad libitum. The adult male rats castrated 2–5 months before and the intact male rats of 5 months old were
used respectively for the donors of anterior pituitaries (AP) and for the recipients of intratesticular treatments. The LRF specimen (18AFS-130) used in the present experiments was kindly endowed by Dr. C. P. Fawcett. This was prepared from the acidic extract of ovine hypothalami followed by successive fractionation and purification by gel filtration, and having the FSH releasing activity in addition to LRF activity.

The methods utilized were almost the same as reported previously (Suzuki et al., 1970). A piece of mid-sagittally sectioned, freshly removed AP was implanted into a testis through a pin-hole made on the albuginous membrane with an aid of a fine glass Pasteur pipette and a plunger. The implanted AP was recovered from the testis 2, 8 or 25 hr after the implantation to trace the effect of the ITLR action. Their LH contents together with the intact AP were assessed by the intrabursal OAAD method (Takahashi and Suzuki, 1968). The AP was made into 1% homogenate with 0.9% saline, and after centrifugation the supernatant, adjusted as equivalent to 1/200 AP to be contained in 0.005 ml, was applied into the ovarian bursa of an adult pseudopregnant rat. The LH content was expressed as either net OAAD % or a term of pituitary equivalent of LH (pit. eq. LH), which was defined as the ratio of LH content against the control (pre-implanting level), calculated by fitting to the dose-response curve prepared by the graded doses of the intact homogenate.

For 1 hr just prior to the withdrawal of the implant, testicular vein blood (TVB) of the testis bearing an AP implant was collected according to the method of Suzuki and Eto (1962). Testosterone was extracted and quantitated as described previously (Suzuki et al., 1970).

Table 1. LH content of intratestricularly implanted pituitary

<table>
<thead>
<tr>
<th>Treatment</th>
<th>No. of assays*</th>
<th>OAAD %**</th>
<th>Pituitary equivalent***</th>
</tr>
</thead>
<tbody>
<tr>
<td>not implanted</td>
<td>8</td>
<td>22.9 ± 0.9§</td>
<td>100 ± 7.8§</td>
</tr>
<tr>
<td>implanted for 2 hrs</td>
<td>7</td>
<td>15.7 ± 0.7</td>
<td>21 ± 5.0</td>
</tr>
<tr>
<td>implanted for 8 hrs</td>
<td>3</td>
<td>8.9 ± 1.8</td>
<td>4.5 ± 1.0</td>
</tr>
<tr>
<td>implanted for 25 hrs</td>
<td>4</td>
<td>2.7 ± 0.3</td>
<td>1.2 ± 0.2</td>
</tr>
<tr>
<td>implanted for 30 hrs and LRF infusion</td>
<td>3</td>
<td>24.5 ± 1.8</td>
<td>141 ± 22</td>
</tr>
</tbody>
</table>

* 5-6 animals were used for each assay.
** 1/200 of pituitary was assayed.
*** The intact value, equivalent to 16 µg NIH-LH-S15/gland, was designated as 100.
§ mean ± s.e.

The LRF effect upon the implanted AP was started to investigate at 28 hr after the implantation. The LRF, equivalent to one ovine hypothalamus was dissolved in 0.1 ml of saline solution, and was infused for 20 min into the testis. For the infusion, a fine polyethylene tubing attached to the syringe of a micro-infusion device was introduced into the testis through a pin-hole which had already been made for the implantation. The tip of tubing was placed near to the implant. From 29 hr after the implantation, TVB was collected for 1 hr. The implant was recovered at the end of the blood collection, that is, 30 hr after the implantation. The LRF solution in the same volume was infused into the testis without AP implant to see the direct effect of this preparation upon the output of testosterone.

Results

The change of LH content of the intratesticularly implanted AP was traced temporally as shown in Figure 1 and Table 1. Only by 2 hr of the implantation, the LH content...
decreased extremely as reported previously (Suzuki et al., 1970). Further decrease was observed with the lapse of time, and by 25 hrs of the implantation the LH content declined to about 1/100 of the pre-implanting level.

Testosterone secretion of the testis bearing AP implant was stimulated corresponding to the decrease of the LH content. The testosterone concentration increased significantly and reached at the maximum by 7-8 hr after the implantation somewhat lagging from the rapid decrease of LH content. It, then decreased again at 24-25 hr of the implantation and showed a slightly higher level than that of the intact animal (Fig. 1, Table 2).

Thus, at the end of one day's implantation, almost all of LH reservoir in the AP implant had been released, and even the stimulatory effect on the testosterone output evoked by the intratesticularly released LH mostly disappeared. The LRF activity was, thence investigated using this condition. The infusion of LRF brought about a marked increase of LH content in the AP implant; the assessment of 2 hrs after the start of LRF infusion revealed that the LH content elevated from nearly zero up to the amount surpassing even the pre-implanting level by 40% as seen in Figure 2. A concommitant maximal increase of testosterone output from the testis bearing the AP implant was also observed.

The LRF infusion into a testis bearing no AP implant, for the control experiment, did not give any significant effect upon the testosterone output (Table 2).

**Discussion**

As previously reported (Suzuki et al., 1970) the internal milieu of rat testis induced a striking LH release from the rat pituitary (ITLR action). This action continued to reduce the LH content and after 25 hr implantation only 1/100 of the original amount of LH was left in AP. Testosterone secretion was also highly stimulated by the LH released from the AP implant. But it was noteworthy that the stimulatory effect did not continue for 25 hr. Since intratesticular injection of only 5 ng of LH (NIH-LH-S15) caused a significant increase of testosterone output in 2 hr (Suzuki et al., 1970), this disappearance of stimulated pattern of testosterone output here observed, indicates that the LH release has
ceased at latest before 25 hr of the implantation. Therefore, it is assumed that the ITLR action on the rat pituitary is promoting only the releasing process of LH, so that the change in LH content of AP implant shown in Figure 1 is regarded as the direct reflection of the release of LH. If the synthetizing activity of LH in the pituitary tissue is merely the secondary event successive to the decrease of LH reservoir in the pituitary, as is claimed by Wakabayashi and McCann (1970), why the the synthesis of LH had not occurred in this case? Though the time factor must be taken into consideration, of course, our data indicate that the synthesis of LH does not necessarily take place after a marked release of LH from the pituitary.

The results obtained in the LRF infusion experiment clearly demonstrate that the LRF used has LH synthetizing activity as a primary action. Because the LRF is capable of raising nealy zero level of LH content in AP beyond the intact level only in 2 hr.

In this connection, several workers, as mentioned before, have suggested a promoting effect of LRF on de novo synthesis of LH in addition to the release of LH. However, the matter seems not to be settled. Samli and Geschwind (1967) have failed to demonstrate the LH synthetizing effect by hypothalamic extract in in vitro study of incorporation of labeled amino acid and hexosamine into LH. Recently Wakabayashi and McCann (1970) have investigated the LRF activity in vitro, using a low LH content AP of castrated rats pretreated with testosterone propionate (TP) for 2–3 weeks, and found none of appreciable effect on the total amount of LH (LH in medium plus in AP). They, therefore, have concluded that de novo synthesis of the hormone is only the secondary effect. Though there is a possibility that long and high dose treatment of TP would cause a change in receptivity of AP to LRF, they also examine the short and low dose of TP treatment and fail to show the LH synthetizing activity of LRF.

In our experiment, on the other hand, AP was put in the milieu of rather high concentration of testosterone secreted from the adjacent interstitial cells of testis. Yet, LRF caused a highly significant and rapid increase of LH content, that is, de novo synthesis of LH. The discrepancy found in the effect of LRF would not be reconciled merely by the effect of testosterone, but the different test systems utilized would be one of causes.

Simultaneously with the raise of LH content in AP implant, a marked raise of testosterone concentration in the effluent blood from the testis bearing AP implant was induced by LRF infusion, indicating the release of LH. Since no release of LH by ITLR action became observable before the infusion of LRF probably owing to the lack of LH reservoir in AP, no more release of LH would not be expected unless de novo synthesis of LH occurred. Therefore, this raise of testosterone after the infusion of LRF also provides a strong evidence for the stimulated synthesis of LH by LRF. The release of LH in this case may be brought about by a combined effect of ITLR action in the testis and the releasing action of the infused LRF.

At least in the LRF preparation we used, it is likely that LRF possesses the dual action of release and synthesis of LH as its primary action.

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