Changes of Serum and Pituitary TSH, LH and FSH Concentrations Following the Slow Infusion of TRH and LRH*

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Synopsis

Wistar-Imamichi male rats were slowly infused with synthetic TRH (T), LRH(L) and T+L in combination for of 1, 3, 24, 48 and 72 hr. Doses of the releasing hormones were 1 μg/hr. For each duration of infusion, pituitary and serum TSH, LH and FSH concentrations were radioimmunoassayed. When T is infused, the pituitary TSH concentration increases at 72 hr. When L is infused, it increases at 48 hr but decreases at 72 hr. When T and L are combinely infused, it decreases at 24 hr, but increases at 72 hr. The serum TSH concentration increases at all the durations of T infusion, but is not changed after the L infusion. The T+L infusion results in a rise of the serum TSH concentration at 1, 24 and 72 hr. The pituitary LH concentration increases at 48 and 72 hr of the T infusion, but decreases for the whole duration of the L infusion. The T+L infusion increases the pituitary LH concentration at 24 and 48 hr. The serum LH concentration does not tend to rise after the T infusion, but increases at 24, 48 hr after the L infusion. The T+L infusion increases less progressively the serum LH concentration at 24, 48 and 72 hr than the L infusion at the corresponding hours. The pituitary FSH concentration declines with the prolongation of T infusion, and a tendency of slight decrease is observed after the L and T+L infusions. It is postulated from these results that T may be either an inhibitor to the FSH-release or a stimulator to the pituitary LH-synthesis. It is also speculated that T may act antagonistically upon the releasing effect of L, and that L may suppress the FSH-release.

Needless to say, the secretion of TSH and LH is under the control of TRH and LRH, respectively. It has been generally accepted that the target cell of TRH or LRH is independent from each other, and that TSH and LH are secreted by TSH-cells and LH-cells, respectively. Arimura et al. (1972), Redding and Schally (1972), Redding et al. (1972), Shiino et al. (1972), Schally et al. (1972) and Mendoza et al. (1973) established a concept of LH/FSH-releasing hormone, based on the radioimmunoassay and morphological evidence that the chronic injections or slow infusion with synthetic LRH caused the hypersecretion of FSH. The previous immunohistochemistry demonstrated that the same basophil was stained with the antibodies to LH and FSH (Nakane, 1970; Phifer et al., 1973; Phifer and Spicer, 1973; Robyn et al., 1973) and that the basophils with the characteristic features as the LH- and FSH-gonadotrophs were stained with anti-β TSH (Tougar et al., 1973; Moriarty, 1975 and

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Materials and Methods

Slender polyethylene cannula was inserted into the femoral vein of the Wistar-Imamichi male rats of 60 days of age, and then made operatively to pass straight through the subcutaneous tissues of dorsal skin to prevent its translocation. The other end of the cannula free from the neck was connected with the syringe set up in the slow infusion instrument (Natsume Seisakusho Co., Ltd., Tokyo). The free part of cannula was covered with the steel spring wire to prevent the bite of a rat which was allowed to move freely in the cage to avoid the stress. Immediately after cannulation, the infusion was begun. The animals were divided into four groups consisting of (1) those treated with synthetic TRH (supplied from Protein Institute of Osaka Univ.), (2) those with synthetic LRH (Dai-ichi Seiyaku Co., Ltd., Tokyo), (3) those with am ixture of TRH and LRH, and (4) those with saline as the control.

Dose of T and L was 1 µg/hr dissolved in physiological saline. A mixture for the combined infusion consisted of 1 µg of each RH. The solution was slowly infused at a speed of 0.0344 ml/hr. The control rats were infused with saline for 1, 3, 24 and 72 hr. The subgroups receiving the infusion of each hormone or saline for 1, 3, 24, 48 and 72 hr, consisted of 5–7 rats. After infusion, they were laparotomized under urethan anesthesia, and blood samples were collected from the abdominal aorta, allowed to clot at a room temperature and then centrifuged. The whole pituitary glands were immediately removed and weighed. They were cut into halves medially with a razor blade. A half of the gland was homogenized in phosphate buffer saline (pH 7.5). The sera and pituitary extracts were stored at −80°C until the radioimmunoassay. LH, FSH and TSH concentrations were determined using NIAMDD radioimmunoassay kits supplied by Rat Pituitary Hormone Program which consisted of NIAMDD rat LH I-4, anti-rat LH S-3, rat LH RP-1, rat FSH I-3, anti-rat FSH S-6, rat FSH RP-1, rat TSH I-2, anti-rat TSH S-3 and rat TSH RP-1 according to the method of Daane and Parlow (1971) slightly modified by Wakabayashi et al. (1972). Assay results are expressed as the equivalent of NIH-LH-S, or NIH-FSH-S, for LH or FSH, respectively, and as the amount of NIAMDD-rat TSH RP-1 for TSH.

Results

Pituitary weight

Pituitary wet weights of all the groups which received TRH (T), LRH (L) and T +L in combination treatment showed no significant changes, compared with those of the controls.

Pituitary and serum TSH, LH and FSH concentrations of the sham operation control rats with the slow infusions of saline

After 1-, 3-, 24- and 72-hr infusions of saline, the pituitary TSH and LH concentrations did not change, but pituitary FSH concentration slightly increased at 3, 24 and 72 hr (p<0.01), as seen in Fig. 1. No changes of the serum TSH, LH and FSH concentrations were detectable for any duration of saline infusions (Fig. 2).

Pituitary and serum TSH concentrations in the rats infused with the releasing hormones

As shown in Fig. 3, when T was infused, pituitary TSH concentration increased significantly (p<0.01) at 72 hr; when L was infused, pituitary TSH concentration increased at 48 hr (p<0.01) but decreased at 72 hr (p<0.05); when T and L were infused in combination, the pituitary TSH concentration decreased at 24 hr (p<0.01),
but increased at 72 hr (p<0.01). Serum TSH concentration rose after the initiation of T infusion, especially, at 3 hr, to the highest value of $1,296 \pm 157 \text{ ng/ml}$ which was about 6 times higher than the normal value ($169 \pm 47 \text{ ng/ml}$), as shown in Fig. 4. Subsequently, the serum TSH concentration declined as evidenced by the values at 48 and 72 hr. The rats treated with L showed no significant changes in serum TSH concentration (Fig. 4). The changes in serum TSH concentration of the rats treated with T+L were comparable to those in the rats treated with T only, and a tendency of remarkable decline in serum TSH concentration of the rats treated with a 48-hr infusion of T+L was comparable to that of the rats treated with a 48-hr infusion of L only.

Pituitary and serum LH contents in the rats infused with the releasing hormones

Pituitary LH content showed no significant change after the 1-, 3- and 24-hr infusion of T, but increased after a 48- and 72-hr infusion, reaching the value of $3,434 \pm 275 \text{ ng/pit}$ which was above 1.5 times higher than the control (Fig. 5). On the
other hand, pituitary LH content was commonly low in all the L treated groups. After the 1-hr infusion of L, it decreased to 1,781 ± 27 ng/pit, reaching the lowest value (624 ± 39 ng/pit) which was about 30% of the saline control at 72 hr. The T + L treated groups showed a tendency of slight decrease in the pituitary LH content at 3 hr (1,750 ± 22 ng/pit), but showed a recovery to the normal level at 72 hr after a temporal rise at 24 and 48 hr (Fig. 5).

While serum LH concentration did not change remarkably after the T infusions, it increased 2.5 times higher than the control value after the 24-hr L infusion, and thereafter increased conspicuously to the level of 11.30 ± 0.69 ng/ml which was 7 times higher than the control value after the 48- or 72-hr infusion (Fig. 6). Serum LH concentrations in the T + L treated rats increased 1, 24 and 72 hr after the initiation of infusion, but the values at 24, 48 and 72 hr were significantly lower than the values in the rats infused with L only for the corresponding duration. Thus, despite no conspicuous response of T infusions on the serum LH concentration except for at 24 hr, the T + L treatment suppressed the effect of L to elevate the serum LH concentration.
Pituitary and serum FSH contents

As shown in Fig. 7, pituitary FSH concentration of the rats receiving a 3-hr T infusion decreased significantly (p<0.05), and that of the rats receiving the 24-hr and 72-hr L infusions decreased significantly (p<0.05 and p<0.01, respectively), but that of the rats given the T+L infusion was not changed. Serum FSH concentration was diminished with the prolongation of T infusion (Fig 8). When compared with the control value (820±60 ng/ml), serum FSH concentration decreased to a half after the 72-hr T infusion (480±24 ng/ml). A tendency of slight decline was observed in both groups receiving the L infusion and T+L infusion. No increase in the serum FSH concentration was observed following the L infusion in the present study.

Discussion

Slow infusion of the releasing hormones (RH) was adopted for the first time by Arimura et al. (1972), who carried out a 4-hr infusion of the total dose of either 0.2 µg or 0.3 µg of synthetic LRH. They reported the considerable rise of both serum LH and FSH levels and that the magnitude of rise for both serum LH and FSH contents was dose-related. A 4-hr infusion of the total dose of either 1 or 3 µg of LRH, according to Arimura et al., stimulated LH and FSH release near maximally. They found a pronounced increase both in the serum LH concentration (12 times more than the control value) and in the serum FSH concentration (10 times more than the control value). The additional reports (Redding et al., 1972; Shiino et al., 1972; Arimura et al., 1973; Mendoza et al., 1973) presented evidence that LRH decapptide was also the only or principal FSH-RH, proposing a concept of LH/FSH-RH. According to Yoshimura et al. (1973), a single iv injection of LRH (1 µg/ml) to the immature male rats of 30 days of age induces an increase not only in the serum LH but also in the serum FSH concentration. Following an injection serum FSH concentration showed a peak (1.5 times more than the normal value) after 30 min followed by the subnormal drop after 180 min thus confirming the coordinated rises of the serum LH and FSH concentrations through the acute experiments. In this study, after a slow infusion of LRH into the femoral veins of the young rats, however, no significant rise of serum FSH concentration was observed during any duration. This finding is incompatible with the elevation of serum FSH concentration reported by Arimura et al. (1972). One reason may be the young age of the used rats. The other reasons are unknown. The dose of LRH used in this experiment does not seem to be very small.

The interpretations are made as to the results obtained from this study as follows: (1) T may be an inhibitor to the FSH-release from the gland, because T infusions for 1, 3, 24 and 72 hr diminish the serum FSH concentration (Fig. 8). (2) T may possibly be a kind of stimulator to the synthesis of pituitary LH, because T infusions for 48 and 72 hr increase the pituitary LH concentration (Fig. 5), despite no remarkable increase of serum LH concentration (Fig. 6). (3) T may act antagonistically upon the LH releasing activity of L, because the T+L infusions diminish the original effect of L on LH release from the gland (Figs. 5 and 6). (4) L may be an inhibitor to the release of the FSH from the gland, because L infusions decrease significantly the serum FSH concentration (Fig. 8). Thus, the present results are inconsistent with the common concept that one releasing hormone completely corresponds with one kind of trophic hormone.

The present author here compares the present results after the continuous infusion with our previous data after the chronic discontinuous injections of T and L in
separation or in combination (Soji et al., 1977): 1) Chronic subcutaneous injections of T (10 μg/0.2 ml) for 3 or 7 days reduced the serum LH concentration, but those for 21 days contrarily cause a slight rise of the serum LH. On the other hand, a slow T infusion fails to affect it clearly (Fig. 6). 2) Chronic T injections for 7 days result in a decrease of the serum FSH concentration, and coincidently a slow infusion of T diminishes the serum concentration (Fig. 8). These suppressive effects may favorably suggest a possibility that T is an inhibitor to the FSH-release. 3) Chronic infusion of L (5 μg/0.2 ml) for 21 days diminishes the pituitary FSH concentration, and concomitantly the L infusions remarkably diminish it (Fig. 7). This diminishment of pituitary FSH concentrations after the chronic injections and slow infusions may suggest that L is an inhibitor to the FSH-release. 4) Chronic T+L injections for 7 or 21 days diminish more progressively the pituitary and serum LH concentrations than the chronic L injections for the corresponding days. A slow infusion of T+L for 72 hr increases less progressively the serum LH concentration than that of L only for 72 hr (Fig. 6), but does not evidently change the pituitary LH concentration, although an infusion of L only for 72 hr decreases remarkably the pituitary LH concentration (Fig. 5). Thus, the chronically injected T may synergistically act upon the LH releasing effect of L, while the slowly infused T may antagonistically act upon the LH releasing effect of L.

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