A New More Potent Analogue of LH-RH: [Des-Gly-NH₂¹⁰, Pro-ethylamide⁹]-LH-RH

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Synopsis

The effect of a new analogue [Des-Gly-NH₂¹⁰, pro-ethylamide⁹]-LH-RH (TAP-031) on the release of LH and FSH was compared with the effect of synthetic LH-RH by both the *in vivo* and *in vitro* assay method. The concentration of FSH and LH in serum and medium was determined by NIAMD-rat hormone radioimmunoassay kits. Single intravenous injections of 1, 10, 100 and 1,000 m μ g of TAP-031 in ovariectomized-estrogen-progesterone blocked rats (O.E.P.), induced the increases of serum LH 5 to 60 min after injection. In contrast, single injections of the same four doses of LH-RH in O.E.P. rats induced the increase of serum LH at 5 to 30 min after injection. Intravenous single injections of 1 and 10 m μ g of TAP-031 and LH-RH induced no significant elevations of serum FSH in O.E.P. rats. However, injections of 100 and 1,000 m μ g of TAP-031 and LH-RH induced significant elevations of serum FSH 1 to 2 hrs after injection of TAP-031, and 10 to 60 min after the injection of LH-RH, respectively. The appearance of peak in serum LH and FSH was remarkably postponed in administration of over 100 m μ g of TAP-031. The increase in serum LH and FSH was larger in administration of TAP-031 than in administration of LH-RH.

In *in vitro* rat pituitary incubation experiments, LH and FSH releasing activities of TAP-031 were shown to be stronger than those of LH-RH.

These data offer the evidences demonstrating that TAP-031 has the stronger and more prolonged LH and FSH releasing activities both *in vivo* and *in vitro* than synthetic LH-RH.

Since the chemical structure of hypothalamic LH-RH was determined by Matsuo *et al.* (1971), several LH-RH analogues have been synthesized and their structure-activity-relationships have been discussed (Stievertson *et al.*, 1971; Rivier *et al.*, 1972; Yanaihara *et al.*, 1972; 1973). These synthetic analogues of LH-RH had no or far less activity than natural LH-RH. However, Fujino *et al.* (1972) reported a new analogue of LH-RH having five-times greater potency than the natural LH-RH in the LH-RH activity assayed by the ovulation-induction method.

The object of this study is to investigate LH

and FSH releasing activity of this new compound (TAP-031) by both *in vivo* and *in vitro* assay methods.

Materials and Methods

The decapeptide of LH-RH and the nanopeptide, [PGlu-His-Trip-Ser-Tyr-Gly-Leu-Arg-Pro-ethylamide], (TAP-031) were synthesized by Fujino *et al.* (1972) of Takeda Pharmaceutical Co., Osaka, Japan.

Adult male rats weighing 300–400 g and female rats weighing 200–300 g of Holtzman strain, were maintained in an animal room with constant room temperature (25 $\pm$ 2°C) and lighting (lights on 5:00 a.m.-7:00 p.m.).

In vitro assay for LH- and FSH-releasing activities was performed according to the modified Mittler and

Meites method (1964). The one half anterior pituitary of adult male rats was incubated for 30 min in 1.0 ml of Krebs-Ringer glucose bicarbonate medium. The medium was then decanted and replaced with a fresh medium containing various doses of LH-RH or TAP-031. After the 2 hr incubation, the media were separated from the pituitary and stored at -20°C until radioimmunoassay. The weight of rat pituitary half was measured after incubation. The amount of FSH and LH released into the medium was assayed with NIAMD rat FSH and LH radioimmunoassay kits.

The *in vivo* assay for LH- and FSH-releasing activities was performed according to the slightly modified McCann-Ramirez's method (1963). Three days after the injection of 100 μg estradiol benzoate and 10 mg progesterone in ovariectomized Holtzman rats, about 3 ml of blood was collected from the jugular vein under ether anesthesia 5, 10, 30 min, 1, 2, 3, 4 and 6 hrs after the intravenous injection of one ml of various doses of LH-RH or TAP-031. The serum was stored at -20°C until radioimmunoassay. Serum FSH

and LH concentrations were measured with NIAMD rat FSH and LH radioimmunoassay kits. Results were expressed in terms of the NIAMD rat FSH-RP-1 and the NIAMD rat LH-RP-1, respectively. In both *in vivo* and *in vitro* studies, five rats were used in each group. Statistical significance was evaluated by Student's test.

Results

In vitro release of FSH and LH:

Figure 1 and 2 show the ratio of LH and FSH, respectively, released in the medium, when LH-RH or TAP-031 was added in the medium. The doses over 0.001 μg of both TAP-031 and LH-RH induced a remarkable increase of LH and FSH. LH and FSH releasing activities were stronger in TAP-031

Fig. 1. Effect of varying doses of TAP-031 and LH-RH on the release of LH from rat pituitaries incubated *in vitro*. The vertical bars represent standard error.

Fig. 2. Effect of varying doses of TAP-031 and LH-RH on the release of FSH from rat pituitaries incubated *in vitro*. The vertical bars represent standard error.

group than in LH-RH group.

In vivo release of LH and FSH:

Single intravenous injection of one μg of TAP-031 and LH-RH induced LH release and FSH release *in vivo* as shown in Figure 3 and Figure 4. Serum LH peak was observed 5 min after intravenous injection of either LH-RH or TAP-031. The serum LH peak was slightly higher in TAP-031 group than in LH-RH group, but the difference was not significant. The effect of TAP-031 was prolonged until 2 hrs after injection, while the effect of LH-RH disappeared within one hr. No significant

changes in serum FSH were observed in both LH-RH and TAP-031 injected groups (Fig. 4).

Injection of 10 μg of LH-RH and TAP-031 induced LH- and FSH-release *in vivo* as shown in Figure 5 and Figure 6. The manifest peak in serum LH was observed at 10 min after injection of either LH-RH or TAP-031, and the LH peak was significantly higher ($p < 0.001$) in the TAP-031 group than in the LH-RH group. Serum LH levels in TAP-031 group were retained at significantly higher levels until 1 hr after injection than those in the LH-RH group. No significant changes in

Fig. 3. Time course of LH release in the O.E.P. rat following an i.v. dose of 1.0 μg TAP-031 and LH-RH. The vertical bars represent standard error. Mean $\pm$ SE of 5 animals per treatment.

Fig. 4. Time course of FSH release in the O.E.P. rat following an i.v. dose of 1.0 μg TAP-031 and LH-RH. The vertical bars represent standard error. Mean $\pm$ SE of 5 animals per treatment.

Fig. 5. Time course of LH release in the O.E.P. rat following an i.v. dose of 10.0 μ g TAP-031 and LH-RH. The vertical bars represent standard error. Mean $\pm$ SE of 5 animals per treatment.

Fig. 6. Time course of FSH release in the O.E.P. rat following an i.v. dose of 10.0 μ g TAP-031 and LH-RH. The vertical bars represent standard error. Mean $\pm$ SE of 5 animals per treatment.

serum FSH were found between the 10 μ g LH-RH group and the 10 μ g TAP-031 group (Fig. 6).

Injection of 100 μ g of TAP-031 raised sharply serum LH from 5 min to 2 hrs, while injection of 100 μ g of LH-RH raised serum LH only from 5 to 30 min after injection (Fig. 7). The LH peak value at 1 hr in the TAP-031 group was significantly higher ($p < 0.01$) than the peak at 10 min in the LH-RH group. As shown in Figure 8, a single injection of 100 μ g TAP-031 induced prolonged higher serum FSH levels from 30 min

to 2 hrs than the 100 μ g LH-RH injection, although the difference was not significant.

A single injection of 1,000 μ g of TAP-031 raised serum LH levels over 1 μ g/ml from 5 min to 3 hrs, while the same dose of LH-RH induced an increase of serum LH levels over 1 μ g/ml from 5 min to 1 hr (Fig. 9). The difference in LH levels was highly significant ($p < 0.01$). Serum FSH levels were also significantly higher ($p < 0.001$) in the 1,000 μ g TAP-031 group than in the 1,000 μ g LH-RH group from 1 to 4 hrs after injection (Fig. 10).

Fig. 7. Time course of LH release in the O.E.P. rat following an i.v. dose of 100.0 μ g TAP-031 and LH-RH. The vertical bars represent standard error. Mean $\pm$ SE of 5 animals per treatment.

Fig. 8. Time course of FSH release in the O.E.P. rat following an i.v. dose of 100.0 μ g TAP-031 and LH-RH. The vertical bars represent standard error. Mean $\pm$ SE of 5 animals per treatment.

Discussion

In the *in vivo* experiment of Rippel *et al.* (1973) showed that this analogue of LH-RH (TAP-031) released a greater amount of LH and FSH and induced a higher percentage of ovulation in the Nembutal-blocked proestrous rat than the LH-RH. Our present results not

only confirmed the *in vivo* effect reported by Rippel *et al.* (1973), but also demonstrated the *in vitro* effect of TAP-031.

In the *in vivo* experiment, it is noteworthy that the difference in increase of serum LH between the TAP-031 group and the LH-RH group is dose-dependent. Injection of one μ g of TAP-031 and LH-RH in O.E.P. rats induced increases of LH of the same levels in

Fig. 9. Time course of LH release in the O.E.P. rat following an i.v. dose of 100.0 m μ g TAP-031 and LH-RH. The vertical bars represent standard error. Mean $\pm$ SE of 5 animals per treatment.

Fig. 10. Time course of FSH release in the O.E.P. rat following an i.v. dose of 1,000.0 m μ g TAP-031 and LH-RH. The vertical bars represent standard error. Mean $\pm$ SE of 5 animals per treatment.

both groups and the only prolonged effect was observed in the TAP-031 group. As the dose of injection was increased, the higher peak of serum LH was observed in the TAP-031 group than in the LH-RH group. Although the time of beginning of increase in serum LH was always 5 min in every dose in both TAP-031 and LH-RH groups, the prolongation of high serum LH levels was always more remarkable in the TAP-031 group than in the LH-RH

group and was dose-dependent in both groups.

The duration of high LH levels was 30 min in 1 and 10 m μ g, 1 hr in 100 m μ g, 2 hrs in 1,000 m μ g injection of LH-RH, while the duration of high LH levels was 1 hr in 1 and 10 m μ g, 3 hrs in 100 m μ g, and 4 hrs in 1,000 m μ g of TAP-031 injection. The range and duration of increased serum FSH level were also dose-dependent. There was no increase of serum FSH in the injection of 1 m μ g of

either TAP-031 or LH-RH. The dose-dependence of serum FSH peak was more remarkable in the TAP-031 group than in the LH-RH group from 10 to 1,000 μg . The duration of high serum FSH was more dose-dependent, that is, 1 hr in 10 μg , 3 hrs in 100 μg , 4 hrs in 1,000 μg of TAP-031 injection, while 30 min in 10 μg , 1 hr in 100 and 1,000 μg of LH-RH injection. These results obtained from the ovariectomized estrogen-progesterone blocked rats, were consistently in agreement with the Rippel *et al*'s results obtained in Nembutal-blocked proestrous rats.

Present *in vitro* experiment clearly shows dose-dependent release of both LH and FSH *in vitro* from the hemisected rat pituitary. It is noteworthy that the doses over 0.01 μg of TAP-031 always induced the release of more FSH and LH *in vitro* than the same doses of LH-RH. Our results demonstrated the more potent LH and FSH releasing activities of TAP-031 than those of LH-RH not only *in vivo*, but also *in vitro*.

Although the reason why the TAP-031 is far more potent than natural LH-RH is unknown so far, the longer half life of TAP-031 than natural LH-RH in blood may contribute the greater potency *in vivo* as recently reported by Kumasaka *et al.* (1974). However, further studies are required to elucidate the mechanism of the greater potency of TAP-031 *in vitro* than natural LH-RH.

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