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Stimulation by a TRH Precursor, TRH-GLY, of TSH and PRL Secretion in Rats: Effect of Starvation

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Abstract—The hypophysial activities of a possible direct precursor of thyrotropin (TSH) -releasing hormone (TRH), TRH-Gly, were evaluated in estrogen, progesterone-primed rats under urethane anesthesia. Intravenous administration of TRH-Gly in doses of 2-200 μg caused a significant and dose-dependent increase in blood TSH and prolactin (PRL). The stimulatory activity of TRH-Gly was 170 to 400-times less potent than that of TRH. The lower potency was confirmed by the action of TRH-Gly on the anterior pituitary cells in vitro. In starved rats, TRH-Gly apparently stimulated TSH and PRL secretion in a dose-dependent manner, and the stimulatory activity increased in starved rats as compared to normal controls. TRH-Gly did not affect [^{3}H-MeHis]TRH binding in pituitary plasma membranes. These data imply that large amounts of TRH-Gly may have significant biological activities and these are potentiated in the starved condition.

Introduction

Recent observations (1, 2) have demonstrated the complete amino acid sequence of the precursor of thyrotropin (TSH)-releasing hormone (TRH, pGlu-His-ProNH_2). The precursor contains five TRH sequences. The precursor is processed to produce a number of precursor forms. Eventually, TRH arises from its direct precursor, pGlu-His-Pro-Gly (TRH-Gly) via the action of an α-amidating enzyme. This enzyme requires ascorbate, copper ion and molecular oxygen, and α-amidates the C-terminal proline residue cleaving the C-terminal glycine as the NH_2 donor (3, 4).

Recently, significant concentrations of TRH-Gly have been found in the hypothalamus of fetal and adult rats, and also in the rat anterior pituitary and peripheral blood (4, 5). In human cerebrospinal fluid, large amounts of TRH-Gly were detected; the concentration of TRH-Gly was reported to be over 100-times higher than that of TRH (6). In addition, intracisternal injection of TRH-Gly stimulates gastric secretion in rats (7), and some TRH precursors may regulate TSH gene expression (8). Therefore, the possibility has been

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raised that the glycine-extended precursor of TRH may have biological actions on the anterior pituitary.

The intriguing proposal that the starved condition exceptionally enhances secretion of pituitary hormones has arisen from observations that TRH and LHRH stimulated growth hormone (GH) secretion and GH-releasing hormone stimulated prolactin (PRL) secretion in patients with anorexia nervosa (9-11). The present study was designed to evaluate the action of TRH-Gly on TSH and PRL secretion from the rat anterior pituitaries and scutinize the changes in the stimulatory potency of TRH-Gly in starved rats.

Materials and Methods

TRH was supplied by Tanabe Pharmaceutical Co., Osaka, Japan. [^{3}H-MeHis]TRH was purchased from New England Nuclear, Boston, Mass (specific activity = 57.8 Ci/mmol). Estradiol benzoate and progesterone were purchased from Wako Pure Chemical Industries Ltd., Osaka, Japan.

Experiment 1, TRH-Gly synthesis

TRH-Gly was synthesized by solid-phase methodology (Tanabe Pharmaceutical Co.) and purified by high performance liquid chromatography (HPLC, Ultron S-C_{18} column 4.6 × 150mm, Shinwa Chemical Industrial Co., Kyoto, Japan, solvent system = 0.02M KH_2PO_4 pH 2.5, flow rate = 1ml/min at 50°C).

Experiment 2, in vivo administration of TRH-Gly

Adult male Wistar rats weighing 200-250g were housed in a temperature-controlled room (22-23°C) with lights on from 5:00am to 7.00pm. Because administration of TRH stimulates PRL secretion in male rats when they are primed with estrogen and progesterone, each animal received daily subcutaneous injections of 50μg estradiol benzoate and 2.5mg progesterone in sesame oil for 3 days. One hour after the estradiol/progesterone injected animals were anesthetized with urethane (150mg per 100g body weight, intraperitoneally), TRH (0.02-2.0μg) or TRH-Gly (0.2-200μg) was given in saline into the jugular veins. Blood samples were centrifuged at 1500 × g for 30 min and the plasma samples were stored at −20°C until assay.

Experiment 3, in vitro administration of TRH-Gly

Adult male rats were decapitated and anterior pituitaries were immediately removed. According to the method as described elsewhere (12), pituitary cells were dispersed using 5mg/ml trypsin (type III, Sigma Chemical Co., St. Louis. MO). The pituitary cells were suspended in culture medium (medium 199 with 25mM $NaHCO_3$, 100U/ml penicillin, 0.1mg/ml streptomycin and 10% fetal calf serum), and cultured at a density of 0.5 × 10^6 cells per well at 37°C in 5% CO_2 for 3 days. The cells were washed twice with 1ml of culture medium and incubated with varying doses of TRH or TRH-Gly in culture medium for 20h. The supernatants were saved and stored at −20°C.

Experiment 4, effects of starvation on TRH-Gly-stimulated hormone secretion

Animals which were injected with estradiol benzoate and progesterone as described above were deprived of food for 1 or 3 days. TRH-Gly was then injected intravenously under urethane anesthesia. Blood samples were taken using heparinized syringes 0, 5 and 15 min after peptide injection. The plasma samples were stored at −20°C.

TSH and PRL in the blood and media were assayed by radioimmunoassay using kits supplied by the Rat Pituitary Hormone Program, NIADDK, NIH, as described previously (13). The sample values were expressed as the equivalent of NIADDK rat TSH RP-1 (0.22U/mg) and rat PRL RP-3 (30U//mg).

Experiment 5, TRH receptor assay

Anterior pituitaries of normal rats were homogenized in 10 vol 40mM Tris-HCl buffer (pH 7.4) containing 0.25M sucrose and centrifuged at 1000 × g for 10 min. The supernatants were centrifuged at 5400 × g for 30 min. The precipitates (crude plasma membranes) were reconstituted in Tris-HCl buffer and used for assay. The TRH receptor assay was done according to the method as described previously (14). In brief, 200μl of membranes were incubated with 10μl 1.73pmol

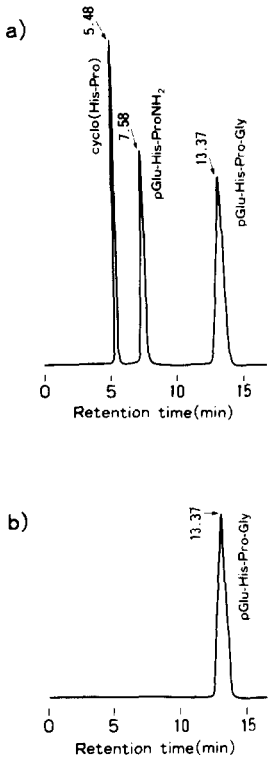

Fig. 1 An HPLC analysis of synthetic TRH-Gly. A mixture solution containing TRH-Gly, TRH and cyclo(His-Pro) was analyzed by HPLC (a). An analysis of TRH-Gly alone (b).

[^{3}H-MeHis]TRH in the presence of varying doses of TRH or TRH-Gly at 0°C for 2h. After addition of 0.5ml chilled Tris-Hcl buffer, the incubation mixtures were filtered under reduced pressure through GF/B filters (Whatman). The filters were washed with 5ml Tris-HCl buffer, transferred to scintillation vials containing 5ml Aquasol 2 (New England Nuclear) and [^{3}H] counted in a scintillation spectrometer (Aloka LSC-700, Tokyo, Japan).

The statistical analysis was done by Duncan's multiple range test.

Results

Figure 1 shows an HPLC analysis of synthetic TRH-Gly. The TRH-Gly migrated as a single peak on an HPLC.

Figure 2 shows the sequential changes in blood TSH and PRL levels after TRH or TRH-Gly administration. As shown in Figure 2 (a-d), intravenous injections of TRH doses of 0.2 and 2.0μg significantly increased blood TSH and PRL levels, and secretion of these two hormones was also stimulated by 200μg TRH-Gly. As shown in Figure 2 (e and f), blood TSH and PRL secretion

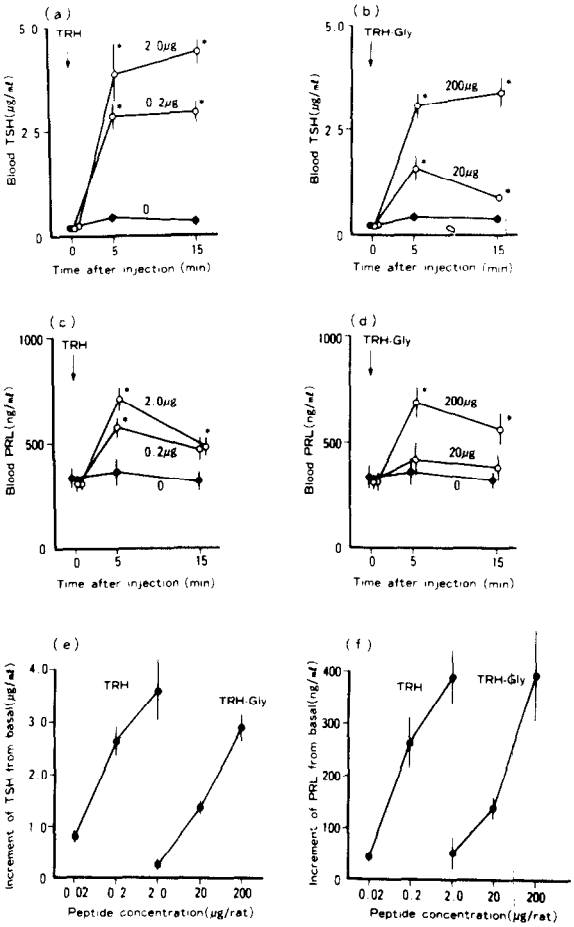

Fig. 2 The sequential changes in blood TSH and PRL secretion in response to TRH and TRH-Gly. The estradiol progesterone-primed rats were intravenously injected with TRH and TRH-Gly under urethane anaesthesia. Blood samples were drawn 0, 5 and 15 min after injection, and assayed for TSH and PRL by RIAs. Each point represents mean ± S.E.M. in five rats. *($P < 0.01$) differs from the basal levels. (a) and (b), the blood TSH levels; (c) and (d), the blood PRL levels; (e), dose-response curve for secretion of TSH; (f) dose-response curve for secretion of PRL.

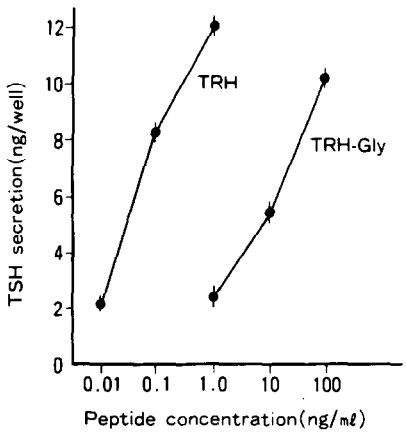

Fig. 3 The in vitro TSH secretion in response to TRH and TRH-Gly. The anterior pituitary cells were incubated with varying doses of TRH and TRH-Gly at 37°C for 20h. The supernatants were assayed for TSH by RIA. Each result point represents mean ± S.E.M. in five tubes.

increased with increasing doses of TRH and TRH-Gly, and the stimulatory activity of TRH-Gly on TSH and PRL secretion was calculated to be 0.25% (0.09-0.59%, 95% fiducial limit) and 0.60% (0.22-1.51%), respectively, of TRH activity.

Figure 3 shows in vitro TSH secretion by anterior pituitary cells in response to TRH and TRH-Gly. Because in this cell culture system addition of TRH did not significantly stimulate PRL secretion, only TSH was estimated after addition of TRH and TRH-Gly. TRH-Gly stimulated TSH secretion from pituitary cells and the stimulatory efficiency was 0.69% of that of TRH (0.57-0.81%, 95% fiducial limit).

Figure 4 shows the changes in TRH-Gly-stimulated secretion of TSH and PRL in starved rats. The animal body weights decreased progressively during starvation (0 day, 243.2 ± 2.7, N = 20; 1 day, 215.6 ± 1.9, N = 18; 3 days after starvation, 180.2 ± 2.2g, N= 20). TRH-Gly stimulated TSH and PRL secretion in a dose-dependent manner in starved rats. Two hundred μg of TRH-Gly had a significantly greater effect on both hormones in the 3-day-starved rats than in the normal control animals (TSH: normal group, 2.91 ± 0.22 vs. 3-day-starved group, 5.37 ± 0.84 μg/ml, $P < 0.01$; PRL: control group, 394 ± 88 vs. 3-day-starved group, 598 ± 22ng/ml, $P < 0.01$).

As shown in Figure 5, 5.0pmol TRH significantly decreased [^{3}H-MeHis]TRH binding by 75%, while as much as 200pmol TRH-Gly did not significantly affect the binding. The dose of TRH required to displace half of the [^{3}H-MeHis]TRH binding was 14 pmol per tube (63.6nM).

Discussion

The present study demonstrated that the glycine-extended biosynthetic precursor of TRH significantly stimulated blood TSH and PRL secretion in

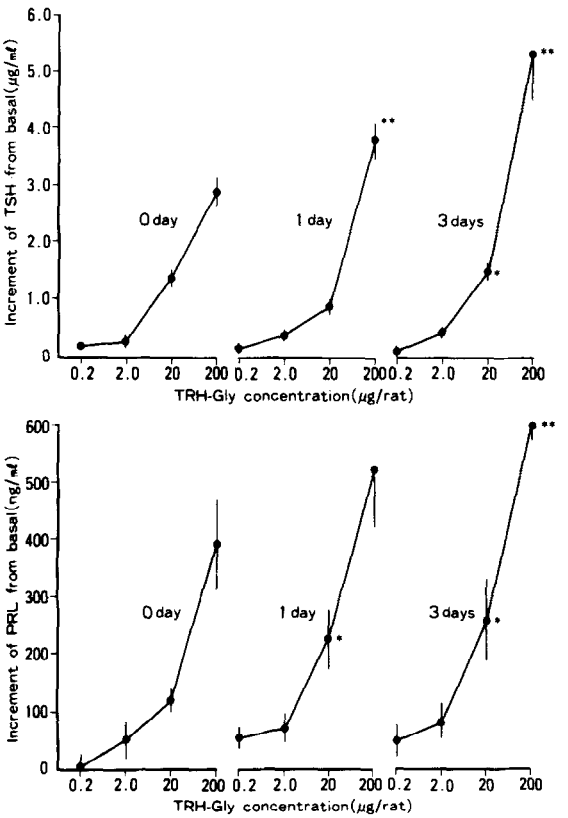

Fig. 4 The changes in TRG-Gly-stimulated secretion of TSH and PRL in starved rats. The estradiol, progesterone-primed rats were deprived of food for 0, 1 or 3 days. The animals were intravenously injected with varying doses of TRH-Gly under urethane anaesthesia. The blood samples were drawn 0 and 5 min after injection, and used for TSH and PRL by RIAs. Each result point is expressed as increment from the basal level with mean ± S.E.M. in five rats. *($P < 0.05$) and **($P < 0.01$) differ from the hormone levels at 0 day. The upper panel, the blood TSH level; the lower panel, the blood PRL level.

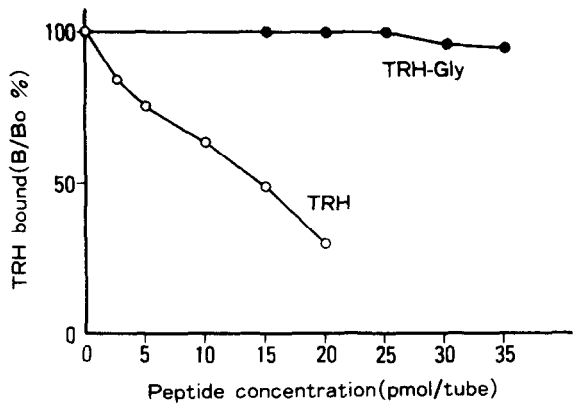

Fig. 5 Effects of TRH-Gly on TRH binding in rat anterior pituitary plasma membranes. The crude plasma membranes were incubated with [^{3}H-MeHis]TRH and varying doses of peptides at 0°C for 2 h. [^{3}H-MeHis]TRH binding was estimated in duplicate determination. TRH bound is expressed as percentage inhibited radioactivity (B) of total bound radioactivity (Bo).

estradiol, progesterone-primed rats, and the stimulatory effect was dose-dependent. TRH-Gly-dose dependent secretion of TSH also occurred in cultured anterior pituitary cells. However, a 150-fold greater dose of TRH-Gly was required to produce a same hormone secretory response equivalent to that induced by TRH. Similarly, TRH-Gly is less potent in stimulating gastric acid secretion than TRH (7). Although significant concentrations of TRH-Gly-immunoreactivity were found in the hypothalamus, pituitary and peripheral blood of the rat (5), the present data imply that TRH-Gly may have a minor role in regulating the secretion of anterior pituitary hormones. However, Pekary et al (6) measured 100-fold higher amounts of TRH-Gly than TRH in human cerebrospinal fluid. Therefore, TRH-Gly may have some biological effects in humans.

Based on the previous observations showing that abnormal and increased secretion of pituitary hormones occurs in patients with anorexia nervosa (9-11), changes in blood TSH and PRL secretion in response to TRH-Gly were evaluated in starved rats, an animal model of anorexia nervosa. The stimulatory action of TRH-Gly on pituitary hormones had a tendency to increase with the period of starvation, and blood TSH and PRL levels in response to 200 μg TRH/Gly were significantly greater in the starved group than in normal controls. These observations suggest that TRH-Gly may have significant biological effects in pathological conditions such as starvation, even if its actions are minimal in normal subjects.

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