PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 154, 573-577 (1977)

Release of Growth Hormone by TRH in Intact Rats or in Intact or Hypophysectomized Rats bearing a Heterotopic Pituitary (39721)

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Introduction. Thyrotropin-releasing hormone (TRH) is capable of releasing growth hormone (GH) in several pathologic conditions in man, e.g., acromegaly, uremia, mental depression, and anorexia nervosa (1-4), and in the laboratory animal especially when the anterior pituitary (AP) lacks its normal neurohormonal influences (5-9). This led us to postulate that in the pathologic conditions of the human, the anomalous GH response to TRH also might be due to the existence of a functional disconnection between the central nervous system (CNS) and the AP (8, 9). However, another possibility could not be excluded, i.e., that alterations in peripheral hormone titers due to the existence of the CNS-AP disconnection might induce a sensitization of the AP to the GH-releasing effect of the tripeptide. To rule out this possibility in this study the ability of TRH to induce GH release was evaluated in either intact rats or in intact or hypophysectomized rats bearing an AP graft under the kidney capsule. In addition, electron microscopy studies were performed on the *in situ* or transplanted pituitary in basal conditions or following TRH stimulation.

Materials and methods. In all experiments female and male Sprague-Dawley rats (Carlo Erba S.p.A., Milano) were used. Some rats of both sexes were hypophysectomized (10) and 10 days later they received under the kidney capsule an entire AP obtained from rats of the same sex and weight (11) (HT). Intact rats of both sexes also received an entire AP under the kidney capsule (IT). All experiments were performed 15 days after transplantation in either HT or IT rats (body weight, 120-140 g). As controls for both groups weight-matched intact female and male Sprague-Dawley rats were used.

Blood samples. Throughout all studies, blood samples (0.3 ml) were collected from the exposed jugular vein with rats under urethane anesthesia (1.5 g/kg body wt), with the exception of the first blood sample (0.4 ml) which was obtained in unanesthetized animals by puncture of the retro-orbital venous plexus (12). The second blood sample (0 min), was collected 60 min after urethane and immediately thereafter 0.2 ml of either normal saline solution or TRH (0.15, 0.6, and 1.2 μg) dissolved in saline was injected into the jugular vein. Five and ten minutes after injection blood samples were obtained from each rat and the blood removed was replaced with an equal volume of normal saline.

Hormone determinations. Blood samples were immediately centrifuged; the plasma was separated and kept frozen at −20°C until assayed. GH and PRL levels were measured by radioimmunoassay as previously described (13) employing materials generously supplied by the NIAMDD. All plasma samples for a given experiment were tested in a single assay to eliminate interassay variation in the interpretation of treatment effects.

Electron microscopy (EM) studies. EM studies were performed on 16 pituitaries from eight IT female rats (two from each dose group, randomly selected). Immediately after the last blood sample was taken, rats were killed and both the *in situ* and the ectopic pituitary were quickly removed and fixed in cold Karnowsky solution for about 4 hr and then washed in Sørensen buffer, 0.12 *M*, pH 7.4. From each pituitary a paramedial section of AP was obtained and divided in four portions, as described elsewhere (G.

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ISSN 0037-9727

L. Rossi *et al.*, in preparation). The portions of AP tissue were then embedded in Epon 312 as previously described (14) and blocks were properly positioned under a stereomicroscope. From one block randomly taken from each pituitary, ultrathin sections were cut, mounted on 300-mesh grids, and contrasted with uranyl acetate and lead citrate for EM. At × 14,000, three grid fields per block were examined and all figures of exocytosis found to occur in 10 somatotrophs randomly selected among those fullfilling the criteria elsewhere reported were counted.

Statistics. Absolute GH increases above pretreatment values observed after TRH injection were compared to those following saline administration. In all experiments the significance of differences was evaluated according to Duncan's multiple range test or analysis of variance (ANOVAR).

Results. Baseline GH values. No significant difference in plasma GH values was reported between intact and IT rats (49.9 ± 2.1 and 63.9 ± 13.3 ng/ml, respectively), while in accordance with previous results (8), plasma GH values were significantly lower in HT rats (9.0 ± 0.7 ng/ml). Urethane anesthesia significantly lowered plasma GH values in intact and IT (11.8 ± 0.7 and 6.5 ± 1.1 ng/ml, respectively), but not in HT rats (8.7 ± 0.7 ng/ml).

GH response to TRH. Administration of TRH at doses of 0.15, 0.6, and 1.2 μg induced a significant rise in plasma GH levels in HT and IT female rats at both 5 and 10 min, except in IT rats with the two lower doses at 10 min. The GH rise after 1.2 μg of TRH was more consistent in the IT than in the HT rats ($F = 7.50$, $P < 0.05$) (Fig. 1, mid and right sections). In the intact female rats, of the three TRH doses used, only the highest (1.2 μg) induced a significant increase in plasma GH at both 5 and 10 min. However, this dose was less effective than in IT rats ($F = 8.82$, $P < 0.05$) (Fig. 1, left section).

A similar trend was present in male rats, in which the two doses of the tripeptide used, 0.15 and 0.6 μg, increased plasma GH levels in HT and IT rats at both 5 and 10 (Fig. 2, mid and right sections). In intact rats TRH was effective as a GH-releaser only at the dose of 0.6 μg (Fig. 2, left section).

Electron microscopy studies. EM observations showed that in IT rats the ectopic pituitary had undergone the known changes

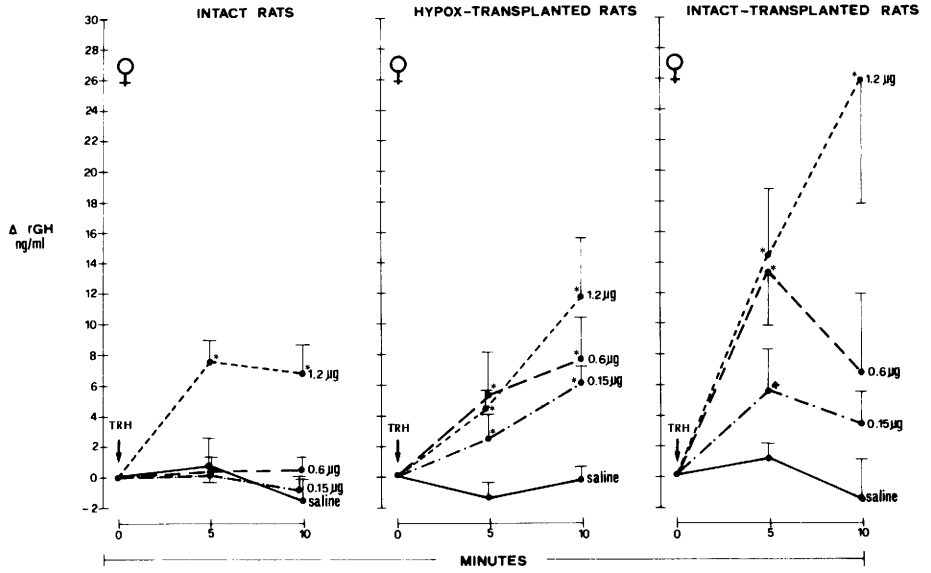

FIG. 1. GH-releasing effect of TRH in intact female rats, hypophysectomized rats with pituitary grafts, and intact rats with pituitary grafts. Six to seven animals per group were used. Data are expressed as Δ values (mean + SEM) from baseline; asterisks denote a difference statistically significant from corresponding Δ values of saline-treated rats (Duncan's test). The same description applies to Fig. 2.

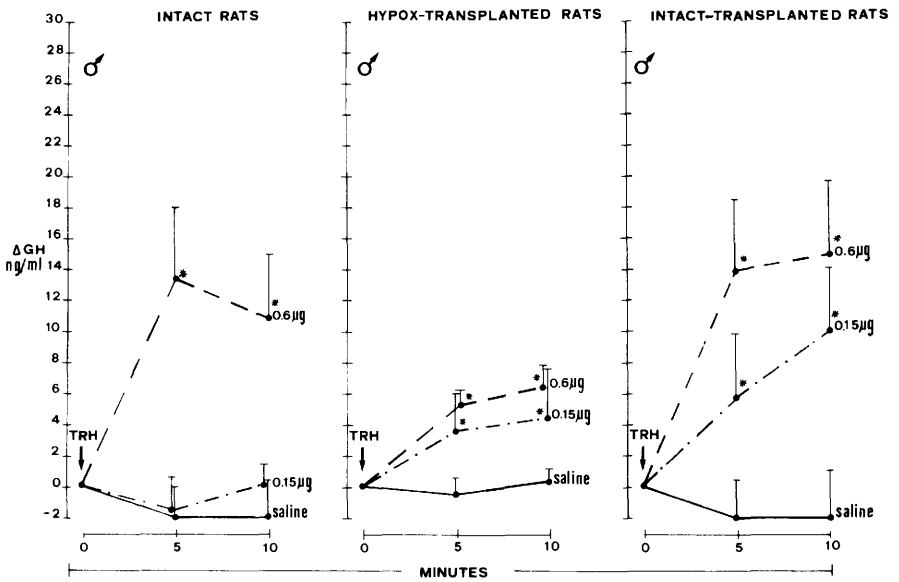

FIG. 2. GH-releasing effect of TRH in intact male rats, hypophysectomized rats with pituitary grafts and intact rats with pituitary grafts. See Fig. 1 legend for details.

due to transplantation already demonstrated in HT rats (14). Briefly, the area of necrosis, which appears in the central portion of the transplanted pituitary in the immediate posttransplantation period, had been invaded by mesenchimal cells. In the zone bordering the necrotic area, all cell types though somewhat modified were still easily recognizable. Somatotrophs, although containing predominantly small secretion granules, were still abundant. Administration of TRH at the highest dose (1.2 μg) was capable of eliciting exocytosis both from the *in situ* (Fig. 3A) and the ectopic (Fig. 3B) pituitary.

Quantitative studies (Table I) demonstrated that in IT female rats, TRH stimulated exocytosis from the ectopic pituitary at all doses used (0.15, 0.6 and 1.2 μg), whereas only the highest TRH dose (1.2 μg) was capable of increasing the frequency of exocytosis from the pituitary *in situ*. The extent of exocytosis from the ectopic pituitary was significantly higher than that occurring from the pituitary *in situ* with the 0.15- and 1.2-μg doses. Because of large variation and the small number of animals examined, the effect of the 0.6-μg dose was not statistically significant.

Discussion. In agreement with previous reports by our group (8, 9), experiments performed in this study point out the preferential release of GH by TRH from an anterior pituitary devoid of CNS influences. A relatively small rise in plasma GH induced by TRH in HT rats agrees with previous findings (8, 15) and is likely attributable to the presence of a small GH pool in the ectopic pituitary (14, 15). TRH at the doses of 0.6 and 0.15 μg in female and male rats, respectively, induced GH release in animals bearing an ectopic pituitary, irrespective of the presence of the pituitary *in situ*. TRH at the same doses was completely ineffective in intact rats and only with the highest TRH dose (1.2 and 0.6 μg in female and male rats, respectively) was a GH release elicitable. The more consistent rise in plasma GH after the highest TRH dose present in IT female rats indicates that in this instance both the ectopic and *in situ* pituitary glands were contributing to the GH increase. This pattern was less evident in the IT male rats.

Results of EM studies corroborated these findings by showing that exocytosis from the somatotrophs of the ectopic AP was highly stimulated after all TRH doses whereas only with the highest dose was exocytosis increased also in the somatotrophs of the pituitary *in situ*.

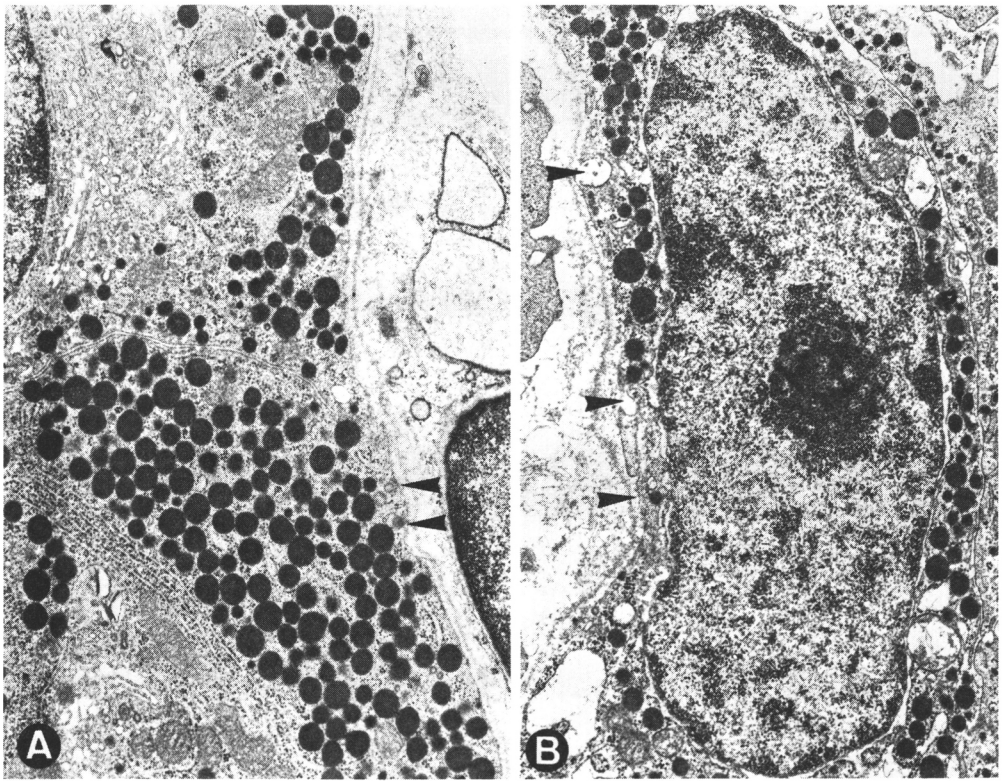

FIG. 3. Ultrastructural aspects of somatotrophs of the *in situ* (A) and ectopic (B) pituitary after injection of 1.2 μg of TRH, showing occurrence of exocytosis (▲).

TABLE I. EFFECT OF TRH ON THE OCCURRENCE OF EXOCYTOSIS IN SOMATOTROPHS OF INTACT FEMALE RATS BEARING AN ECTOPIC PITUITARY.

Treatment	Number of rats	Exocytosis/somatotroph (mean ± SE)	
		In situ pituitary	Ectopic pituitary
Saline	2	1.95 ± 0.15	2.25 ± 0.25
TRH, 0.15 μg	2	2.20 ± 0.10	4.08 ± 0.22*,**
TRH, 0.6 μg	2	2.50 ± 0.90	9.90 ± 3.90
TRH, 1.2 μg	2	5.45 ± 0.25[a]	16.05 ± 1.55*,**

* $P < 0.05$ vs saline control (ANOVAR).
** $P < 0.05$ vs *in situ* pituitary (ANOVAR).

The observation that TRH releases GH preferentially from an ectopic gland also when the latter is transplanted into an intact recipient seems to rule out the possibility that an impaired secretion of target gland hormones, e.g., gonadal steroids, thyroid hormones etc., with ensuing sensitization of the pituitary gland to the action of the tripeptide, may account for the high susceptibility to TRH of the HT rat. An intervention of "peripheral" hormones, e.g., estrogens, was made likely by the observation that the intact male is more susceptible than the intact female rat to TRH-induced GH release (13, 16, and this study) and that no GH rise or blunting of the GH response was present in intact male rats given huge doses of estrogens after systemic (17) or intraportal vessel (6) administration of TRH. An exaggerated GH response to TRH, which was inhibited by thyroxine pretreatment, has been reported in rats made hypothyroid (18). Similarly hypothyroid human beings respond to TRH with a GH rise. This re-

sponse was abolished following thyroid therapy (19).

In spite of these findings, the higher susceptibility to TRH of the ectopic pituitary gland also when placed in a physiologic endocrine milieu indicates that target gland hormones do not play a crucial role except at the extremes of the secretory spectrum, i.e., when present in supraphysiologic amounts or when totally absent.

A more sound reason for the preferential release of GH from the somatotrophs of an ectopic pituitary after TRH seems to be the existence of a CNS–anterior pituitary disconnection per se. Supporting this view is the observation that a preferential release of GH after TRH is also present in rats with extensive hypothalamic lesions (9) and is clear-cut in the infant rat in which CNS–anterior pituitary links have been weakened by destruction of catecholamine nerve terminals in the hypothalamus by 6-hydroxydopamine (20).

Summary. Thyrotropin-releasing hormone (TRH) was administered iv using urethane anesthesia to either intact female and male controls or intact (IT) or hypophysectomized (HT) rats of both sexes bearing an anterior pituitary (AP) graft under the kidney capsule. TRH at all the doses used (0.15, 0.6, and 1.2 μg) elicited a clear GH rise in both IT and HT rats, while only at the highest does (1.2 μg) was it effective in intact controls. Qualitative and quantitative electron microscopic studies performed on the *in situ* and ectopic pituitaries from IT female rats demonstrated that TRH stimulated exocytosis from the ectopic pituitary at all doses used, whereas only the highest TRH dose was capable of increasing the frequency of exocytosis from the pituitary *in situ*. The similar responsiveness to TRH of the ectopic pituitary irrespective of the presence of an *in situ* AP, rules out the possibility that peripheral hormonal factors (e.g., lack of estrogens or thyroid hormones) may play a crucial role in the preferential GH response to TRH present in HT rats.

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Received November 18, 1976. P.S.E.B.M. 1977, Vol. 154.