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TRH-Induced Growth Hormone (GH) Release in Rats of Both Sexes: Changes in Pituitary Response after Gonadectomy and During the Estrous Cycle[1] (39649)

S. R. OJEDA, A. CASTRO-VAZQUEZ,[2] AND S. M. McCANN

Department of Physiology, The University of Texas Health Science Center at Dallas, Southwestern Medical School, Dallas, Texas 75235

Thyrotropin-releasing hormone (TRH) is known to stimulate at high doses the release of pituitary GH both *in vivo* and *in vitro* (1-4). TRH can also stimulate GH secretion in patients with anorexia nervosa, renal failure, mental depression or acromegaly and in lactating cows (5-9).

To determine if the gonads might influence the pituitary GH response to TRH, the neurohormone was injected intravenously into ovariectomized or intact female rats at different phases of the estrous cycle and in intact or castrated male rats and its effect on pituitary GH release was evaluated by measuring radioimmunoassayable plasma GH levels following the injection.

Materials and methods. Young adult male and female rats (55-60 days of age) of the Holtzman strain (Holtzman Co., Madison, Wisconsin) were used. They were housed under controlled conditions of lighting (14 hr on, 10 hr off) and temperature (24-26°). Tap water and Purina laboratory chow were available *ad libitum*.

Gonadectomy. Males were castrated 6 days before TRH injection through a single midline incision in the scrotum. Female rats were castrated at day 22-23 of age via the dorsal approach. Ether anesthesia was used for all operative procedures.

TRH injections. Animals were anesthetized with tribromoethanol (10) at a dose of 25 mg/100 g body wt administered ip 60 min before injecting the TRH. Maintenance doses of the anesthetic (1/2 the initial dose) were administered sc 45 and 5 min before TRH injection. The tripeptide (Beckman) was dissolved in 0.9% NaCl solution at a concentration of 8.5 μg/ml and injected iv at a dose of 850 ng/100 g body wt. Control animals were injected with saline solution (0.1 ml/100 g body wt). Blood samples were drawn from the jugular vein into heparinized syringes immediately before or at 2, 5, and 10 min following the injection of TRH or the diluent. When cyclic females were used, vaginal smears were obtained daily and only those rats exhibiting at least two consecutive 4-day cycles were injected with TRH. All experiments were performed in the morning.

Pituitary GH measurements. In another experiment, different groups of animals were decapitated, their pituitaries dissected out, the neurohypophysis removed and the anterior lobe placed on dry ice. Thereafter, the glands were weighed to the nearest 0.1 mg and homogenized in cold 0.9% NaCl. Following low speed centrifugation, the supernatants were separated and stored at −20° until assayed. When pituitary GH of intact female rats was determined, the animals were classified according to the vaginal cytology they presented on the day of sacrifice. All animals were killed in the morning.

Radioimmunoassay. Plasma and pituitary GH were measured by radioimmunoassay using a kit supplied by the NIAMDD.[3] To avoid interassay variation, samples from a complete experiment were assayed in the same assay. Intra-assay variation was 9.5%. Interassay variation was 12.7%. Results are expressed in terms of the NIAMDD rat GH-RP-1 standard supplied with the kit.

Statistics. Significance of differences between means of two groups was determined by Student's *t* test. Differences between pre-

[1] Supported by grants from NIH (AM 10073 and HD 05151), the Ford Foundation, and the World Health Organization.

[2] Postdoctoral Research Fellow of the World Health Organization. Present address: Instituto Latinoamericano de Fisiologia de la Reproduction, Universidad del Salvador, C.C. 10, San Miguel, Argentina.

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and postinjection hormone values in the same group were analyzed with the paired *t* test.

Results. Initial plasma GH levels. One hour after initiation of tribromoethanol anesthesia, plasma GH levels were significantly higher ($P < 0.005$) in intact male rats (151 ± 25 ng/ml) than in castrated males (69.1 ± 9.1 ng/ml) or than those in intact and castrated females ($P < 0.025$-$P < 0.005$). Although GH levels of estrous females (72 ± 10 ng/ml) tended to be greater than those of females in proestrus (47 ± 5 ng/ml), diestrus I (48 ± 8 ng/ml), diestrus II (60 ± 10 ng/ml), or in ovariectomized animals (46 ± 4 ng/ml), these differences were not statistically significant.

Effect of TRH on GH release in female rats. The injection of TRH into intact female rats induced a significant increase in plasma GH at all phases of the estrous cycle with the exception of diestrus day 2 (Fig. 1). Maximal mean responses were observed in all cases at 2 min following the injection. Although the GH release induced by TRH at 2, 5, and 10 min after its injection was greater in estrus than at the other phases of the cycle, these differences were not statistically significant. Long term ovariectomy (at day 22) decreased the GH response to TRH of 55-day-old rats, the increase in plasma GH being significant only at 5 min ($P < 0.025$).

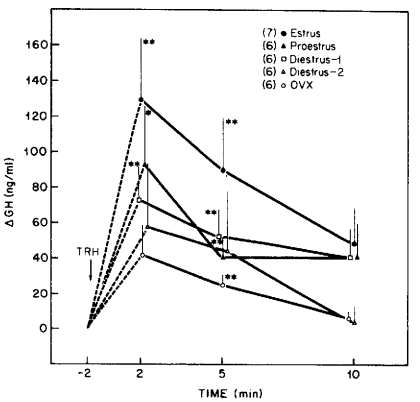

FIG. 1. Growth hormone release induced by TRH (850 ng/100 g body wt) at different phases of the estrous cycle and in ovariectomized rats. * = $P < 0.05$ with respect to initial value, ** = $P < 0.025$ with respect to initial value. In this and subsequent figures, vertical lines represent standard error of the mean and numbers in parentheses next to the key indicate number of animals used.

Effect of TRH on GH release in male rats. Intact male rats injected with TRH showed an increase in plasma GH levels greater than that of females injected with the neurohormone at any stage of the cycle (Fig. 2). GH titers were maximal at 2 min ($P < 0.01$) and decreased rapidly thereafter, although they were still significantly elevated at 5 min ($P < 0.05$). When TRH was injected in castrated male rats, the GH response to the tripeptide was significantly ($P < 0.005$) blunted. The small increase in plasma GH observed after TRH in these animals was significant only at 2 min ($P < 0.05$).

Maximal increment in plasma GH levels induced by TRH in male and female rats. The maximal increase in GH levels following TRH was not always attained at the same time in animals of the same group. Therefore, the maximal GH increment induced by TRH in each animal was determined and the mean values obtained for the different groups were compared. Figure 3 illustrates these results. Ovariectomized rats released less GH than rats in estrus ($P < 0.05$) but not significantly less than animals in proestrus, diestrus day 1 or diestrus day

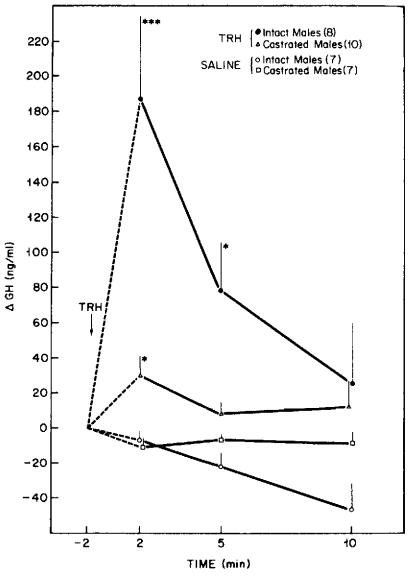

FIG. 2. Effect of gonadectomy on the GH release induced by TRH injection (850 ng/100 g body wt) in male rats. * = $P < 0.05$ with respect to initial value, *** = $P < 0.01$ with respect to initial value.

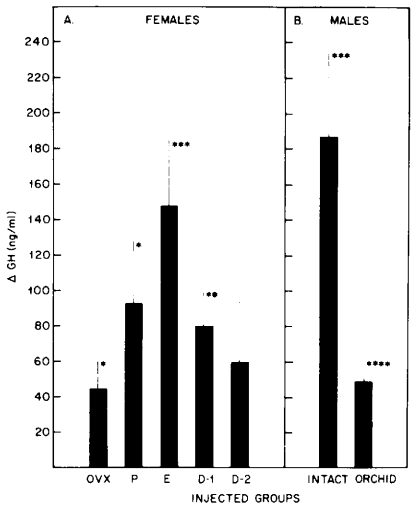

FIG. 3. Comparison of the effect of TRH (850 ng/100 g body wt) on GH release in intact or castrated female and male rats. The figure depicts the maximal increment in GH observed at any of the postinjection times studied (2, 5, and 10 min). Abbreviations: OVX, ovariectomized; P, proestrus; E, estrus; D-1, diestrus day 1; D-2, diestrus day 2; Orchid, orchidectomized. **** = $P < 0.005$. For other statistical significances, see Figs. 1 and 2.

2. The GH response to TRH was greater ($P < 0.05$) in estrus than in diestrus day 2.

Intact male rats released much more GH in response to TRH than castrated animals ($P < 0.01$). In spite of the dramatic decrease in GH response after castration, TRH was still able to induce a significant ($P < 0.005$) release in the castrated animals.

Pituitary GH in intact or castrated male and female rats. Pituitary GH content and concentration tended to be higher in intact females in diestrus than in animals in estrus (Table I). However, this difference was not statistically significant. Long-term ovariectomy resulted in a significant increase in both content ($P < 0.01$) and concentration ($P < 0.005$) of GH in comparison to the GH content in female rats in estrus. Pituitaries of male rats had significantly greater GH content ($P < 0.005$) and concentration ($P < 0.001$) than intact female rats. Orchidectomy resulted in a decrease in both content ($P < 0.005$) and concentration ($P < 0.01$) of GH in the gland.

Discussion. The present results show that GH release induced by TRH is facilitated by the presence of the gonads in both male and female rats and that in the female, cyclic variations in ovarian activity which occur during the estrous cycle of the animal also result in changes in pituitary GH responsiveness to the tripeptide. Although it is conceivable that the anesthetic procedure used may have influenced the results, other authors (4) have demonstrated that the GH response to TRH injection is not different between animals maintained constantly anesthetized or animals that were briefly etherized during the collection of blood samples. We have recently found that the pattern of GH release induced by TRH in conscious, free moving female rats is essentially the same as that found in tribromoethanol anesthetized rats.

That TRH acts on the pituitary to induce GH release has been convincingly demonstrated by the observations that TRH can induce GH release in rats with extensive hypothalamic ablation (4) when infused into the portal vessels (2) or *in vitro* from perifused rat hemipituitaries (3).

Although after column chromatography of hypothalamic extracts, GH-releasing factor (GH-RF) was found to have an elution volume similar to that of TRH (11–13), TRH alone could not account for the whole GH-releasing activity of the purified hypothalamic extract (12). This and other observations (1–3) have led to the suggestion (3,

TABLE I. PITUITARY GH CONTENT AND CONCENTRATION OF ADULT (55-56 DAYS OF AGE) INTACT AND CASTRATED MALE AND FEMALE RATS.

Groups	No. of rats	GH content (μg/pit)	GH concentration (μg/mg)
Intact females (estrus)	16	365 ± 27[1]	43.9 ± 3
Intact females (diestrus)	12	427 ± 37	54.3 ± 5
Castrated[2] females	4	542 ± 35[a]	70.5 ± 11.8[b]
Intact males	9	632 ± 41[c]	86.9 ± 6.7[c]
Castrated[3] males	7	381 ± 58[d]	56.9 ± 7.5[e]

[1] Mean ± SEM
[2] Ovariectomized at day 22–23 of age.
[3] Orchidectomized 6 days before decapitation.
[a] $P < 0.01$ vs estrus.
[b] $P < 0.005$ vs estrus.
[c] $P < 0.005$ vs females in diestrous phase.
[d] $P < 0.005$ vs intact males.
[e] $P < 0.001$ vs intact males.

4) that TRH and GH-RF may share certain common structural features which can account for their stimulatory action on GH release.

The diminished GH response to TRH following gonadectomy is in all likelihood due to removal of gonadal steroids. A facilitative effect of sex steroids on basal growth hormone release has been suggested earlier (14–16), and, in fact, estrogen treatment has been shown to increase circulating GH levels in female rats (17). Malacara and Reichlin (13) previously reported that treatment with estrogen and progesterone sensitized the pituitary gland to the GH-releasing effect of porcine hypothalamic extracts. In further support of the concept of a modulatory action of gonadal steroids at the pituitary level on GH secretion are the observations that following gonadectomy there were marked changes in pituitary GH content and concentration. Interestingly, pituitary GH increased after ovariectomy and decreased after orchidectomy, a finding that confirms the earlier report of Birge *et al.* (18) who also showed that testosterone treatment resulted in an increase in pituitary GH content and concentration, whereas estrogen led to a decrease in pituitary concentration of the hormone. It is unlikely that the saline extraction of pituitary GH used in the present experiments influenced the measurement of GH because the results were in complete agreement with those of Birge *et al.* (18) who homogenized the glands in 0.01 *N* NaOH. Moreover, these authors found that the mean yield of GH in homogenates of NaOH, acetic acid or phosphosaline buffer as measured by RIA did not significantly differ between the different groups.

Although the administration of both estrogen and androgens enhances the GH response to provocative stimuli (14–16, 19), the mechanism by which these steroids exert their effect on GH release is probably different since they have opposite effects on pituitary GH stores. The greater GH responsiveness of male than female rats to TRH may be related to the fact that pituitary GH content and concentration in males is greater than in females. Interestingly enough, in the human, the GH response to provocative stimuli is clearly greater in females than in males (14–16, 19). In the rat, the existence of a sex difference in GH content was suggested by Jones *et al.* (20) and clearly established with the use of radioimmunoassay by Birge *et al.* (18). Lower pituitary GH values in female rats are probably related to an increase release of the hormone induced by circulating estrogen levels (17) without a concomitant increase in the rate of synthesis. Higher basal plasma GH levels in female than in male ambulatory patients have been reported (21) as well as an increase in plasma GH levels during the preovulatory period (22), a time at which estradiol levels also rise (23).

Dickerman *et al.* (17) reported that plasma GH levels were higher in estrus than at other phases of the cycle. The present finding that GH responsiveness to TRH is also more pronounced in estrus suggests that an increased GH response to GH-RF and/or TRH can at least in part account for the elevated GH levels observed at that phase of the cycle.

The fact that TRH can induce GH release and that the magnitude of this response is modified by altered thyroid (24) or gonadal status raises the possibility that TRH can be used as a tool to study the capacity of the pituitary gland, to release GH under varied physiological or pathological conditions.

Summary. In both intact adult male and female rats anesthetized with tribromoethanol, the iv injection of thyrotropin-releasing hormone (TRH) evoked GH release within 2 min following the injection. The GH response to TRH was greater in males than in females and this response was significantly diminished by short-term (6 days) orchidectomy. In females, the GH response to TRH was maximal in estrus and minimal in diestrus day 2, showing intermediate values at proestrus and diestrus day 1. Long-term ovariectomy decreased the response. Pituitary GH content and concentration were higher in males than in females. Orchidectomy was followed by a significant decrease in pituitary GH values, whereas ovariectomy resulted in increased GH content and concentration. The results indicate that the gonads can modify pituitary GH response to TRH and, therefore, play a physi-

ological role in modulating the stimulatory effect that the hypothalamus exerts on GH release through the secretion of substances with GH-releasing activity.

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