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Effects of Intravenous Infusion of TRH on Plasma TSH and Prolactin Concentrations in Rats (39718)

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Recent studies employing iv injection(s) of LH-releasing hormone (LHRH) have suggested that both endogenous (1) and exogenous (2) LHRH can prime the adenohypophysis to release an increased quantity of LH in response to additional stimulation with LHRH. In additional studies (3, 4) which support this view, two stages of pituitary LH and FSH release were clearly revealed by continuous constant-rate iv infusion of LHRH. Plasma LH and FSH concentrations rose a relatively small amount for approximately 40 min during LHRH infusion. This was followed by "augmented response" in which a rapid and marked increase in plasma LH and FSH concentrations occurred. The lag period of approximately 40 min could not be advanced by increasing the LHRH infusion rate, and the response did not require intact ovaries during infusion. These studies (3, 4) also revealed that a pituitary "refractory" mechanism exists, at least in the case of LH release. After a period of continued stimulation, the pituitary releases only minimal amounts of LH even though it contains substantial stores of LH and continues to be stimulated by LHRH.

It is of importance to determine whether release of other pituitary hormones can be governed by a "priming" and/or "refractory" mechanism. Synthetic TSH-releasing hormone (TRH) is readily available, and administration of TRH has been reported to increase plasma prolactin as well as TSH in female rats (5, 6). The results in male rats are conflicting (6-8). The present investigation was undertaken to determine whether a "priming" and/or "refractory" mechanism exists for pituitary TSH and prolactin release via the TRH stimulation mechanism.

Materials and methods. Sprague-Dawley (Simonsen) rats were housed in a room with the lights on from 0500-1900 hr. The stages of the estrous cycle were monitored by daily vaginal lavage and only rats which exhibited two or more consecutive 4- or 5-day estrous cycles were used. Four-day cyclic rats (220-262 g) were used on proestrus or during the first day of diestrus. Five-day cyclic rats were ovariectomized and used 4 to 6 weeks later (271-322 g). Sixteen male rats (210-232 g) were also used.

In all rats, two cannulas were inserted into the right atrium of the heart between 0530 and 0700 hr, as described previously (3). Saline or TRH (Beckman, lot B0129) in saline was infused through one cannula from 1100-1600 hr and blood (0.35 ml) was withdrawn through the other cannula immediately before and at various times during infusion by the techniques described previously (3).

Plasma TSH and prolactin concentrations were measured by radioimmunoassay, with use of the kits and instructions provided by NIAMDD. Values are expressed as nanograms per milliliter of plasma in terms of the NIAMDD standards, which have biological potencies equivalent to 0.22 USP of (bovine) TSH U/mg and 11 IU/mg for TSH and prolactin, respectively. Student's t test (unpaired samples) was used to analyze the data. A P value < 0.05 was considered to be the acceptable level of significance.

Although TRH was diluted in sterile saline immediately prior to all infusions, the possibility existed that the TRH might decompose significantly over the infusion period. The following bioassay suggests that no significant decomposition took place. Additional rats on the first day of diestrus were fitted with a single indwelling atrial cannula and were injected with 10 or 100 ng of TRH with a freshly made TRH solution or a TRH solution which was allowed to stand at room temperature for 5 hr be-

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fore injection. The rises in plasma TSH concentration after injection of the two TRH preparations at either dose level were not significantly different from each other and were similar to the values plotted in Fig. 4 of a previous paper (5). Four rats were used for experimentation in each of the four groups.

Results. Effects of TRH on plasma TSH. In rats on the first day of diestrus, infusion of 1 μg of TRH/hr caused a marked elevation ($P < 0.001$) in plasma TSH concentration within 15 min (Fig. 1, top). Mean plasma TSH concentration remained high and showed no significant change over the next 105 min. Plasma TSH declined during the next 3 hr of infusion but was still significantly elevated ($P < 0.001$) at the end of the 5-hr infusion period. Infusion of TRH at the 100 ng/hr rate also produced a significant ($P < 0.01$) increase in plasma TSH concentration. However, plasma TSH in the 100 ng/hr group remained at elevated levels around a plateau for the entire 5-hr infusion period. Plasma TSH concentration remained fairly constant and did not change significantly for the 5-hr period in saline-infused rats and in rats infused with 1 or 10 ng of TRH/hr. The TSH values in the groups of rats given saline or 1 ng of TRH/hr were combined to facilitate plotting of the data. Very similar patterns in plasma TSH concentration were seen in rats infused with saline or the different dosages of TRH in proestrous rats (top of Fig. 2) and in four ovariectomized and four male rats (not illustrated).

Effects of TRH on plasma prolactin. Plasma prolactin concentration showed minor fluctuations during infusion of saline or 1, 10, or 100 ng of TRH/hr in rats on the first day of diestrus but no statistically significant differences were measured during the 5-hr period (Fig. 1, bottom). On the other hand, infusion of 1 μg of TRH/hr caused a small but statistically significant increase ($P < 0.05$) in plasma prolactin, but only at 15 and 30 min after the start of infusion. Similar patterns in plasma prolactin concentration were seen in proestrous rats during the first 2 hr of infusion except that the brief elevation in plasma

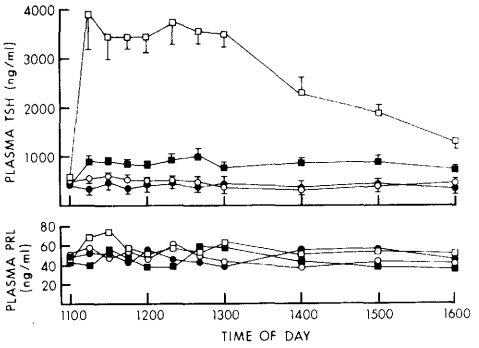

FIG. 1. Mean plasma TSH and prolactin concentrations in rats on the first day of diestrus. Rats were infused with saline or 1 ng of TRH/hr (shaded circles), 10 ng of TRH/hr (unshaded circles), 100 ng of TRH/hr (shaded squares), or 1 μg of TRH/hr (unshaded squares) from 1100–1600 hr. Plasma TSH and prolactin were measured on the same samples. Each group consisted of four rats. Standard errors are not plotted on mean TSH values at 1100 hr and on all mean PRL values to make display of the data more clear.

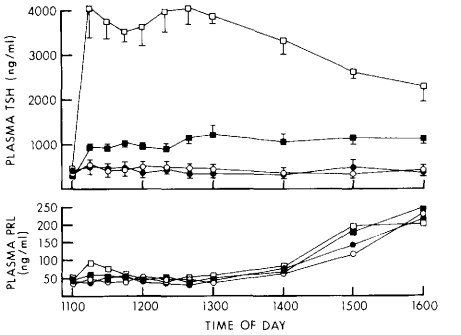

FIG. 2. Mean plasma TSH and prolactin concentrations in proestrous rats. See legend to Fig. 1 for details.

prolactin in the 1 μg/hr group was more pronounced ($P < 0.05$) at 15 min after the start of infusion (Fig. 2, bottom versus Fig. 1, bottom). Within 3 hr of infusion plasma prolactin concentration had started to rise and it was markedly elevated ($P < 0.001$) at 1500 and 1600 hr in all groups of proestrous rats (Fig. 2, bottom). Plasma prolactin was not elevated after the start of infusion of TRH at 1 μg/hr in both ovariectomized and male rats (not illustrated).

Discussion. The results of this study strongly indicate that TRH does not prime the adenohypophysis to release increased amounts of TSH or prolactin in response to

additional stimulation by TRH. Maximal or near-maximal levels of plasma TSH and prolactin were reached within 15 or 30 min after the start of infusion of TRH at 1 μg/hr and, in the case of TSH, at the 100 ng/hr infusion rate as well. Infusion of TRH at lower rates did not elevate plasma TSH or prolactin initially, and these hormones did not rise in plasma after continued infusion. Since TRH failed to "prime" the pituitary in females with widely different steroidal background milieu and in males, it is unlikely that TRH can prime the pituitary to itself under other *in vivo* conditions.

The rise in plasma prolactin concentration during TRH infusion at 1 μg/hr was similar to the rise seen after a rapid injection of TRH. The rise in the cyclic rats was small and brief. The fact that plasma prolactin did not remain elevated very long, even though the infusion of TRH was continued, indicates that either only a small amount of the total radioimmunoassayable prolactin in the pituitary can be released by TRH and that this pool is not replenished rapidly, or that some factor released during TRH infusion is blocking TRH-induced prolactin release. Were it not for the failure of saline and low dosages of TRH to elevate plasma prolactin during infusion, one might consider the TRH-induced prolactin release to be only a "stress" response. Nevertheless, it is possible that some (9, 10) but not all (5) types of stress- or drug-induced prolactin release may be mediated by TRH release. In any event, prolonged exposure of the pituitary to TRH did not interfere with the onset or magnitude of the spontaneous prolactin surge on proestrus.

Since plasma prolactin did not rise in ovariectomized and male rats infused at the 1 μg/hr rate, it is suggested that estrogen may facilitate TRH-induced prolactin release. The fact that the increase in plasma prolactin was greater in proestrous than in diestrous rats supports this view since circulating estrogens are highest during proestrus in this strain of rat (11). It is possible that had TRH been infused at considerably higher rates, an increase in plasma prolactin might be measured in ovariectomized and male rats. The need for a high dosage of TRH to elevate plasma prolactin in males has been pointed out by Mueller *et al.* (6).

Although all releasing hormones do not prime the pituitary to themselves, as shown in the present study, the results should not be interpreted to mean that other prolactin-releasing hormones or other releasing hormones in addition to LHRH may not produce priming effects on pituitary cells. On the other hand, it may very well be that the LHRH priming mechanism is unique. Although the LHRH priming response was induced experimentally, it now appears to be of physiological significance. The evidence is strong that the preovulatory surges in plasma LH and FSH which start during the afternoon of proestrus involve priming of the pituitary to additional LHRH by LHRH itself (3, 4).

Plasma TSH concentration rose rapidly to high levels and was maintained at a plateau until at least 2 hr after the start of infusion of TRH at 1 μg/hr. Thereafter, there was a diminution of TSH release in response to presumably constant blood levels of TRH. This decrease was similar to the decline in plasma LH after approximately 3.5 hr of continuous LHRH infusion at a rate which simulated the proestrous LH surge (3). In the case of LH, it was demonstrated that the pituitary refractory mechanism did not appear to be the result of a depletion of total radioimmunoassayable pituitary LH stores or any gonadal feedback mechanism (3). Since pituitary TSH concentration was not measured and since TRH was not infused in thyroidectomized rats, it is not possible to ascertain whether the decline in plasma TSH concentration is a reflection of reduced pituitary TSH stores or the result of a negative feedback mechanism of thyroid hormone(s) on the adenohypophysis. Alternatively, the decrease in plasma TSH could be due to some other mechanism, as in the case for LH release.

In contrast to pituitary refractoriness to LHRH-induced LH release which plays a role in ending the preovulatory surge of LH in plasma (3), the physiological signifi-

cance, if any, of pituitary refractoriness to TRH-induced TSH release remains to be determined. Studies utilizing ultrastructural immunocytochemical techniques coupled with measurements of pituitary TSH content in normal and thyroidectomized TRH-infused rats are underway to elucidate the mechanism responsible for decreased pituitary TSH output.

Summary. In rats, blood was withdrawn through one of two indwelling atrial cannulas while saline or TRH in saline was infused at a constant rate for 5 hr through the other. Plasma TSH and prolactin were measured by radioimmunoassay. Infusion of TRH at 100 ng or 1 μg/hr in ovariectomized and male rats as well as 4-day cyclic rats on proestrus and the first day of diestrus caused a rapid rise in plasma TSH which remained at a plateau for 5 and 2 hr, respectively. In the 1 μg/hr group, plasma TSH declined progressively during the last 3 hr of infusion. No increases in plasma TSH were measured during the 5 hr period after infusion of saline or TRH at 1 or 10 ng/hr. Plasma prolactin rose a small but significant amount only in cyclic rats infused at the 1 μg/hr rate but it decreased to basal values after 30 min of infusion even though infusion was continued. The results suggest that TRH does not prime the pituitary to release greater quantities of TSH or prolactin in response to additional stimulation by TRH. They also suggest that a pituitary refractory mechanism for TRH-induced TSH release exists, but it remains to be determined whether any physiological significance can be attributed to this refractory mechanism.

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