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Plasma Growth Hormone Concentrations after Cerebroventricular and Jugular Injection of Thyrotropin-Releasing Hormone[1] (39949)

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Vascular injection of thyrotropin-releasing hormone (TRH) not only elevates plasma thyrotrophic hormone (TSH) levels, it also increases plasma growth hormone (GH) and prolactin concentrations (1-3). This TRH stimulation of GH secretion has been observed in the cow (2, 4) and rat (3, 5, 6) and in humans with certain neural and pituitary disorders (7-10). Since TRH stimulation of GH secretion occurs in pituitary gland cultures (11-13) and in hypophysectomized rats bearing ectopic pituitary transplants (14-16), the primary site of TRH action appears to be the pituitary gland. However, TRH is also capable of altering neural function after systemic injection (3, 17, 18), thus suggesting that the central nervous system (CNS) might also be a site for TRH action. To test the possible CNS effect of TRH, we compared plasma GH levels in unanesthetized calves after cerebroventricular and jugular injection of TRH.

Materials and methods. The three Guernsey bull calves (40-60 kg, 4-6 months old) used in these studies were housed in a controlled environment with 20 ± 1° temperature and 14 hr of light per day. A maintenance ration was fed twice daily, and water was provided *ad libitum*. The calves were prepared with lateral ventricle guide cannulae at least 1 month before the start of the experiments [for details, see (19)]. The patency of the lateral ventricle route was determined by withdrawing clear fluid at the beginning and end of the study. Also, cannula locations were confirmed by gross dissection during autopsy.

Intraventricular and intravascular injections of synthetic TRH (Abbott Laboratories, North Chicago) in 0.9% saline were given once to each calf at doses of 0, 12.5, 50, 100, and 200 μg. Injection volumes were 0.1 ml ventricular and 1 ml jugular. All injection solutions were sterilized by filtration. Chronic jugular vein catheters, installed several days before the start of the experiment, were used for this blood sampling and injection. For lateral ventricle injection, the injector cannula, tubing, and syringe were completely prefilled with the treatment solution. After injection, the cannula was left in place for at least 1 min. Following jugular injection of the treatment solution, the catheter was flushed with 3 ml of saline. Six milliliters of blood were withdrawn from the jugular vein with heparinized syringes 10 min before injection and 10, 20, 30, 60, 90, and 180 min after either ventricular or jugular injection. A minimum of 2 days lapsed between the start of individual animal treatments. After centrifugation, the plasma was stored frozen until radioimmunoassay.

Bovine GH (NIH-B1003A) was used for standards and for iodination. Iodination was done by the methods of Greenwood *et al.* (20) and Sinha *et al.* (21). The antibody to bovine GH was prepared from baboon antisera and was a gift from Dr. L. Machlin, Monsanto Co., St. Louis, Missouri. Duplicate determinations were made on each plasma sample, while standards were assayed in triplicate. Each assay tube contained 100 μl of plasma or standard, 200 μl of antibody (1:90,000 dilution), and 200 μl of ^{125}I-labeled bovine GH containing 10,000 cpm. The antibody and labeled hormone solutions were prepared in 0.025 *M* barbital buffer (pH 8.6) and 1% bovine serum albumin. After 24 hr of incubation at 4°, the antibody was precipitated with polyethylene glycol (22). With zero standards, the antibody bound 65% of the total

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labeled hormone. Plasma estimates were calculated from counts of bound hormone by log-logit transformation method (23). Assay sensitivity for plasma GH was 0.5 ng/ml. The coefficient of intraassay variation was 9.6%, while the interassay variation was 12.0%.

Data were analyzed by analysis of variance and Duncan's new multiple range test (24).

Results. GH concentrations averaged (±standard error) 9.1 ± 0.6 ng/ml (N = 30) for all preinjection samples and 10.2 ± 0.9 ng/ml (N = 30) for samples taken 180 min after injection. None of the lateral ventricle injections had any effect on plasma GH (Fig. 1), while 50-, 100-, and 200-μg doses of TRH by jugular injection elevated plasma GH levels (Fig. 2). Within 10 min of jugular injection of TRH, plasma GH reached maximal levels; these increases in GH were approximately proportional to the TRH dose, with the exception of the 200-μg dose. By comparison with the preinjection concentrations, plasma GH levels were significantly elevated 10 and 20 min following jugular injection of 50, 100, and 200 μg of TRH ($P < 0.05$), and 30 min after 200 μg of TRH ($P < 0.05$). Sixty minutes after jugular injection, GH levels averaged 8.2 ng/ml, compared to the preinjection level of 9.7 ng/ml. None of the TRH injections had any effects on gross behavior.

Discussion. These observations corroborate previous reports indicating that vascular injection of TRH rapidly (within 5 to 10 min) elevates plasma GH concentrations. Convey *et al.* (2), for instance, reported that in cows, jugular injection of 100 μg of TRH increased plasma GH from an average

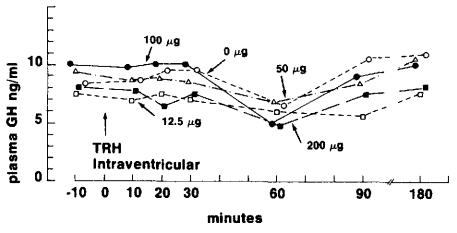

FIG. 1. Concentrations of plasma growth hormone after various doses (micrograms) of thyrotropin-releasing hormone (TRH) were injected into the lateral ventricles.

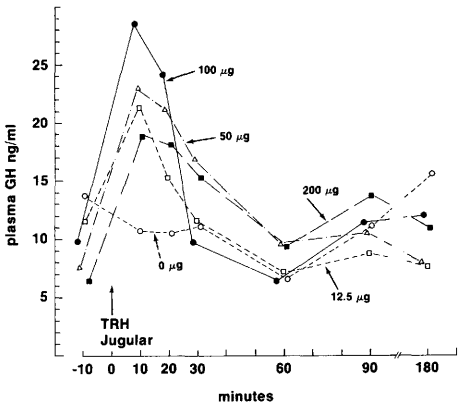

FIG. 2. Concentrations of plasma growth hormone after various doses (micrograms) of thyrotropin-releasing hormone (TRH) were injected into the jugular vein.

basal level of 8.2 ng/ml to a peak concentration of 25 ng within 10 min. This is quite similar to the threefold increase in GH levels that we observed in the calf. In anesthetized rats, Chihara *et al.* (3) observed a maximum GH response within 5 min of intravascular injection of TRH, whereas Ojeda *et al.* (6) reported that peak GH concentrations occurred within 2 min of TRH injection. This pattern of GH response to TRH is similar to changes in plasma levels of other adenohypophysial hormones following the injection of their respective hypophysiotropic hormones (25–27), and thus suggests that TRH may act directly on the pituitary gland to elevate plasma GH.

A direct effect of TRH on pituitary GH secretion is supported by several studies in rats. TRH stimulates GH secretion in hypophysectomized rats with ectopic pituitary transplants (14–16) and in rats with lesions of the median eminence (3). In some cases, TRH also stimulates GH release from the pituitary gland *in vitro* (11–13).

Although it is clear from data on the rat that TRH stimulation of GH secretion is primarily by direct action on the pituitary gland, there still remains a sufficient amount of evidence to suggest that TRH might also act directly on the CNS to alter anterior pituitary secretion (3, 17, 18, 28). For instance, neural effects may be involved when intraventricular or intravascular injec-

tion of TRH alters chlorpromazine-induced elevation of plasma GH levels in rats (3). Furthermore, when TRH is injected into the lateral ventricles of rats, it increases plasma GH levels, possibly by action on the CNS (3); however, because TRH can pass from cerebrospinal fluid to the hypophysial portal vessels (31), this effect could also be due to direct TRH stimulation of the pituitary gland. Several reports show that TRH stimulates prolactin secretion in certain animals *in vivo* but not from their pituitary glands *in vitro* (2, 29), thus suggesting that non-specific effects of TRH might initially involve extrapituitary sites such as those responsible for neural control of releasing hormones. Our results, however, suggest that it is unlikely that exogenous TRH stimulates GH secretion by direct action on the CNS because even a high dose of TRH (200 μg) injected into the lateral brain ventricles was without effect on plasma GH concentrations.

Finally, our data also suggest that the sensitivity of pituitary somatotrophs to TRH is much less than the sensitivity of thyrotrophs to TRH. We previously found, with these same calves, that as little as 25 μg of TRH injected into the lateral ventricles stimulated an increase in plasma thyroxine, and this stimulatory effect of TRH on thyroxine was dose dependent (30). It was concluded that TRH passed from the ventricles into the vascular circuit, possibly the hypophysial portal vessels, to stimulate thyrotrophin secretion. In the present study, even the highest intraventricular dose of TRH was not sufficient to stimulate GH secretion. It seems quite possible, therefore, that the "nonspecific" stimulation of GH secretion by TRH requires unnaturally high systemic concentations of TRH likely to be achieved only after vascular injection.

Summary. To determine if thyrotropin-releasing hormone (TRH) stimulates growth hormone (GH) secretion by acting on the central nervous system (CNS), we measured plasma GH concentrations in unanesthetized calves at various times after cerebroventricular and jugular injection of TRH. We found that even after the highest dose of TRH (200 μg), lateral ventricle injection of TRH had no detectable effect on plasma GH levels. With jugular injection, on the other hand, 50-, 100-, and 200-μg doses of TRH were effective in significantly elevating plasma GH levels. Maximum increases occurred within 10 min of injection and usually involved a threefold increase in GH from preinjection levels. In all cases, plasma GH concentrations returned to basal levels within 60 min postinjection. These results indicate that stimulation of GH secretion by exogenous TRH is probably due only to direct stimulation of the pituitary gland and does not involve action of TRH on the CNS.

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