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Effects of Thyrotropin-Releasing Hormone on Plasma Thyroxine in the Calf: Comparison of Intraventricular and Intravenous Injection Routes[1] (40007)

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Several investigators have examined the possible role of cerebrospinal fluid (CSF) in the transport of thyrotropin-releasing hormone (TRH) to the pituitary gland by comparing the relative effectiveness of intraventricular and intravascular TRH in altering pituitary or thyroid function. On the basis that CSF offers a smaller dilution volume and a longer half-life for TRH (1), it was reasoned that, if CSF is involved in TRH transfer, intraventricular TRH injection might be more effective in stimulating the pituitary gland than intravenous injection. However, Gordon *et al.* (2) found that intraventricular TRH was much less effective in elevating plasma thyrotropin (TSH) than jugular injection, while Oliver *et al.* (3) reported that the two routes were about equal. Kendall *et al.* (1) concluded that lateral ventricular and jugular injections of TRH were equal in stimulating iodine release from the thyroid. These observations indicate that, although intraventricularly injected TRH does reach the pituitary gland, TRH by this route is not more effective than by the intravenous route. This suggests that CSF may not be important for the transport of TRH.

Each of these studies utilized rats anesthetized with agents known to alter the concentrations of plasma hormones including TSH (4–7). Thus, important differences between the two routes of TRH administration may have been masked in these studies by the anesthetic agents. The present study compares the relative effectiveness of various doses of intraventricular and intravascular TRH to alter thyroid gland function in unanesthetized calves in order to examine the possibility of CSF transport of TRH.

Materials and methods. The Guernsey bull calves (40–60 kg, 4–6 months old) used in these studies were housed in a controlled environment at 20 ± 1° and with 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 ventricular guide cannulae at least 1 month before the start of the experiments (8). The patency of the ventricular cannula was confirmed before and after each study by removing clear CSF. Cannula locations were also confirmed by dissection at autopsy.

In the first study, three calves were treated with intraventricular and intravascular injection of synthetic TRH (Abbott Laboratories, North Chicago) in 0.9% saline at doses of 0, 12.5, 25, 50, 100, and 200 μg. A minimum of 2 days lapsed between the start of these individual treatments. After a 200-μg dose, however, the next treatment was not given until 4 days later. Examination of the data indicated that these intervals were sufficient to allow for return of thyroxine to basal levels. All injection solutions were sterilized by filtration. Chronic jugular vein catheters, in place several days before the start of the experiment, were used for blood sampling and injection. Injection volumes were 0.1 ml for the ventricular route and 1 ml for the jugular route. For lateral ventricular injection, the injector cannula, tubing, and syringe were completely prefilled with the treatment solution. After ventricular 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 then 10, 20, and 30 min and 1, 1.5, 2, 3, 4, 5, 6, 8, 10, 12, and 23 hr after

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either ventricular or jugular injection. After centrifugation, the plasma was stored frozen until assay.

In the second study, five calves (three from Study I) were treated by the two routes with saline and 100 μg of TRH. Except for 8- and 10-hr samples, blood was withdrawn at the times listed for Study I. The other procedures used were as described above.

Thyroxine was measured in duplicate 25-μl plasma samples using the radioimmunoassay method of Premachandra and Ibrahim (9) as modified by Vanjonack (10), including the use of the charcoal separation technique of Herbert *et al.* (11). The coefficients of variation for pooled plasma samples were 9.1% between assays of 11.1% within assays. Data were analyzed by analysis of variance; individual sample means were compared at the 0.05 level by the least-significant difference procedure in Study I and by Duncan's new multiple-range test in Study II (12). The linear regression between thyroxine levels and first 11 sampling times was also calculated for each treatment group (13). All TRH injection groups, except the 12.5-μg intraventricular group, showed significant ($P < 0.0001$) linear regressions. Goodness of fit tests on these regressions indicated that the linear equations were an acceptable representation of the data.

Results. In the dose-response study (I), thyroxine levels reached their maxima 4 to 8 hr after intraventricular injection of TRH (Fig. 1A). Only in the 200-μg dose group did thyroxine levels remain significantly elevated 8, 10, and 12 hr after treatment. Twenty-three hours after injection, none of the groups showed significantly elevated thyroxine levels. On the basis of comparing means, there was no indication that the 12.5- and 25-μg dose groups were effective

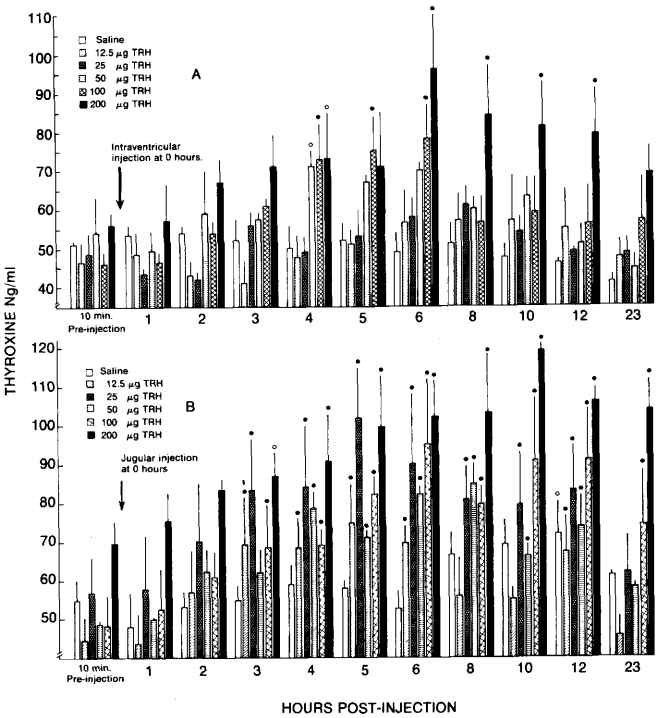

FIG. 1. Plasma thyroxine concentrations at various times following intraventricular (A) and jugular (B) injection of saline and five dose levels of thyrotropin-releasing hormone (TRH). Each bar represents the mean of duplicate determinations from three calves. Standard error of the mean is shown as a vertical line. A significant difference between a postinjection mean and a corresponding preinjection mean is shown either as a solid ($P < 0.05$) or an open circle ($P < 0.06$). Postinjection means for the 10-, 20-, 30-, and 90-min samples are shown on Fig. 2.

at any time. There was also no indication that thyroxine levels in any group at 10, 20, 30, or 90 min after injection were significantly changed from preinjection levels (these means are plotted on Fig. 2A).

Using only the first 6 hr of intraventricular data, regression analysis indicated that thyroxine concentrations increased linearly with time in the 200-, 100-, 50-, and 25-μg dosage groups (Fig. 2A). Slopes of the regression lines for these groups were different from zero ($P < 0.0001$). With saline and 12.5-μg TRH treatments, no significant linear regression was found. This analysis suggests that intraventricular TRH at dosages of 25 μg or more are effective in elevating plasma thyroxine levels.

Following jugular injection of TRH (Study I), thyroxine concentrations increased significantly ($P < 0.05$) from preinjection level to peak at 10 hr with 200 μg of TRH, at 6 hr with 100 μg, at 8 hr with 50 μg, at 5 hr with 25 μg (N.S.), and at 5 hr with 12.5 μg (Fig. 1B). At the 23-hr sample, only the 100- and 200-μg groups showed significantly elevated thyroxine levels ($P < 0.05$). The earliest significant ($P < 0.05$) elevations were seen at 3 hr when levels were increased above the preinjection levels in all groups except the 50-μg TRH.

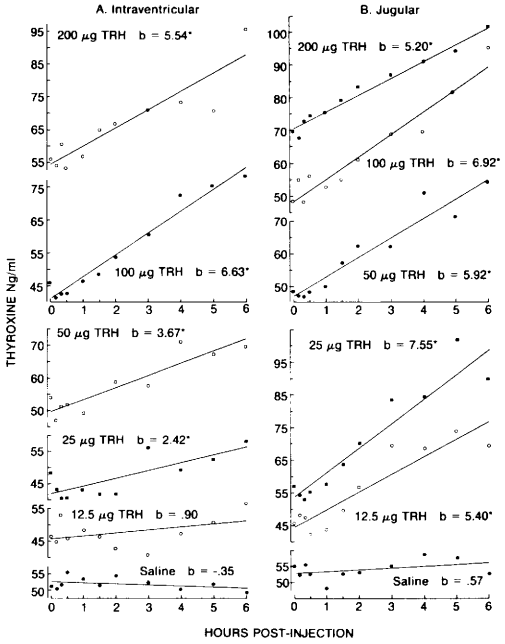

FIG. 2. Plasma thyroxine concentrations for the first 6 hr after intraventricular (A) and jugular (B) injection of saline and five dose levels of thyrotropin-releasing hormone (TRH). Each of the data points is the mean of determinations from three calves. Solid lines were fitted to individual values by the least-squares method. Asterisks show slopes that are different from zero ($P < 0.0001$).

Regression analysis of the first 6 hr of jugular data indicated that plasma thyroxine concentrations increased linearly with time with all TRH dosage groups (Fig. 2B). Slopes of all the TRH linear regression lines were different from zero ($P < 0.0001$) but none were different from each other, while saline injection data did not yield a significant linear regression. A t test comparison of regression slopes for the same doses between the two routes of TRH administration indicated significant differences ($P < 0.05$) between the routes with the 12.5-, 25-, and 50-μg dosage groups, but no differences with the 100- and 200-μg.

In the second study (II), involving five calves, comparisons were made between routes after injection of saline and 100 μg of TRH. By comparison to preinjection levels, thyroxine was elevated in both TRH groups at all sampling times from 1.5 to 23 hr (Fig. 3). Regression analysis indicated that thyroxine concentrations in both TRH-injected groups varied linearly with time for the first 6 hr. The slopes of the lines were 7.10 for the intraventricular route and 6.18 for the jugular route. These slopes were not significantly different from each other or from the slopes of 100-μg dose groups in Study I; however, they were different from zero ($P < 0.0001$). Lines fitted by least-squares method for the saline groups had slopes of 1.78 for the intraventricular route and .75 for the jugular routes; neither was significantly different from zero.

Discussion. The thyroxine response to TRH appears to be directly related to the TRH dosage. This relationship is clearest with ventricular injection at the 6-hr sample time (Fig. 1A) and with the rank order of change of thyroxine concentrations from preinjection to peak levels. Except for the 25-μg dose group, equivalent data from the jugular route treatment also shows a dose-response relationship (see Fig. 1B). Comparing the two routes, Kendall *et al.* (1) noted that thyroidal iodine release varied

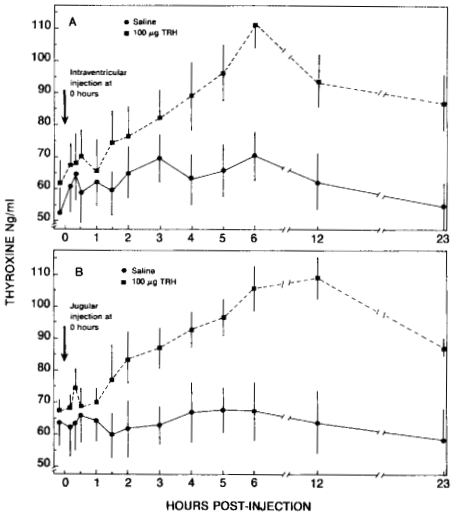

FIG. 3. Plasma thyroxine concentrations at various times after intraventricular (A) or jugular (B) injection of saline and 100 μg of thyrotropin-releasing hormone (TRH) (Study II). Each point shows the mean of five calves and the standard error.

directly with the TRH dose; while Oliver *et al.* (3) found dose-related changes in plasma TSH concentration.

At the highest dose levels (100 μg ≡ 2 μg/kg; 200 μg ≡ 4 μg/kg), the effectiveness of TRH to elevate plasma thyroxine was quite similar for the intraventricular and jugular routes (compare Figs. 1, 2, and 3). Kendall *et al.* (1), using etherized rats and 1.5 to 3 μg of TRH/kg, also found that the ability of TRH to stimulate thyroidal iodine release was equal for the two routes. Similarly, Oliver *et al.* (3) observed little difference between the two routes in terms of the effectiveness of 300 ng of TRH/kg to elevate plasma TSH levels in rats anesthetized with pentobarbital. These observations indicate that intraventricular TRH is transferred out of the brain and is effective in stimulating the pituitary–thyroid axis. The similarity of responses with anesthetized and unanesthetized animals suggests that, at these high TRH dose levels, anesthesia does not alter the ability of intraventricular TRH to stimulate the pituitary–thyroid axis.

At the lower dose levels, the ability of TRH to elevate thyroxine concentrations was clearly dependent upon the route of administration. All TRH dose levels by the jugular route were effective in elevating thyroxine concentrations, whereas only the three highest doses were clearly effective by the intraventricular route. Intraventricular 12.5-μg TRH (≡250 ng/kg) was not effective and the potency of 25 μg (≡500 ng/kg) was only marginal. Oliver *et al.* (3) reported that with anesthetized rats, 30 ng of TRH/kg injected into the cerebral ventricles produced a much smaller increase in plasma TSH levels than when the same dose was given by jugular injection. Using pentobarbital-anesthetized rats, Gordon *et al.* (2) injected 50 ng of TRH (≡170 ng/kg) at various sites and found that intrapituitary injection resulted in the greatest increase in plasma TSH while the intraventricular route produced the smallest increase. These observations indicate that when lower doses of TRH are used, ventricular TRH injection is clearly less effective than intravascular injection. Since the results from anesthetized animals are similar to those seen with the unanesthetized calves, anesthesia is apparently not a major factor in impeding the action of these lower doses of intraventricular TRH.

It is not possible, at the present time, to evaluate critically the relative importance of CSF transport of TRH because we do not know what portion, if any, of the total hypophysiotropic TRH actually travels through CSF to reach the pituitary gland. What the present study offers are answers to several questions important to the transport issue. Our results are consistent with previous reports (see above) which show that TRH can transfer from CSF to blood to stimulate pituitary and thyroid secretion. This transfer process is apparently not affected by anesthetics known to influence TSH secretion. The effectiveness of TRH to stimulate the pituitary–thyroid axis is clearly not identical for the two routes of administration. If the two routes were equal, this would suggest that movement of TRH from CSF to the pituitary gland is dependent primarily upon the same physical forces governing distribution in the circulatory system. Since the transfer of ventricular TRH to the pituitary gland appears to be impeded by comparison to vascular injection, cellular activity might be involved at some point in the transfer of TRH from CSF to blood.

Several observations suggest that the median eminence is involved in the transfer of substances from CSF to portal capillaries. Goldgefter (14) found that tritiated LH-RH injected into the lateral ventricle penetrated into subependymal areas of the median eminence by ordinary diffusion and accumulated in cells in the external zone by a nondiffusion process. Ependymal cells of the median eminence have been demonstrated to take up labeled LH-RH (15) and TRH (16, 17) following intraventricular injection. Labeled TRH is also detected in the anterior pituitary gland, as well as the median eminence, shortly after third ventricle infusion (18). Oliver *et al.* (3) found that after lateral ventricle injection of labeled TRH, both the appearance of TRH in portal blood and the increase in plasma TSH were delayed by comparison to intravenous TRH injection. Although our results do not indicate a route or mechanism for TRH action, the above studies suggest that transport of TRH by the median eminence may impede TRH transfer from CSF to blood and thus could account for the reduced effectiveness of the intraventricular route.

Summary. Various doses of TRH (12.5, 25, 50, 100, and 200 μg) were injected into the lateral ventricle and jugular vein of unanesthetized calves to examine the possibility of CSF transport of thyrotropin-releasing hormone (TRH). At higher doses (100 and 200 μg), there was no major difference in effectiveness of jugular and ventricular TRH to elevate thyroxine levels. Thyroxine levels were significantly elevated as early as 1.5 hr and peaks occurred 6 to 12 hr after 100 μg of TRH. At the lower doses (<100 μg), the effectiveness of TRH to elevate thyroxine levels was clearly route dependent. All TRH doses (<100 μg) by jugular injection were effective, while only the 50-μg intraventricular dose was clearly effective. These observations indirectly indicate that TRH is transferred from CSF to the vascular system. This transfer process, possibly involving cells of the median eminence, may impede the movement of TRH to blood and thus account for the reduced effectiveness of intraventricular TRH.

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