BOVINE SERUM HORMONE CONCENTRATIONS AFTER THYROPROTEIN AND THYROTROPIN RELEASING HORMONE

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SUMMARY

Twenty-four Holstein heifers were assigned randomly to one of four treatment groups to be given: 1) thyroprotein (TP) for 6 days; 2) casein for 6 days; 3) TP for 27 days; or 4) casein for 27 days. Thyrotropin releasing hormone (TRH, 33.3 µg/100 kg BW) was given at 1030 and 1330 hr on day 6 or 33 to heifers treated for 6 or 27 days, respectively.

Prior to TRH treatment, serum thyroxine (T4) averaged 133.8 and 82.3 ng/ml in heifers given TP or casein for 6 days and 24.0 and 82.2 ng/ml six days after feeding TP or casein for 27 days. Six hours following TRH, average serum T4 concentration had increased (35 ng/ml) in heifers given casein for 6 or 27 days, and 4.8 ng/ml in heifers given TP for 27 days, but remained unchanged in heifers fed TP for 6 days.

Basal serum thyrotropin (TSH), growth hormone (GH) and prolactin (PRL) concentrations were not affected by TP treatments, averaging 6.7 ng TSH/ml, 13.8 ng GH/ml, and 7.0 ng PRL/ml. Following TRH injection, TSH concentrations were increased to 26 ng/ml in heifers given casein for 6 or 27 days and to 20 ng/ml in heifers given thyroprotein for 27 days. However, TSH was unchanged after TRH given on the 6th day of TP feeding. Serum GH and PRL concentrations (ng/ml) were increased by TRH to 36 and 100, respectively, and responses were independent of TP treatments.

Magnitude of GH and PRL increases after the first TRH injection were greater than after the second. In addition, increase in serum PRL concentration after TRH was greater in heavier older than in lighter younger heifers.

We conclude that 1) changing serum T4 concentration does not alter basal serum TSH, GH or PRL concentrations; 2) increased T4 in serum inhibits TSH release by TRH but under our experimental conditions low serum T4 concentrations did not influence TSH release by TRH; 3) serum T4 concentrations do not alter the magnitude of increase in serum GH or PRL following TRH; 4) there is a reduction in the magnitude of increase in GH and PRL concentration following a second TRH challenge as compared with the first which is independent of serum T4 concentrations; and 5) PRL release by TRH is greater in heavier older than in lighter younger heifers.

(KEY WORDS: Thyroxine, TRH, Thyrotropin, Prolactin, Growth Hormone, Bovine.)

INTRODUCTION

In addition to causing secretion of thyrotropin (TSH), thyrotropin releasing hormone (TRH) causes release of prolactin (PRL) and growth hormone (GH) from pituitaries of several species (Tashjian et al., 1971; Jacobs et al., 1971; Irie et al., 1972; Fell et al., 1973; Convey et al., 1973). The thyroid hormones, thyroxine (T4) and triiodothyronine, participate in control of TSH secretion via a direct negative feedback on the pituitary (Vale et al., 1967; Schally and Redding, 1967). Thyroid hormones may also affect GH and PRL secretion. Changing thyroid hormone availability will alter basal serum GH and/or PRL concentrations in sheep and rats (Peake et al., 1973; David et al., 1973; Eisenberg et al., 1972; Nicoll et al., 1963) and their release by exogenous TRH in humans and rats (Snyder et al., 1973; Vale et al., 1973).
The role of thyroid hormones in control of pituitary hormone secretion in cattle is poorly defined. Objectives of this experiment were to describe effects of exogenous T₄ on basal serum concentrations and TRH-induced release of TSH, PRL and GH in prepubertal heifers.

Thyroprotein (TP), an iodinated casein containing about 1% T₄, was fed as an exogenous source of T₄. By feeding TP, a constant, relatively high serum T₄ concentration is maintained (Shaw et al., 1975), thus avoiding fluctuations in serum T₄ concentrations that characterize injection of this hormone. In addition, a marked decrease in serum T₄, to concentrations characteristic of hypothyroid conditions, followed cessation of TP feeding.

MATERIALS AND METHODS

Experimental Design. Twenty-four Holstein heifers, age 5 to 11 months, were assigned on the basis of body weight (BW) to one of six blocks of four heifers each. Body weight ranged from 115 to 290 kilograms. One heifer from each block was randomly assigned to one of four treatment groups. Twelve heifers were fed TP for either 6 or 27 days. Thyroprotein was fed at 6.6 g/100 kg BW/day on days 0 to 3 (to quickly establish high serum T₄ concentrations), then at 3.3 g/100 kg BW/day thereafter. The remaining 12 heifers served as controls, receiving casein in amounts and according to schedules described for TP above. The date that TP or casein treatments were begun was designated day 0. Casein and TP were given orally via capsule at approximately 1600 hours.

Thyrotropin releasing hormone (TRH, 33.3 μg/100 kg BW) was given via jugular cannula at 1030 and 1330 hr on day 6 or 33 to heifers treated for 6 or 27 days, respectively. Heifers were started on TP or casein treatments were begun was designated day 0. Casein and TP were given orally via capsule at approximately 1600 hours.

Thyrotropin was assayed using antisera previously described for measurement of ovine TSH (Borger and Davis, 1974). Antisera was absorbed, before use in immunoassay, with bovine LH (NIH-LH-B₈) and FSH (NIH-FSH-B₈) to remove nonspecific antibodies which reacted with these gonadotropins. Procedures for antisera absorption were described previously (Borger and Davis, 1974). Highly purified bovine TSH was used for iodination and NIH-TSH-B₄ was used for the standard.

The useful range of assay sensitivity for measuring bovine TSH was between .3 and 10 ng per tube. Dilution curves for two pools of bovine sera, containing low or high concentrations of TSH, were parallel to the TSH standard curve. Known quantities of NIH-TSH-B₄ added to bovine sera were quantitatively recovered (98.7%). Linear regression of amount of NIH-TSH-B₄ added on amount of TSH recovered gave a line with a slope of 1.20 and an intercept of -.16. Neither the slope nor the intercept were different (P>.4) from the ideal of 1.0 and 0, respectively. Bovine GH (NIH-GH-B₁), follicle stimulating hormone (NIH-FSH-B₁), LH (NIH-LH-B₈) at concentrations up to 500 μg/500 μl reaction volume caused negligible reductions in binding of labeled TSH. The minor degree of displacement that did occur could be accounted for on the basis of known TSH contamination of the NIH preparations used.
A few heifers began to demonstrate estrual activity during this experiment. Therefore, selected serum samples were assayed for progesterone (Louis et al., 1973) to identify heifers that had ovulated.

Analysis of variance of these repeat measurement data was patterned after that described by Gill and Hafs (1971). Significance of differences between main effects or blocks were determined by using Bonferroni's t-test (Miller, 1966).

RESULTS AND DISCUSSION

Serum T<sub>4</sub> concentrations ranged from 58 to 83 ng/ml over the treatment period in heifers fed casein for 6 or 27 days (figure 1). Serum T<sub>4</sub> concentrations in heifers given TP for 6 or 27 days averaged 75.9 and 60.5 ng/ml, respectively, before TP feeding (days -3, and 0) and increased (P<0.05) to 138.8 and 116.2 ng/ml, respectively, by day 6 of TP treatment. In heifers given TP for 27 days, serum T<sub>4</sub> concentrations on days 8, 12 and 16 were greater (P<0.05) than corresponding concentrations for controls. Cessation of TP feeding on day 28 resulted in a marked decline in serum T<sub>4</sub> concentrations comparable in duration and magnitude to that seen under similar circumstances in lactating cows (Shaw et al., 1975). On day 33, serum T<sub>4</sub> averaged 24.0 ng/ml in heifers fed TP for 27 days, which was lower (P<0.05) than corresponding concentrations for controls (82.2 ng/ml). The marked decrease in serum T<sub>4</sub> concentrations observed in lactating cows (Shaw et al., 1975) and heifers following cessation of TP feeding may result from increased T<sub>4</sub> metabolic clearance rate (Premachandra et al., 1961) and/or a decrease in endogenous T<sub>4</sub> secretion due to latent suppression of the thyroid after negative feedback inhibition of TSH release by increased serum T<sub>4</sub> concentrations (Krugman et al., 1975). These alterations in serum T<sub>4</sub> concentration created by TP feeding was utilized to determine what effect serum T<sub>4</sub> concentration had on TRH-induced changes in serum TSH, T<sub>4</sub>, PRL and GH concentrations. Accordingly, TRH was given when T<sub>4</sub> concentrations were increased (day 6 TP feeding) or reduced (6 days after TP feeding was ended), relative to corresponding controls.

Differences in TSH release after the first or second TRH injection were not significant and therefore only the change in TSH after the first TRH injection is shown (figure 2). Basal serum TSH concentrations (−30, −15 and 0 min) were not different among the four treatment groups; the overall average was 6.7 ng TSH/milliliter. Hopkins et al. (1975) observed no change in basal serum TSH concentrations of sheep in which serum T<sub>4</sub> concentrations were increased but noted an increase in serum TSH after thyroidectomy. Similarly, Davis and Borger (1973) observed increased serum TSH concentrations in thyroidectomized sheep. Failure in the present study to detect a change in basal serum TSH concentrations of sheep in which serum T<sub>4</sub> concentrations were increased but noted an increase in serum TSH after thyroidectomy. Similarly, Davis and Borger (1973) observed increased serum TSH concentrations in thyroidectomized sheep. Failure in the present study to detect a change in basal serum TSH concentrations of sheep in which serum T<sub>4</sub> concentrations were increased but noted an increase in serum TSH after thyroidectomy. Similarly, Davis and Borger (1973) observed increased serum TSH concentrations in thyroidectomized sheep. Failure in the present study to detect a change in basal serum TSH concentrations of sheep in which serum T<sub>4</sub> concentrations were increased but noted an increase in serum TSH after thyroidectomy.
24 ng T₄/milliliter. Alternatively, chronic high concentrations of serum T₄ may have suppressed TSH synthesis by the thyrotrophs to the extent that they could not increase secretion rate in response to the low concentrations of T₄ that follow TP withdrawal.

Serum TSH concentrations in heifers given casein for 6 or 27 days were increased (P<.05) within 4 min after TRH injection relative to the average TSH concentration of pretreatment samples and reached peaks of 26.0 and 26.4 ng/ml, respectively, within 30 min after TRH (figure 2). In contrast, serum TSH concentration was unchanged by TRH given on day 6 of TP feeding. These results suggest that increased serum T₄ concentrations (6 days TP feeding) exerted a negative feedback on the pituitary, inhibiting TRH-induced TSH release, similar to the T₄ inhibition of TSH release described for rats (Schally et al., 1967; Vale et al., 1967). However, TRH given when serum T₄ concentrations were decreased (6 days after 27 days of TP feeding) did not cause an exaggerated TSH release. Thus, the maximum serum TSH concentration attained was 20 ng/ml, which occurred 20 min after TRH. The average TSH concentration over the 3-hr sampling period was less than that of the appropriate casein-treated control, although the differences only approached significance (P<.10). Humans given prolonged thyroid hormone therapy required approximately 2 weeks to regain normal TSH release in response to TRH challenges (Krugman et al., 1975). Thus, it is conceivable that negative feedback effects of T₄ on pituitary thyrotrophs for 27 days were not totally reversed by 6 days after cessation of TP. Although serum T₄ is low following TP feeding in cattle, it is unlikely that these animals are functionally hypothyroid and should not be equated with hypothyroid or thyroidectomized animals.

Serum T₄ concentrations in heifers given casein for 6 or 27 days averaged 82.3 and 82.2 ng/ml, respectively, prior to TRH (0 min) and increased to 119.2 and 117.6 ng/ml, respectively, 3 hr after the second TRH injection (figure 3). This increase in T₄ was probably in response to TSH released by TRH because when TSH was not released by TRH, T₄ did not increase in serum. Thus, in heifers given TRH on day 6 of thyroprotein feeding, serum T₄ averaged 133.8 ng/ml at the time TRH was given and was not increased by TRH. Although the increase in serum TSH was significant (P<.05) in heifers given TRH 6 days after 27 days of TP feeding, serum T₄ only increased 4.8 ng/ml in these heifers. These data indicate that thyroid gland inhibition by chronic T₄ treatment causes a decreased ability of the thyroid to respond to TSH and this inhibition persists for at least 6 days after cessation of TP treatment.

Average basal serum GH (−30, −15 and 0 min) concentrations were not altered by TP or casein feeding (figure 4), although there was a tendency for GH in serum of heifers given TP for 6 days to be less than the respective concentrations for the other groups (P= .10). Similar results have been reported for the cow (Shaw et al., 1975) and lamb (Davis and Borger, 1973). However, in rats, thyroid insufficiency, rather than excess, causes re-
duced basal serum GH (Eisenberg et al., 1972; Peake et al., 1973).

Following TRH, serum GH consistently increased in all groups of heifers from an overall average basal concentration (-30, -15 and 0 min) of 13.8 ng/ml to an overall average of 36.5 ng/ml at 12 min following TRH (figure 4); differences between treatment means after TRH were not significant (P>0.05). However, analysis of variance revealed a difference (P<0.05) in GH response from the first to second TRH injection, regardless of treatment (figure 5). Thus, the magnitude of increase in serum GH after the first TRH injection (Δ=36.8 ng/ml) was greater (P<0.05) than the comparable response after the second injection (Δ=9.6 ng/ml). Similarly, the increase in serum PRL concentration induced by a second TRH challenge (Δ=51.7 ng/ml) was less (P<0.05) than the comparable increase after the first injection (Δ=132.6 ng/ml) regardless of treatment (figure 6). In view of the fact that TP or casein feeding did not alter the magnitude of GH or PRL (see below) release by TRH in these heifers, it is unlikely that reduced GH or PRL release by the second TRH injection was due to increased serum T4 resulting from the first TRH injection. Rippel et al. (1974) and Mongkonpunya et al. (1975) observed a similar phenomenon with gonadotropin releasing hormone-induced LH release; increased serum LH was greatest after the first GnRH injection. Tucker et al. (1975) showed that, following an initial increase, serum GH and PRL concentrations declined in the presence of infused TRH. However, a subsequent injection of prostaglandin F2α induced a further significant increase in serum GH and PRL concentration. This suggests that reduced GH and PRL responses to TRH probably were not due to depletion of pituitary GH or PRL content, but to a "refractoriness" of the pituitary to the TRH stimulus.

In agreement with previous results from this laboratory (Shaw et al., 1975), TP feeding did not effect basal serum PRL concentrations (-30, -15 and 0 min), which averaged 7.0 ng/ml (figure 7). However, Davis and Borger (1973) reported that the marked reduction in thyroid hormone availability that occurs after thyroidectomy in lambs is accompanied by an increase in serum PRL concentration.

Changes in serum PRL concentration after the first TRH injection are shown in figure 7. Following TRH injections, PRL increased from basal concentrations to maximum values of 60 to 140 ng/ml at 4 minutes. Although there was
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Figure 8. Serum PRL before and after iv injection of TRH (33.3 µg/100 kg BW) into Holstein heifers blocked according to weight, lightest (block 1) to heaviest (block 6).

a trend toward decreased PRL release by TRH in heifers given TP for 6 days, treatments did not alter the PRL releases (P>.05). In contrast, in humans (Snyder et al., 1973; Yamaji et al., 1974) and rats (Vale et al., 1973) increase in serum PRL following TRH is inhibited by elevated serum T4 and stimulated by low T4.

Heifers in this experiment ranged in age from 5 to 11 months, and were therefore grouped by weight. Analysis of variance of serum PRL concentration after TRH revealed an effect (P<.05) due to body weight (figure 8). This was not true for TSH or GH. Furthermore, a positive correlation (r = +.49, P<.05) exists between heifer weight and average TRH induced PRL response. Thus, magnitude of PRL release by TRH should be grouped so distinctly into age-weight groups, particularly since there was no distinct break in age or weight between blocks 1 to 3 and 4 to 6, is not known. Some of these heifers were near puberty at the time of this experiment. However, progesterone determinations in serum collected on alternate days for 17 days, centered for all heifers on the day of TRH challenge, suggested that only the three heaviest heifers had ovulated. These heifers did not exhibit unusually high PRL responses to TRH.

Hence, in prepubertal heifers, alteration of serum T4 concentration, as demonstrated in this experiment, was neither effective in altering basal serum TSH, GH or PRL concentra-tions nor the TRH-induced release of PRL or GH. However, increased serum T4 concentration did prevent the TRH induced release of TSH.

LITERATURE CITED


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