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Effects of TRH and Ergocryptine on Mammary Arterial and Venous Concentrations of Growth Hormone in Lactating Cows[1] (40436)

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Growth hormone (GH) increases milk yields in cows (1–5). Although basal serum concentrations of GH are relatively stable throughout lactation (6, 7) capacity of the anterior pituitary to release GH after thyrotropin releasing hormone (TRH) is enhanced in cows during early as compared with late lactation (8, 9). If GH is required for lactation one might expect the mammary gland to remove GH from blood. In the present study we report arterial (A) and venous (V) concentrations of GH in serum from two experiments conducted originally (10) to test whether A-V differences exist for prolactin in cows with acutely increased prolactin (TRH, milking) or chronically decreased concentrations of prolactin (ergocryptine). Although ergocryptine (11) and milking (7, 12, 13) do not affect concentrations of GH in cattle, TRH increases GH (9, 14). Thus, we measured A-V differences in GH when GH concentrations were unstimulated or acutely increased, and when prolactin was acutely increased or markedly reduced.

Materials and methods. A pudendal artery and a subcutaneous abdominal mammary vein of six Holstein cows in early lactation (5–12 weeks) and six in late lactation (37–57 weeks) were cannulated as described (10). Throughout these experiments cows were stanchioned and fed daily 18 kg of corn silage, 4.5 kg alfalfa-grass hay and 1 kg of grain concentrate per 2.5 kg of milk produced. Water was supplied *ad libitum.*

The first experiments began 3 to 5 days after surgery when milk production approximated presurgery levels. On the day of each experiment cannulas were flushed with 3.5% sodium citrate at 15 min intervals for approximately 2 hr to accustom the cows to sampling procedures. On three alternate afternoons (3 replicates) of the first experiment each cow was injected iv with 200 μg TRH in 2 ml of 0.85% NaCl (Abbott Laboratories, Chicago, IL) followed 30 min later by milking for 3–5 min. Arterial and venous samples of blood were drawn simultaneously before and after TRH and milking as in Fig. 1.

At the end of the first experiment five cows in early lactation were injected sc with 30 mg of 2-bromo-α-ergocryptine methanosulfate (CB-154, Sandoz, Basle, Switzerland) in 5 ml of 95% ethanol. Five cows in late lactation were injected with 80 mg of ergocryptine (Sigma, St. Louis, MO). The doses of CB-154 and ergocryptine reduced basal secretion of serum prolactin an average of 67 and 81%, respectively, for the remainder of this experiment (10). It is not known whether the greater suppression of prolactin with ergocryptine was associated with differences in potencies of the drugs or with physiological differences between early and late lactating cows. The second experiment began 4 days after CB-154 or ergocryptine. Cows were injected iv on three alternate afternoons (3 replicates) with 500 μg TRH in 0.85% NaCl. Blood was collected simultaneously from the arterial and venous cannulas before and after TRH as in Fig. 2.

Blood was stored at approximately 25° for 2 hr followed by 5° for 24 to 36 hr at which time samples were centrifuged at 2500 *g* for 15 min. Serum was stored at −20° until assayed for GH as described in (15). Bovine GH (NIH B_{12}) was used as reference standard. The lower limit of sensitivity of the GH assay was 0.1 ng.

Hormone concentrations were determined in duplicate, and accepted when agreement was ±5%. Hormone concentrations at each sampling time within each experiment were

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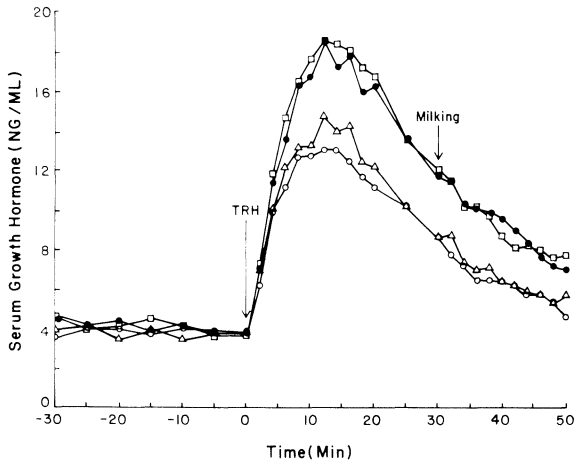

FIG. 1. GH concentrations in serum samples from early (5–12 weeks postpartum) lactating (arterial □——□; venous ●——●) and late (37 to 57 weeks postpartum) lactating (arterial △——△; venous ○——○) cows before and after injection of 200 μg TRH followed 30-min later by milking which lasted 3–5 min. Each point is the mean GH concentration from three replicates in each of six cows. Pooled SE among early lactating cows within the 30-min before TRH, 30-min after TRH and 20-min after milking were 0.2, 2.4, and 1.0, for arterial and 0.2, 2.4, and 1.0 ng/ml for venous samples, respectively. Among late lactating cows the pooled SE were 0.7, 2.0, and 1.1 for arterial samples and 0.8, 2.0, and 1.2 ng/ml for venous samples, respectively.

averaged over the three experimental replicates for each cow. These values were analysed using a split plot analysis of variance (16). In the first experiment data were grouped into periods which consisted of the 30 min before TRH, the first 30 min after TRH, and the 20 min after the beginning of milking. In the second experiment the periods were: the 30 min before TRH, the first 20 min after TRH, and the last 50 min of the experiment. Effects of TRH, milking, stage of lactation and period on GH were tested in the split plot analysis. The t-test was used to determine if overall mean A-V difference within a period was different from zero.

Results. In the first experiment overall mean serum GH in arterial and in venous sera collected during 30 min before TRH from cows in early lactation each averaged 4.0 ± 0.2 ng/ml (Fig. 1). Serum GH increased ($P < 0.01$) to maximal concentrations of 18.6 ± 3.1 in arterial and 18.5 ± 3.3 ng/ml in venous sera 12 min after TRH; thereafter GH steadily declined to values of 7.7 ± 0.8 (arterial) and 7.0 ± 0.6 ng/ml (venous) 50 min after TRH. During the 30 min immediately after TRH mean A-V difference was 0.6 ± 0.2 ng/ml and different from zero ($P < 0.05$). No other A-V differences were significant in cows in early lactation.

Mean GH concentrations in arterial and venous sera of cows in late lactation 30 min before TRH were 3.7 ± 0.6 and 3.8 ± 0.7 ng/ml, respectively (Fig. 1). TRH increased ($P < 0.01$) GH to peaks of 14.8 ± 2.7 (arterial) and 13.1 ± 2.3 ng/ml (venous) at 12 min. Subsequently GH decline to 5.7 ± 0.8 and 4.6 ± 0.8 ng/ml 50 min after TRH in arterial and venous samples, respectively. Mean A-V differences averaged 0.7 ± 0.2 and 0.3 ± 0.1 ng/ml during 30 min immediately after TRH and during the 20 min postmilking period in cows during late lactation, and each mean was greater than zero ($P < 0.05$).

Milking early or late lactating cows 30 min after TRH did not affect serum GH ($P > 0.05$). Although cows during late lactation appeared to release less GH than those in early lactation following TRH, the difference was not significant ($P > 0.05$).

In a second experiment during the 30 min before injection of TRH concentrations of GH averaged 4.9 (arterial) and 5.0 ± 0.9 ng/ml (venous) in cows in early lactation treated with CB-154 (Fig. 2). Injection of TRH increased ($P < 0.01$) arterial and venous concentrations of GH to peaks of 25.2 and 24.7 ± 4.5 ng/ml respectively within 12 min. Thereafter, arterial concentrations declined to 7.1 ± 1.1 and venous concentrations to 7.7

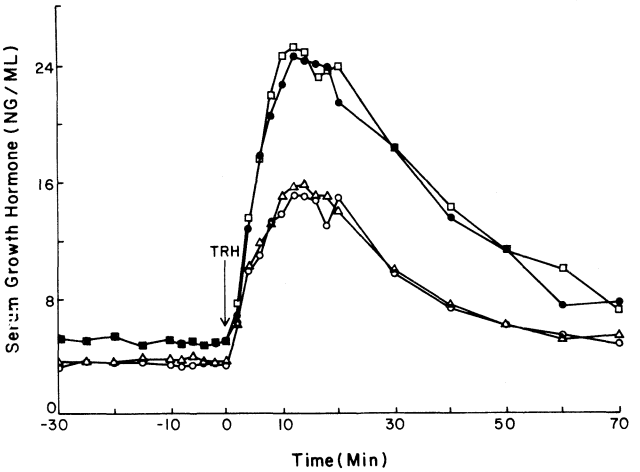

FIG. 2. GH concentrations before and after injection of 500 μg TRH at 0-min to five early (5–12 weeks postpartum) lactating (arterial, □——□; venous, ●——●) treated with 80 mg CB-154 and five late 37–57 weeks postpartum) lactating (arterial, △——△; venous, ●——●) cows treated with 80 mg ergocryptine. Each point is mean GH concentration from three replicates in five cows. Pooled standard errors among early lactating cows within the 30 min before TRH, 20 min after TRH and 30–70 min after TRH were 0.9, 3.6, and 2.6 for arterial samples, and 1.0, 3.5, and 2.5 ng/ml for venous samples, respectively. Among late lactating cows the pooled standard errors were 0.8, 2.0, and 0.8 for arterial samples and 0.8, 2.1, and 0.6 ng/ml for venous samples, respectively.

± 1.8 ng/ml at 70 min post TRH. In the 20 min period immediately after TRH and during the 30 to 70 min post-TRH injection period mean A-V differences averaged 0.7 ± 0.4 and 0.5 ± 0.3 ng/ml, but neither value was different from zero ($P > 0.05$).

In cows during late lactation (pre-treated 4 to 10 days earlier with ergocryptine) GH in arterial (3.6 ± 0.7 ng/ml) and venous (3.4 ± 0.7 ng/ml) sera during 30 min prior to TRH were not different ($P > 0.05$) from concentrations in cows during early lactation (Fig. 2). TRH increased ($P < 0.01$) GH to maximal values of 15.7 ± 2.6 in arterial and 15.1 ± 2.7 ng/ml in venous samples in cows during late lactation within 12 min. GH in arterial and venous sera subsequently declined to 5.4 ± 0.8 and 4.9 ± 0.7 ng/ml, respectively. The overall mean A-V difference during the 20 min immediately after TRH was 0.5 ± 0.1 ng/ml which was greater than zero ($P < 0.05$). During the 30–70 min period after TRH mean A-V difference (0.2 ± 0.3 ng/ml) was not different from zero ($P > 0.05$).

Discussion. Our observations that basal concentrations of GH in sera of cows are not affected by stage of lactation, milking, or ergocryptine is in agreement with previous reports (6–9, 12). The increases in serum GH concentrations observed, following TRH in lactating cows, are similar to previous findings in cows (10), rats (17), and humans (18).

Injection of CB-154 reduced, but did not completely block, the increase in GH following TRH injection in some patients with acromegaly (18), whereas in goats CB-154 increased quantity of GH released during milking (19). In contrast, in cows used here CB-154 or ergocryptine did not affect TRH-induced release of GH, although prolactin was reduced 67 and 81%, respectively (10). As previously reported (10, 11), treatment of cows with ergot drugs did not affect lactation. Thus, the mechanism whereby lactation is maintained in spite of reduced prolactin in sera of cows does not involve a compensatory increase in secretion of GH.

In the present study, cows in early lactation tended to release more GH in response to TRH, than did cows in late lactation. This is in agreement with our previous reports (8, 9) and may suggest that more growth hormone is available in earlier stages of lactation when milk production is greatest. However, under basal conditions we observed no differences in concentrations of GH in mammary arterial and venous sera of cows during either early or late lactation. On the other hand, when serum GH was acutely increased following TRH, GH concentrations in a subcutaneous

vein in the mammary gland were reduced relative to those in a pudendal artery. We suggest that the mammary gland of dairy cows removes GH from blood when the hormone is acutely increased in blood.

Mammary blood flow (MBF) was calculated from the equation of Kronfeld *et al.* (19) in which MBF = 1.0 + 0.42×, where × equals daily milk yield. Daily milk yields averaged 24.5 kg in cows during early lactation and 13.4 kg during late lactation. In the first experiment, A-V differences in GH averaged 0.6 and 0.7 ng/ml during the 30 min after TRH in cows during early and late lactation, respectively. Mammary uptakes of GH during this interval (estimated by multiplying MBF by A-V differences) averaged 6.5 and 4.8 μg/min for cows in early and late stages of lactation, respectively. The tendency for greater uptake of GH in cows during early lactation also occurred within the first 20 min after TRH was administered to cows pretreated with ergots (7.5 and 2.9 μg/min, respectively). Since the A-V differences in serum GH after TRH were essentially the same in early and late lactating cows, the greater mammary uptakes of GH after TRH during early lactation were most likely associated with greater mammary blood flows during this physiological state. It is known that GH is acutely released in an episodic pattern under normal conditions in cattle (21), and we speculate that greater numbers of acute releases of GH (and potentially greater mammary uptake of hormone) would cause increased synthesis of milk.

Summary. Serum GH concentrations were determined in mammary arterial and venous samples from six early (5–12 weeks postpartum) and six late (37–57 weeks postpartum) lactating Holstein cows. Basal serum GH averaged 4.0 ng/ml in cows during early and late lactation. Treatment of five cows in early lactation with 80 mg CB-154 or five cows in late lactation with 80 mg ergocryptine was without effect on basal serum GH concentrations. Administration of 200 μg TRH to early or late lactating cows increased GH concentrations to maxima of 18 and 14 ng/ml at 12 min post-injection, respectively. Serum GH of cows in early lactation treated with CB-154 and cows in late lactation treated with ergocryptine, averaged 25 and 15 ng/ml respectively, 12 min after administration of 500 μg TRH. Mammary arterial concentrations of GH were greater than venous concentrations only after being acutely increased by TRH. We conclude, that in comparison with late lactating cows, early lactating cows tended to release more GH and mammary uptakes of GH were greater in response to TRH. These responses were unaffected by either CB-154 or ergocryptine. Mammary arteriovenous differences in GH occurred when serum concentrations were acutely increased.

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