EFFECT OF COLLECTION STRESS ON SERUM GROWTH HORMONE LEVELS IN PYGMY GOATS

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SUMMARY

Four experiments were conducted with mature female pygmy goats to determine the effect of blood collection stress and blood collection method on serum growth hormone (GH) levels and the nature of serum GH variability. Repeated blood sample collection via jugular venipuncture did not affect serum GH levels. Comparison of serum GH levels in blood samples collected via jugular venipuncture and jugular cannula indicated a lack of significant difference between methods, although GH levels in samples collected by jugular cannula were higher and more variable. Restraint failed to influence serum GH in cannulated goats. Variability observed in serum GH in samples collected without the goats’ knowledge indicates that GH is secreted episodically at irregular intervals irrespective of the constraints of this experiment. The data in these experiments indicate that, in pygmy goats, release of pituitary GH was not increased when the animals were subjected to stressful stimuli. We have demonstrated the validity of a bovine GH radioimmunoassay system for quantitating GH in the pygmy goat. (Key Words: Pygmy Goats, Growth Hormone, Stress.)

INTRODUCTION

The transient effects of stress on serum growth hormone (GH) levels have been studied in several species. Insulin-induced hypoglycemia, exercise, and surgery stimulate pituitary GH release in humans (Parker et al., 1962; Roth et al., 1963a,b; Hunter and Greenwood, 1964; Glick et al., 1965). In contrast to elevated serum GH in response to stress in man, rats and mice respond with decreased serum GH levels. For instance, repeated ether anesthesia, exposure to cold (4°C) for periods of 1 to 2 hr, insulin-induced hypoglycemia, and forced swimming all decrease plasma GH in rats (Schalch and Reichlin, 1968), and ether stress results in a prompt decrease of plasma GH in mice (Schindler et al., 1972).

Controversy exists concerning the effect of stress on serum GH levels in domestic animals. Machlin et al. (1968) observed that stress and exercise provoked significant increases in plasma GH in swine while Weiss et al. (1970) found that physical stress failed to elevate serum GH levels in swine. Early studies in ruminants suggested that blood collection stress stimulated GH secretion since elevated GH levels were noted in cattle immediately after insertion of jugular catheters (Eaton et al., 1968). More recently, it has been observed that physically or psychologically stressful factors failed to alter GH secretion in sheep (Davis, 1972) or dairy cows (Tucker, 1971). However, Olsen and Trenkle (1973) found that exposure of cattle to cold temperatures tended to increase circulating GH levels.

These experiments were conducted 1) to validate a bovine GH assay system for the pygmy goat, a useful animal model, 2) to determine the effect of physical stress on serum GH and 3) to provide initial observations on basal and temporal GH concentrations in pygmy goats.

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MATERIALS AND METHODS

Mature female pygmy goats (wt = 23 ± 1 kg) were used in each experiment. During the first three experiments the goats were maintained in individual, but adjoining pens. It was felt that this isolation allowed a certain amount of psychological stress as well as the physical stress of being caught and restrained during actual blood sample collection. The goats were fed at the end of each days' experiment and water was available ad libitum. The first three experiments commenced at 0900 hr while the fourth experiment was performed from 1300 to 1400 hr on 2 consecutive days.

Experiment 1. During the first experiment, blood samples (5 ml) were collected from five goats by jugular venipuncture at 20 min intervals for eight collection periods.

Experiment 2. Twenty-four hours prior to initiation of the second experiment, jugular cannulas were inserted in seven of 14 goats selected at random. Blood samples were collected at hourly intervals for 6 hr from each of the 14 goats with sample collection in the uncannulated goats via jugular venipuncture. The split-plot method as described by Gill and Hafs (1971) was used to analyze for differences between collection methods, since repeated measurements were taken.

Experiment 3. For the third experiment, four of the cannulated goats from the previous experiment were tied with short lengths of rope. This restraint appeared to be stressful since the goats struggled vigorously. Blood samples were collected via jugular cannula at 5-min intervals for 1 hr from these tied goats and from two cannulated untied goats.

Experiment 4. Twenty-four hours prior to initiation of the fourth experiment, jugular cannulas were inserted into four goats. Each goat was then placed in a metabolic cage with two cages placed side by side to decrease the stress of isolation. Several hours before initiation of collection, the cannulas were extended to allow collection from behind a partition. Every effort was made to collect blood samples without the goats' knowledge. The cannula failed in one goat, limiting our observations to the remaining three goats.

Growth hormone in goat serum was quantified using a bovine GH double-antibody radioimmunoassay similar to that reported by Purchas et al. (1970). Bovine GH (NIH-GH-B16) was used as the labeled competitor and for standards in the radioimmunoassay. Guinea pig anti-bovine GH serum (Purchas et al., 1970), as received from Dr. Hafs, was precipitated with rabbit anti-guinea pig gamma globulin (Nutritional Biochemicals Corp., Cleveland, OH) and the 1125 quantified in an auto-gamma spectrometer.

The specificity of the bovine GH assay for goat GH was validated in the following manner. Dilutions of goat serum and supernatant of homogenized goat anterior pituitaries closely paralleled the bovine GH standard curve (figure 1), which indicates that the goat possesses a hormone species which reacts with the anti-bovine GH antibody in a manner similar to bovine GH. Recovery of exogenous bovine GH (NIH-GH-B16) added to goat serum was quantitative as illustrated in figure 2. Cross reactivity of NIH sources of bovine (B3) and ovine (S9) prolactin and ovine LH (S17), TSH (S6) and

![Figure 1. Dose response curve for NIH-GH-B16, goat serum and pituitary homogenates.](image1)

![Figure 2. Recovery of exogenous bovine GH added to 10 µl of goat serum. Actual recovery is represented by the regression line.](image2)
FSH (S8) were negligible in this assay when tested at levels of 0.5, 5, 50 and 500 ng/tube. Finally, the assay was used to analyze serum from pygmy goats stimulated with arginine. It has been observed that arginine infusion will significantly increase serum GH in the sheep, pig and cow (Hertelendy et al., 1970) and goat (Stern et al., 1971). Consequently, two pygmy goats were infused, first with saline and then with arginine (.5 g/kg), each over a 30-min period. Analysis indicated that serum GH was unaltered during or following the saline infusion (P>.10) whereas it increased by 137% (P<.01) to 8.3 ng/ml at 30 min and then returned to preinfusion levels 60 to 90 min after the initiation of the arginine infusion. This indicates that the assay responded to physiological changes in the serum which most likely represents GH. Stern et al. (1971) first reported a GH assay system for goats, using ovine GH as the source of antigen and antibody development.

RESULTS AND DISCUSSION

Experiment 1. Results of the first experiment (figure 3) indicate that serum GH levels did not increase during repeated blood sample collection via jugular venipuncture. In fact, mean serum GH levels decreased slightly, but not significantly, during the collection period. Mean (±SE) serum GH level over all collections was 2.5 ± .2 ng/ml. The large standard errors (figure 3) illustrate that GH may be secreted episodically in the pygmy goat, similar to that reported in cattle (Trenkle and Irwin, 1970), sheep (Wallace and Bassett, 1970), man (Finkelstein et al., 1972), goats (Hart et al., 1975), swine (Siers and Trenkle, 1973) and rats (Tanenbaum and Martin, 1976).

Experiment 2. In the second experiment (figure 4), serum GH levels in samples collected by jugular cannula (4.5 ± .8 ng/ml) were higher, although not significantly, and more variable than serum GH levels in samples collected by jugular venipuncture (2.2 ± .1 ng/ml). In an attempt to alleviate the heterogeneity of variance between treatments, animals exhibiting GH levels greater than 2 SD above the treatment mean were removed from the analysis (one from each treatment). Differences between serum GH levels in samples collected via jugular cannulae (3.2 ± .4 ng/ml) and jugular venipuncture (2.1 ± .1 ng/ml) then approached significance (P=.07), indicating a possible suppression of serum GH levels by venipuncture collection stress. However, subsequent (experiment 3) serum GH levels in samples collected via jugular cannulae were still lower (1.5 ± .2 ng/ml), thus minimizing this possibility. The rapid elevation in serum GH levels observed in the cannulated goats between the first and second collections (figure 4) could be interpreted as a result of collection stress, but further collections from this same group of goats (experiment 3) over a 1-hr period failed to show a similar elevation. The lack of relationship between successive samples from the same animal led Siers and Trenkle (1973) to suggest that fluctuations in serum GH on the order of 200 to 300% is within physiological limits.

Experiment 3. Results of the third experiment (figure 5) indicate that restraining the goats during blood sample collection did not affect the serum GH levels. Mean serum GH level for the untied and tied groups were 1.9 ±

![Figure 3](image1.png)

**Figure 3.** Serum GH levels in adult female pygmy goats with repeated blood collections by jugular venipuncture at 20-min intervals. (Mean ± SE, n=5).

![Figure 4](image2.png)

**Figure 4.** Serum GH levels in adult female pygmy goats with repeated blood collections by jugular cannula and jugular venipuncture at 1-hr intervals. (Mean ± SE, n=7).
.4 ng/ml and 1.3 ± .1 ng/ml, respectively. Serum GH levels appear to decline rapidly with repeated blood sample collection in the untied group, but this result is due to a high initial level in one of the two goats in this group. This probably represents the decay of an episodic GH secretion in this goat. Mean GH level in both groups in experiment 3 was 1.5 ± .2 ng/ml as compared to 4.5 ± .8 ng/ml in essentially the same group of goats in experiment 2. The possibility exists that the goats had become accustomed to blood sample collection, thus exhibiting lower (P<.01) serum GH levels in experiment 3 relative to GH levels in experiment 2. Although based on few observations, Eaton et al. (1968) observed that fluctuations in serum GH appeared to decrease with time. It would appear then that sufficient time should be allowed for animals to become accustomed to blood sample collection via jugular cannula before attempting to obtain representative samples.

Experiment 4. Results of experiment 4 (figure 6) indicate the variability in serum GH levels. Each line in figure 6 represents the serum GH level of an individual goat over a 1-hr period with sample collections at 5-min intervals. Since every attempt was made to collect blood samples without the goats' knowledge, the variation shown should be indicative of endogenous changes rather than an effect of outside influences. Two of three goats exhibited a sharp increase in serum GH during the 1-hr sampling period. Based on this admittedly short sampling period, it would appear that, similar to most other species studied, GH secretion occurs episodically at irregular intervals.

Results of the present study indicate that normal collection stress in the form of repeated blood sample collections by jugular venipuncture or physical restraint did not influence serum GH levels in mature pygmy goats. Because GH secretion appears to occur episodically in pygmy goats as in other species, it was not possible to ascertain whether the changes in serum GH observed represented the goats' becoming accustomed to the sampling procedure. In agreement with the observations in other species (Finkelstein et al., 1972; Hart et al., 1975; McAtee and Trenkle, 1971; Tannenbaum and Martin, 1976), we believe the fluctuations observed were caused by episodic secretion of GH.

It is unlikely the feeding regime used in these experiments was responsible for any changes observed. First, it is quite controversial whether feeding or fasting has an effect on GH secretion. Most studies have shown that feeding or fasting has no effect on GH secretion (Trenkle, 1971; Wallace and Bassett, 1970; McAtee and Trenkle, 1971) while a few have indicated that serum GH decreased following feeding (Blom and Hove, 1976; Bassett, 1974). And secondly, any possible influence of feeding should have subsided during the 12- to 18-hr interval between feeding and initiation of experiments.
LITERATURE CITED


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