Journal of Animal and Feed Sciences, 4, 1995, 247–254

Growth hormone secretion in anestrous ewes subjected to prolonged stressful stimuli

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(Received 14 July 1995; accepted 22 August 1995)

ABSTRACT

The study was performed on 6 three-year-old Polish Merino ewes to examine the effects of prolonged intermittent footshock stimuli on the secretion of growth hormone (GH) in anestrous ewes. The state of stress was induced by electrical footshocks of enduring and repetitive character (20 min/h, 9 h daily during 3 consecutive days, current intensity 3 mA). The blood samples were taken at 2 h intervals on the day prior to stimulation (controls), during 3 days of footshock application, and on the post-stress day. The control levels of plasma GH were quite low (from 1 to 6 ng/ml) and in some samples they were at, or near,the limit of detection. The mean 24 h plasma GH level ranged from 0.51 to 3.70 ng/ml. The mean concentration of GH in all ewes prior to the exposure to footshock stress was 2.12 ± 0.55 (SE) ng/ml. Footshock led to marked increase in GH concentration; the mean plasma level of GH in animals reached about 200% of pre-stress values. There were no significant differences between GH responses in the first, second and third day of exposure to footshock stimuli. The mean level of plasma GH on the post-stress day did not differ significantly from the control concentration. However, in 4 out of 6 ewes the mean 24 h plasma GH level was lower as compared to the value found in controls. In conclusion, these results indicate that prolonged intermittent footshock stimulation increases GH secretion in anestrous ewes, after which the concentration of this hormone in the blood plasma in most animals on the post-stress day returns to normal, pre-stress values. It is suggested that changes in GH concentrations, among others, may be to some extent a valuable indicator of the biological cost of environmental challenges.

KEY WORDS: ewe, stress, GH

INTRODUCTION

It is generally accepted that prolonged or chronic stress causes major disorders in many physiological functions, including GH secretion which plays crucial role in the body's metabolic activity. Although certain neuroendocrine responses to stress may be adaptive mechanisms, the analysis of GH secretion in organisms under stressful conditions has an important aspect in understanding the physiology of stress. Numerous observations have shown that stress alters the pattern of GH secretion in a different manner in various species; in hamsters (Borer et al., 1982), monkeys (Brown et al., 1971; Meyer and Knobil, 1987) and men (Schalch, 1967; Schalch and Reichlin, 1968) stress increases GH secretion, while in rats (Day et al., 1983; Armario et al., 1986; Aquila et al., 1991; Benyassi et al., 1992; Franci etal., 1992) GH secretion is inhibited. Little is known about GH secretion under stressful conditions in farm animals. Experiments performed on cows revealed that GH secretion responds in a specific manner to different kinds of stressful stimuli (Munksgaard and Levendahl, 1993). It was found that long-term intermittent footshock stimuli of sheep elicits marked depletion of hypothalamic somatostatin (SRIF) but has no apparent effect on GH content in the growth hormone-producing cells of the anterior pituitary gland (Polkowska, 1989; Polkowska and Przekop, 1989). Lack of changes in GH concentration in the pituitary glands of stressed animals can not be easily interpreted since the hormone level in the pituitary cells reflects only the net result of its synthesis and release. On the other hand, growth hormone secretion in sheep was not influenced by cold exposure (Christensen et al.,1990). In the light of the presented results the species and/or stress specificity in the response of GH probably has different effects on the metabolic activity in the organism. As the first step to studying the mentioned problems, the present experiment was undertaken to follow the influence of prolonged intermittent electric stressful stimuli on the concentration of GH in the blood plasma of seasonally anestrous ewes.

MATERIAL AND METHODS

Animals

The experiment was performed on 6 three-year-old, seasonally anestrous, Polish Merino ewes. They were maintained indoors in individual pens and exposed to natural lighting conditions over the experimental period (March-April). Visual contact was allowed between the animals. To minimize the confounding effect of variable ovarian hormone levels on GH secretion, the

experiment was performed on anestrous ewes. Feeds (hay and concentrates) and water were available ad libitum.

The animals were adapted to the experimental environment for at least six weeks before the study.

Stressing procedure

Stress was induced by applying a series of mild electrical footshocks of an enduring and repetitive character. Footshocks were applied through electrodes attached to both forelegs at the level of the metacarpus and connected to a 50 Hz sine wave generator. The generator was programmed to deliver repetitive trains of 3 mA (rms) alternating current (0.5 sec on,1 sec off), arranged in series of ten. The series were repeated 35 times during the initial 20 min of each hour from 09.00 to 18.00 h for three consecutive days.

Blood sample collection

A catheter was introduced under local anesthesia into the jugular vein 24 h prior to the first blood collection. Twelve blood samples were drawn at 2 h intervals during 5 consecutive days (i.e. one day prior to the onset of the stressful stimulation, during three days of the stimulation, and on the day after the stimulation). The blood was centrifuged at 3.000 g for 10 min at 4^0C and plasma was stored at -20^0C until analysis.

GH assay

GH was determined by routine double antibody radioimmunoassay (Dvorak et al., 1987). In order to increase the precision of estimation, all samples were analyzed within one series of RIA. The assay detection limit was 0.034 ng corresponding to 0.68 ng/ml of plasma sample. The coefficient of variation calculated for control samples at concentrations of 0.1, 0.8 and 6.4 ng/ml of GH was 7.4, 2.6 and 5.5%, respectively. The mean concentration of GH for an individual animal was calculated from the area under the curve (sum of trapezoid areas between curve and abscissa).

Statistics

Data are presented as means $\pm$ SEM. Statistical analysis was performed using the two-tailed nonparametric two-way analysis of variance followed by multiple comparisonson ranks of several related samples as described by Theodorson-Norheim (1987). Significance was defined at a level lower than 0.05.

RESULTS

In non-stressed anestrous ewes, plasma levels of GH were quite low (from 1 to 6 ng/ml) and in some samples they were at, or near, the assay sensitivity limit. In these animals, the mean 24 h plasma GH level ranged from 0.51 to 3.70 ng/ml. The mean level of GH in six ewes before exposure to footshock stress was 2.12 ± 0.55 ng/ml.

Footshock application led to a marked increase in GH concentration in the blood plasma (Figure 1). The mean concentration of GH in six ewes reached about 200% of pre-stress values (Figure 2). There were no significant differences between GH responses to footshock stress on the first, second or third day of exposure (Figures 1 and 2). The mean level of plasma GH on the post-stress day did not differ significantly from the control value (Figures 1 and 2). In four out of six ewes, however, the mean 24 h plasma GH level was lower on the post-stress

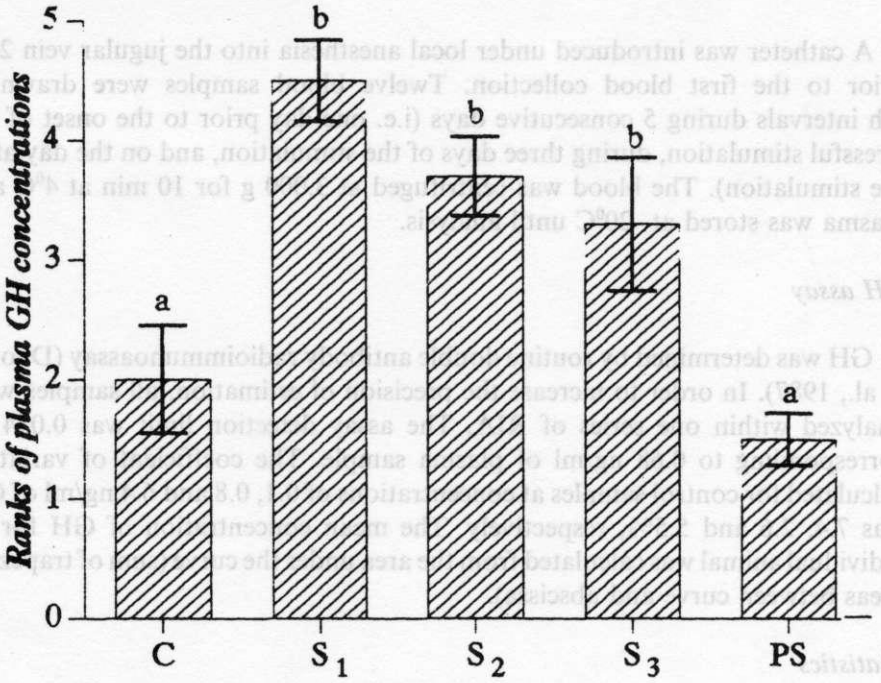

Figure 1. The average ranks of mean daily levels of growth hormone (GH) in the blood plasma of ewes before (C), during (S) and after (PS) footshocking
S_1, S_2, S_3 – consecutive days of footshocking;
different letters denote the significance between groups
S_1 vs – $P \leq 0.01$; S_2 vs – $P \leq 0.025$; S_3 vs – $P \leq 0.05$

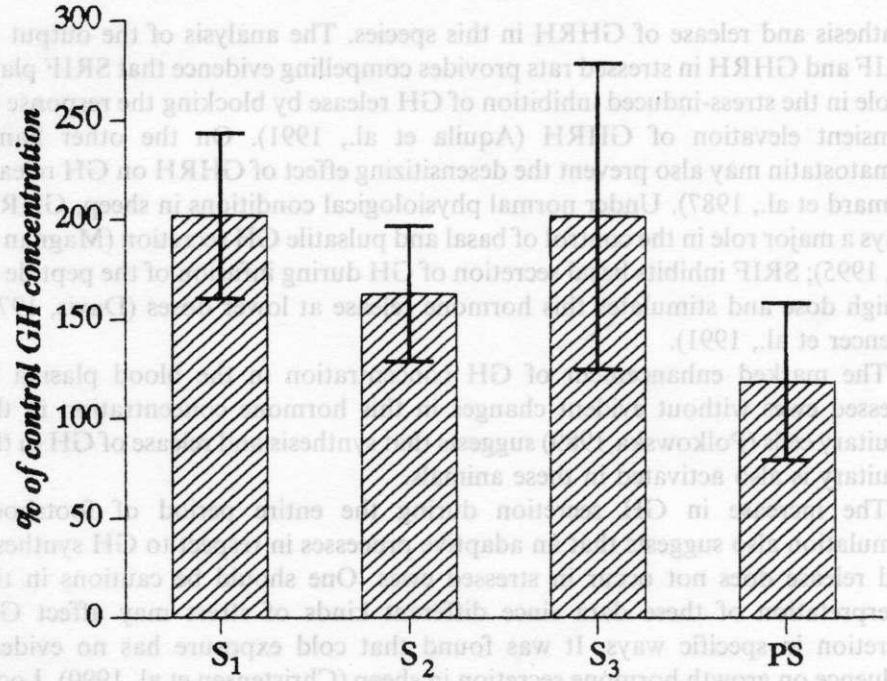

Figure 2. The mean concentrations of plasma growth hormone (GH), expressed as a percentage of respective control value (100 % – the mean daily levels of GH on the day before the onset of footshocks), in ewes during (S) and after (PS) footshocking
S_1, S_2, S_3 – consecutive days of footshocking

day as compared to the one found under control conditions. The mean GH concentration found in these four ewes on the post-stress day attained $64.7 \pm 16.4\%$ of there spective pre-stress value.

DISCUSSION

The increase of GH concentration in the blood plasma of anestrous sheep exposed to prolonged intermittent footshocks provides evidence that stress facilitates GH release.The theoretically, this suggests that the excitatory effect of GHRH and other stimulatory factor(s) released from the hypothalamus of stressed ewes overcomes the inhibitory influence of simultaneously liberated SRIF on GH secretion from pituitary cells. Indeed, it has been found in our Laboratory that this kind of stress elicits activation of SRIF release from the hypothalamus and median eminence (Polkowska and Przekop, 1988). Unfortunately, there is still a lack of studies concerning the effect of stress on the

synthesis and release of GHRH in this species. The analysis of the output of SRIF and GHRH in stressed rats provides compelling evidence that SRIF plays a role in the stress-induced inhibition of GH release by blocking the response to transient elevation of GHRH (Aquila et al., 1991). On the other hand, somatostatin may also prevent the desensitizing effect of GHRH on GH release (Simard et al., 1987). Under normal physiological conditions in sheep, GHRH plays a major role in the control of basal and pulsatile GH secretion (Magnan et al., 1995); SRIF inhibits basal secretion of GH during infusion of the peptide at a high dose and stimulates this hormone release at lower doses (Davis, 1975; Spencer et al., 1991).

The marked enhancement of GH concentration in the blood plasma in stressed ewes without evident changes in this hormone concentration in the pituitary cells (Polkowska,1989) suggests that synthesis and release of GH in the pituitary is also activated in these animals.

The increase in GH secretion during the entire period of footshock stimulation also suggests that an adaptive processes in respect to GH synthesis and release does not occur in stressed ewes. One should be cautions in the interpretation of these data since different kinds of stress may affect GH secretion in specific ways. It was found that cold exposure has no evident influence on growth hormone secretion in sheep (Christensen et al.,1990). Local electrical stimulation of the median eminence in sheep evokes a biphasic effect on GH secretion: inhibition followed by stimulation (Malven, 1975). The consequence of the increased secretion of GH which was observed in our experiments with concomitant enhanced cortisol and prolact in release (Przekop et al., 1985; Wolińska et al., 1986; Polkowska and Przekop, 1988) may, among others, seriously disorder the physiological metabolic processes in the organism.

On the day after stimulation, the GH concentration declined in most animals to below control values. In the light of these results we can not offer a precise explanation for this phenomenon. It seems reasonable only to suggest that the decrease in the plasma GH concentration on the post-stress day may be a result of decreased release, increased utilization or both of these events. Taken together, the above-mentioned results and our data suggest that GH secretion in stressed ewes is dependent to a large degree upon the nature of the stress applied.

In conclusion, these results indicate that prolonged intermittent footshock stimulation in creases secretion of GH in anestrous ewes and leads to a decrease of this hormone concentration on the post-stress day. They suggest that alternations in GH levels, among others, may affect metabolic processes in the organism and may to some extent be a valuable indicator of the biological cost of different environmental challenges.

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STRESZCZENIE

Sekrecja hormonu wzrostu u anestralnych owiec w warunkach długotrwałego stresu

Badania nad wpływem długoterminowego stresu na sekrecję hormonu wzrostu prowadzono na sześciu trzyletnich maciorkach rasy merynos Polski w okresie anestralnym. Stres wywoływano łagodnymi 20 minutowymi impulsami prądu o natężeniu 3 mA w ciągu godziny przez 9 godzin dziennie przez 3 kolejne dni. Próbki krwi do analiz hormonu wzrostu pobierano co 2 godz przez całą dobę przed poddaniem zwierząt stresowi (próby kontrolne), w ciągu trzech dni stymulacji i przez jeden dzień po zakończeniu stresu. Przed stymulacją poziom hormonu wzrostu w osoczu krwi był niski i wahał się od 0 do 6 ng/ml (średnio $2{,}12 \pm 0{,}05$). Stres powodował istotny wzrost stężenia hormonu w osoczu i osiągnął dwukrotną wartość w stosunku do uzyskanej od zwierząt kontrolnych ($P \leqslant 0{,}05$) a stężenie to było podobne w każdych z trzech dni stymulacji. Średnie stężenie hormonu wzrostu po zakończeniu stymulacji w ostatnim dniu doświadczenia powracało do wartości uzyskanych w okresie kontrolnym, a u czterech z sześciu zwierząt było nawet niższe niż przed stymulacją.

Uzyskane wyniki sugerują, że zmiany sekrecji hormonu wzrostu w czasie stresu mogą być jednym z istotnych czynników zmieniających procesy metaboliczne i mogą odzwierciedlać wpływ środowiska na organizm.