Growth Hormone Response to Acute Thermal Exposure in Cattle[1] (36304)

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Plasma growth hormone (GH) changes due to various stressful conditions have been reported (1–4), but acute heat-exposure effects on this hormone are limited to a study in which externally applied heat failed to alter the plasma GH levels in a pentobarbital-anesthetized monkey (5), and another stating that heat exposure accelerated the activity of GH releasing factor (GHRF) and pituitary depletion of GH in normal rats (6). The present study was designed to investigate the effects of acute thermal exposure on plasma GH levels in cattle and to explore the role of hyperthermia in this process.

Experimental Procedures. Six Holstein steers (16 to 18 months old, weighing 298 to 352 kg) were maintained in individual environmental chambers, at an 18°, 50% RH thermoneutral environmental control condition. They were fed a complete BIR cattle ration (Ralston Purina Co., St. Louis, MO) containing 12% crude protein, 20% crude fiber, 2% crude fat, and 3.5% added minerals. The access to food was restricted from 8 a.m. to 6 p.m.; whereas the water supply was withheld from 12 noon to 6 p.m., which was the time period scheduled for experimentation on heat exposure.

After 3 weeks of such conditioning, the individual animal chamber temperature was abruptly raised to 39°, 60% RH at 12 noon and held at this level for 4 hr. During this period, blood samples were withdrawn at 5, 10, and 20 min intervals between 0 to 30, 30 to 120, and 120 to 240 min, respectively, postheat exposure. After 240 min the heating was stopped and the chamber door was opened for rapid cooling; while sampling was continued at 5, 10, and 20 min intervals for 30, 60, and 120 min periods, respectively. Two thermoneutral samples (at 20 and 10 min before the onset of heat exposure) were obtained for control values.

Blood samples were taken from outside the chamber, via a jugular catheter fixed in place 1 day before the trial. About 10 to 12 ml of blood were withdrawn with each sampling and the same volume of 0.85% NaCl was injected into the animal. Blood hematocrit and plasma specific gravity were determined immediately; and aliquots of plasma samples were stored at —20° for determination of GH concentrations by coated-charcoal radioimmunoassay. Rectal temperature was continuously recorded by thermocouple probes; and the respiration rate was measured by visual observation of the flank movements. The heat exposure was replicated following a 1 week rest period at 18°, 50% RH environment. A sham-control experiment, where the frequency, magnitude and the mode of bleeding were the same but without heat exposure, was conducted on each animal 1 week before the first thermal exposure trial.

Radioimmunoassay of GH. Antisera to GH (NIH-GH-B_{12}), produced in guinea pigs, were decomplemented by heat treatment (7), and the immunoglobulin G fraction separated by salt fractionation (8) was used in the assay. A purified bovine GH (2 USP/mg), received as a gift from Dr. L. J. Machlin of Monsanto Company, St. Louis, MO, was used for preparation of standards and for trace labeling. The hormone labeled with ^{125}I by Cambridge Nuclear Corporation, Cambridge, MA (107 mCi/mg sp act) was repurified on a Sephadex G-100 column each time prior to use.

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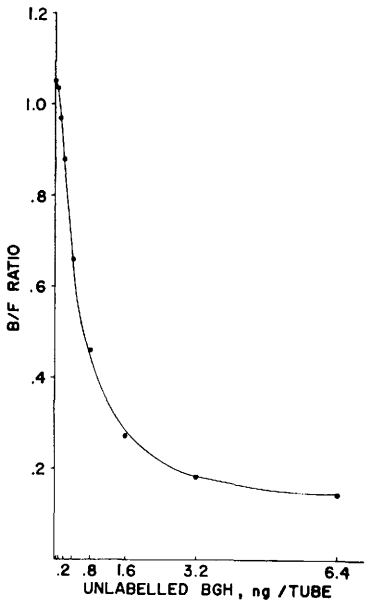

FIG. 1. Standard curve for radioimmunoassay.

Diluted antibody (1:100,000) in 10 × 75 mm glass culture tubes was incubated at 4° with standard GH solution (6.4 to 0.05 ng/tube) or diluted samples (1:50 to 1:100) for 2 days. Purified labeled GH (10,000 cpm) was then added and incubation was extended for 5 additional days. Thereafter, 0.1 ml of normal rabbit sera followed by 2 ml of dextran-coated charcoal suspension [prepared with dextran 80 and Norit A neutral charcoal in barbital–acetate buffer, pH 7.4 (9)] were added, and after a brief mixing on vortex mixer, the tubes were centrifuged for 50 min at 1000*g* (10), to separate the free labeled GH (F) absorbed to charcoal sediment from the antibody bound hormone (B) in the supernatant. The radioactivity of both B and F fractions measured in a Packard autogamma counter were employed to calculate the B/F ratio of each incubate, after correcting for the nonspecific binding as described by Wool and Selenkow (11). The B/F ratio of the standard tubes plotted against the unlabeled GH content constituted the standard curve (Fig. 1) from which the GH concentrations of the unknown were estimated.

With each assay group, a recovery experiment was run by adding known amounts of unlabeled GH to diluted normal sera. In bovine serum, the mean recovery was 103.7%, while recovery of rabbit serum was 100.3%. The mean GH concentration of a pooled plasma sample assayed repeatedly over 6 groups of assay was 18.40 ng/ml ± 0.21 SE, ± 0.52 SD, and 2.8% CV. The duplicate determinations of unknown plasma samples varied between ± 0.32 and 4.5% of their corrected values.

Results. The mean GH values obtained from 6 animals at each sampling time during the sham or control treatment are shown in Fig. 2. No significant changes occurred that could be attributed to sample bleeding. With minor fluctuations, the GH level remained at 32.7 ± 0.39 SE during 6 hr of repeated bleeding at a thermoneutral temperature. Furthermore there was no change in blood hematocrit and plasma specific gravity even during heat-exposure treatment periods that could be related to plasma GH changes in heat-stressed cattle (Table I).

Effect of heat exposure on plasma GH concentrations in cattle following acute exposure to high ambient temperature and the subse-

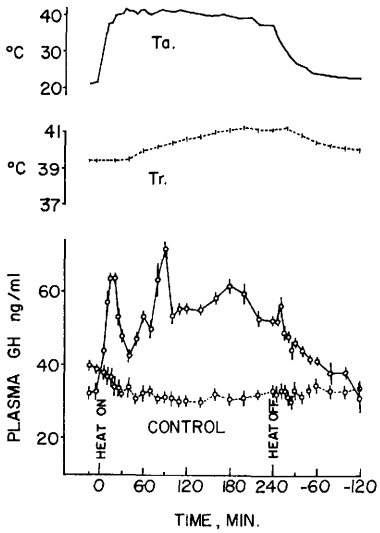

FIG. 2. Environmental chamber temperatures (T_a) and rectal temperatures (T_r), and plasma GH during heat-exposure trial. Control or sham plasma GH values are shown. Each data point of sham (control) is the mean value of 6 steers. Experimental points for T_r and T_a and GH represent 12 observations (6 steers, replicated once).

TABLE I. Plasma Growth Hormone, Rectal Temperature, Plasma Specific Gravity, and Blood Hematocrit in Cattle During Sham Control and Acute Heat-Exposure Experiments.

Item	Sham control[a]	Heat exposure[b]	t[c]
Growth hormone (ng/ml)	32.7	50.8	10.3 ($p < .01$)
Rectal temp (°)	39.4	40.3	7.3 ($p < .01$)
Plasma sp gr	1.025	1.025	1.0 (NS)
Blood hematocrit (%)	28.0	28.1	0.7 (NS)

[a] Mean values for 6 steers, 1 trial, 35 samples/steer.

[b] Mean values for 6 steers, 2 trials, 35 samples/steer.

[c] Students' t test estimated from the differences between sham and experimental values at each sampling time [Snedecor (12), p. 46].

quent changes in rectal temperature are also shown in Fig. 2. With the onset of thermal exposure, the plasma GH levels increased from 32.4 to 44.1 ng/ml within 5 min and continued rising until it reached the first peak of 63.9 and 63.1 ng/ml at 15 to 20 min postheat, respectively. Thereafter, the GH levels declined to 42.6 ng/ml at 40 min followed by an increase to 71.8 ng/ml at 90 min postheat and remained high (between 52.4 and 62.0 ng/ml) for the rest of the heat-exposure period. All these changes were significantly higher ($p < .01$) than the preheat base level, as judged by the method of Snedecor (12).

Fifteen minutes after thermal exposure ceased, the GH level started declining and approached near normalcy only after 80 min of declining chamber temperatures. This decline in plasma GH was characterized by some fluctuations, especially a small peak at 10 min following the cessation of heat exposure.

The acute thermal exposure caused a significant rise ($p < .01$) in rectal temperature; however, this hyperthermic response was evident only after 40 min of continuous heating due to latency of body mass. The initiation of the primary GH response at 5 min postheat occurred long before the rise in deep body temperature. The rise and decline in plasma GH concentrations following the onset and withdrawal of heat exposure preceded the changes in body temperature. The correlation coefficient between changes in plasma GH levels and environmental temperature was + .75 ($p < .01$) while that between plasma GH and body temperature was + .13 (NS).

Discussion. Acute exposure to high ambient temperature resulted in elevated plasma GH levels ($p < .01$) in cattle. This finding is in agreement with the reported rise in GHRF activity in heat-exposed rats (6), but does not support the results (5) of the failure of externally applied heat to alter the GH levels in pentobarbital-anesthetized monkeys. The nonresponsiveness in the latter case could be due to the anesthesia, which has been shown to blunt the usual GH response to a variety of stimuli (2), including that of insulin-induced hypoglycemia (13).

That elevation in plasma GH occurred from increased secretion rather than from a sudden reduction in the hormonal destruction can be assumed from the fact that the biological half-life ($t_{1/2}$) estimated from the declining phases of the two peak GH levels in heat stressed cattle were 25 and 24 min, respectively, a value similar to the normal $t_{1/2}$ of 24.4 min in holstein cows (14) obtained by the labeled GH disappearance technique.

The hyperthermic rise in rectal temperature was not a stimulus to GH secretion in these cattle since the GH response preceded the rise in rectal temperature, similar to the GH response to pyrogen-induced pyrexia that frequently preceded the actual febrile phase (5). The correlation between plasma GH changes and body temperature was positive but nonsignificant in contrast to the highly significant positive correlation between increased GHRF activity and body temperature in heat-exposed rats (6). Such results in

the latter report may be due to the hour-long lapse between the onset of heat exposure and the first sampling, a time significant to elevate body temperature especially in the smaller rat. Kimball *et al.* (15) failed to elicit a GH response by etiocholanolone administration, although a sustained elevation in body temperature was achieved. However, in this study, it is obvious that the environmental temperature surrounding the animal body by some means initiated the secretion of growth hormone into the plasma.

The time course of GH response to thermal stress shows a close resemblance to the reported electrical activity in the preoptic-anterior hypothalamus (PO/AH) area of a heat-exposed cat (17). The initiation of primary GH response at 5 min postheat corresponds with the activation of type II neurons of the PO/AH area which started firing within 2 to 6 min of heat exposure, long before any change in hypothalamic temperature was evident (17); while the initiation of the secondary surge at 40 min postheat, associated with the initiation of hyperthermia, corresponds to the increased firing by both types II and I-A neurons when the hypothalamic temperature was raised (17). Although a direct neuronal connection between the PO/AH area and the premammillary region is not known, the possibility of such connection cannot be disregarded. Since the type II neuron may be an interneuron (17), it may be possible that the neuronal transmission of impulses from peripheral thermoreceptors released NE from the terminal boutons on, or near, the GHRF production site and thereby elicited GH response in heat-exposed animals. Exposure to high ambient temperature accelerates the turnover of hypothalamic NE (16) and stimulates the release of GHRF (6); these modes of action are suggested for possible peripheral thermal effects on GH secretion in heat-exposed animals.

Summary. Plasma GH concentration, as estimated by radioimmunoassay increased significantly in cattle during 4 hr of acute thermal exposure. Blood hematocrit and plasma specific gravity did not change, while rectal temperature increased markedly. However, the rise and decline in plasma GH levels following, respectively, the onset and cessation of thermal exposure preceded the changes in body temperature. Changes in plasma GH were abrupt with the onset of heat exposure and correlated significantly with environmental temperatures suggesting the neural involvement of the skin thermal receptors, and areas of the hypothalamus. The possible adrenergic mechanism and significance of GH secretion during acute thermal stress is discussed.

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