Hypothalamic Growth Hormone Releasing Factor: Release and Synthesis After Exposure to a High Ambient Temperature[1] (37012)

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The regulation of growth hormone (GH) secretion through hypothalamic GH-releasing factor (GHRF) and GH-inhibiting factor (GHIF) has been well established (1). However, very little is known about the hypothalamic content of GHRF as influenced by stress (2–5). Only two reports refer to hypothalamic GHRF content following heat exposure (4, 5).

Most of the work on the physiology and biochemistry of these hypothalamic hypophysiotrophic neurohormones (6) is based on the tibia test which utilizes pituitary GH depletion in rats injected peripherally with hypothalamic extracts (7). The validity of *in vivo* GHRF assay has been further established by this laboratory (8, 9). A linear log–dose response relationship was found in a six-point assay between crude, rat stalk-median-eminence (SME) acid (0.1 *N* HCl) extracts and *in vivo* pituitary STH depletion as based on tibia test. Pressor and antidiuretic activities of these extracts were abolished by treating the extracts with sodium thioglycollate. The precision index of this assay was 0.087 ± 0.15, and it showed a parallelism between a three-point tibial response to a highly purified GH (NIH-GH-B-12). The results reported herein are based on the published (8, 9) six-point assay for GHRF showing parallelism.

The objectives of this study were to describe the temporal responses of GHRF to continuous environmental heat exposures and to determine whether resynthesis, which is denoted by a gradual recovery of GHRF content toward a normal level, is a continuous process. A previous paper (4) reported the environmental heat effects but did not characterize the gradual recovery or synthesis of GH content. A preliminary report of this work has appeared (5).

Materials and Methods. Mature female rats of Sprague–Dawley strain (Holtzman, Madison, Wisconsin) with mean body weights (b.w.) of 239.3 ± 1.6 g were used as experimental animals and were donors of stalk-median-eminence (SME). They were subjected to a high ambient temperature of 35.5° ± 0.5° with relative humidity 50 to 55% for periods of 1, 4, 96, and 240 hr, respectively. Immature female rats of 28 to 34 days with a mean b.w. of 89.1 ± 8.5 g received SME extracts from heat-exposed rats and are referred to as recipient rats. These rats and SME donors belonged to the same strain, source, and sex. They were maintained on Rockland rat pellets and fed *ad libitum*.

Immature female rats of Charles River CD Strain (Charles River Breeding Laboratories, Wilmington, Massachusetts) were hypophysectomized at 28 days of age and were shipped within 24 to 48 hr after the operation. They were given a special diet (10) *ad libitum* (Nutritional Biochemicals, Cleveland, Ohio). All animals were housed in the temperature-controlled (25° ± 1°) small animal climatic laboratory. Further details pertaining to pituitary donors and assay rats are shown in Table I.

SME extracts and pituitary homogenates were prepared as described earlier (8) with the following modification: an equivalent (by weight) of 0.50 SME was the dose used since this concentration of thioglycollate treated SME (thio-SME) yields optimum response as judged in a six-point *in vivo* assay of GHRF (8). Each pituitary was homogenized in 2

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TABLE I. Synthesis of Growth Hormone Releasing Factors in the Rat Hypothalamus After the Depleting Effect of Exposures to a High Ambient Temperature.

	Donors of SME[a]		Recipient rats[b]			Assay rats[c]		
Treatment	Body wt (g) Mean ± S.E.	Rise in rectal temp. (°) Mean ± S.E.[d]	Age (days)	Body wt (g) Mean ± S.E.	Dose AP equivalents	Body wt (g) Mean ± S.E.	Epiphyseal width (μ) Mean ± S.E.	p values vs control
Normal control (16)	233.7 ± 2.5	—	34	108.0 ± 5.23 (10)	1	87.0 ± 3.9	329.0 ± 13.4[h] (7)	
Heat exposed:								
1 hr (16)	232.6 ± 1.7	1.54 ± .13	28	77.2 ± 0.87 (9)	1	92.63 ± 4.2	210.1 ± 7.9[e] (8)	0.01
4 hr (16)	250.5 ± 1.8	1.30 ± .13	33	96.1 ± 1.62 (10)	1	87.85 ± 3.5	242.9 ± 9.0[e,f] (8)	0.01
96 hr (16)	238.5 ± 2.1	0.63 ± .13	32	91.2 ± .74 (10)	1	87.91 ± 4.0	270.9 ± 7.1[f,g] (8)	0.01
240 hr (12)	241.3 ± 2.6	1.62 ± .54	29	73.3 ± 1.43 (9)	1	89.98 ± 1.7	298.4 ± 14.4[g,h] (8)	N.S.

[a,b,c] Numbers in parenthesis indicate number of animals in each treatment group, recipient rats which randomly received SME extracts from each treatment, and hypophysectomized rats used for each assay, respectively.

[d] Rise in rectal temperature of animals on exposure to a high ambient temperature is significantly ($p < 0.01$) higher as judged by Dunnett's procedure for comparing all means with control.

[e,f,g,h] Means having the same superscript are not significantly different from each other as tested by Duncan's Multiple Range Test with Kramer's modification.

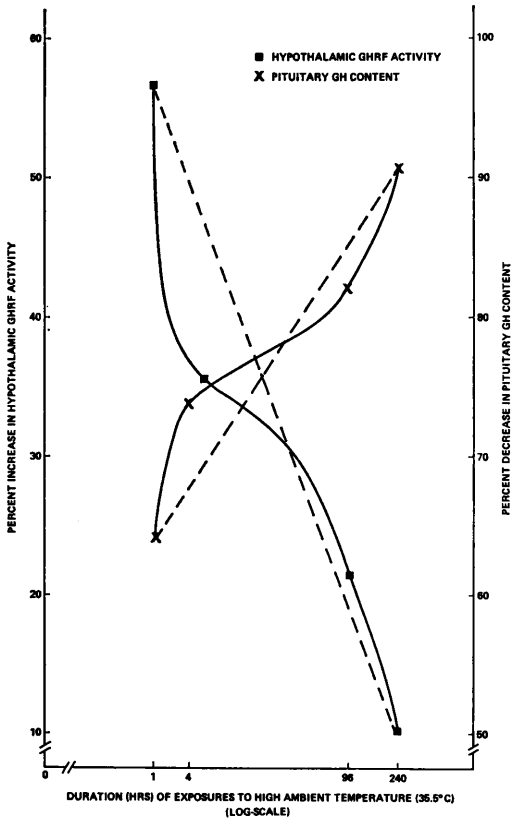

FIG. 1. The effect of exposures to high ambient temperatures (35.5° ± 0.5°) on hypothalamic GHRF activity and pituitary GH content in rats, expressed as percent of those kept at 25° ± 1°.

ml of previously chilled (4°) 0.01 *N* NaOH. Sodium hydroxide (0.01 *N*) was preferred to phospho-saline since it yields optimum extraction as compared to phosphate buffered saline (pH 7.6) and 0.25% acetic acid (11).

Hypothalamic GHRF activity of the heat-exposed rats was evaluated by measuring the depletion in pituitary GH content of the recipient rats through the tibia test of Greenspan *et al.* (12) as detailed earlier (8). Increasing GHRF activity is indicated by decreasing pituitary GH content and, in turn, is reflected in a decrease in epiphyseal cartilage width.

Rectal temperature of heat-exposed rats was measured with a thermistor probe inserted to a depth of 4 cm into the rectum with 1 min allowed for equilibration before readings were taken on a tele-thermometer (Yellow Springs Inst. Co., Yellow Springs, Ohio). Analyses of variance were carried out on the difference (Δ) between pre- and post-exposure rectal temperatures. Experimental means were compared with that of the control by the multiple comparison test of Dunnett (13) and with each other using Duncan's (14) multiple range test.

Results and Discussion. Results are summarized in Table I and illustrated in Fig. 1. These results may be visualized under two main physiological processes: (a) release and (b) synthesis of GHRF. The maximum releasing activity was seen after a 1-hr heat exposure as reflected in reduced epiphyseal cartilage width and thus further confirmed the earlier findings from this laboratory (4). Such a short-term induced release of GHRF is not confined to heat stress but is caused by a variety of stressors such as starvation (15), insulin induced hypoglycemia (3), and cold (2).

A comparison of two consecutive cartilage width means using Duncan's multiple range test (14) with Kramer's modification (16) indicated that two consecutive treatment means (*e.g.*, 1 and 4 hr, 4 and 96 hr, *etc.*) were not significantly different from one another (Table I) which suggests a continuous synthesis of GHRF. The reasons for such synthesis may be postulated as (a) a partial loss of the signaling threshold by the peripheral receptors, through acclimatization, whereby they are unable to elevate firing rates of hypothalamic neurons with the concomitant release of GHRF, since hypothalamic neurons have been recognized as the site for the production of releasing factors (17), and (b) utilization, degradation, and/or decreased production of GHRF. A gradual increase in the somatotrophic release inhibiting factor (18) may account, in part, for the hypothalamic GHRF content approaching near normal levels since the tibial evaluation cannot distinguish the releasing factor for GH from the inhibitive one. Immuno-assayable plasma growth hormone levels were also found below normal levels after prolonged heat exposure (19).

Such a restoration in hypothalamic GHRF content after prolonged stress approaching control levels (Fig. 1) has been reported

with other stressors such as cold (2) and insulin induced hypoglycemia (3).

The possibility that the vasopressin content of the SME extract would alter tibial response and in turn would affect *in vivo* GHRF assay was excluded by treating it with thioglycollate which abolished pressor and antidiuretic activities of vasopressin contained in the SME extracts (20).

The polyamine content of the SME extracts cannot account for its GHRF activity as it does for FSH depleting activity, since 20- to 600-fold concentrations failed to alter GHRF activity (21).

Dexamethasone, which blocks adrenal corticoid response, failed to alter responsiveness of rat pituitary to exogenous GHRF (22). Hence, it is unlikely that elevated plasma corticosterone levels on heat exposure of rats (23) would affect hypothalamic GHRF.

GHRF stimulates mammotrophs (24). However, this would not affect evaluation of GHRF activity through the tibia test since the luteotrophic hormone does not affect the tibia test (12, 25).

Increased GHRF activity caused by pyrexia (Table I) under high ambient temperature is in complete agreement with the GH regulatory responses in humans resulting from pyrogen-induced pyrexia (26, 27).

The physiological significance of enhanced GHRF activity under short-term exposure to heat may be postulated. This may be to compensate for (a) the loss of sodium and potassium in the hot environment, (b) the slow utilization and, in part, increased $t_{1/2}$ time of GH (19), and (c) the catabolic effects of elevated corticoids (23).

Summary. This study was designed to investigate temporal effects of environmental heat stress on the release and synthesis of hypothalamic GHRF in rats. The experiments consisted of five treatment groups; one group of normals at 25° ± 1° and four groups, each exposed to 35.5° ± 0.5° for 1, 4, 96, and 240 hr, respectively. GHRF activity was measured by *in vivo* depletion of pituitary GH content in immature rats. Vasopressor and antidiuretic activities of crude milk median eminence extracts were abolished by treating the extracts with thioglycollic acid. Exposures for 1, 4, and 96 hr significantly ($p < 0.01$) elevated GHRF activity as compared to the control which returned to a normal level by 240 hr. No significant difference existed in GHRF activity for two consecutive periods of exposures, indicating a continuous synthesis of GHRF. Enhanced GHRF activity on heat exposure has been discussed in light of the increased endogenous turnover of hypothalamic norepinephrine on heat exposure and the enhanced firing rate of the hypothalamic neurons. Synthesis of GHRF to near normal levels followed by an initial depletion after a prolonged heat stress has been visualized in terms of loss of peripheral stimulation through acclimatization and/or an increase in the somatotrophin release-inhibiting factor.

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