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Stress Suppression of Growth Hormone Secretion in the Rat: Effects of Disruption of Inhibitory Noradrenergic Afferents to the Median Eminence

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Abstract

The participation of a growth hormone (GH) inhibitory noradrenergic input to the median eminence in stress-induced suppression of rat GH secretion was investigated in animals with median eminence catecholamine lesions produced by intravenous injection of 6-hydroxydopamine (6-OHDA). Unstressed lesioned rats exhibited an enhanced frequency of GH secretory bursts, but both intact and lesioned rats responded to stress with suppression of GH (controls: 56% suppression, 6-OHDA lesioned: 43% suppression, not significantly different). Thus noradrenergic projections to the median eminence, if they participate at all in stress-induced GH suppression, appear to have only a minor role. This study does not exclude the possibility that circulating adrenaline of adrenal medullary origin might obscure defects in GH control produced by noradrenergic denervation of the median eminence.

Introduction

Pulsatile secretion of growth hormone (GH) from the anterior pituitary is regulated by GH releasing factor (Guillemin *et al.* 1982) and a release inhibiting factor, somatostatin (Martin 1976). In the rat, stressful stimuli suppress GH secretion (Krulich *et al.* 1974; Martin 1976). This effect can be prevented by pretreatment with somatostatin antiserum (Terry *et al.* 1976), so that stress-induced suppression of GH secretion is, at least in part, likely to be due to increased median eminence somatostatin release.

Central catecholamine structures play a major role in the regulation of basal patterns of GH secretion. Although their influence is primarily facilitatory, it has been demonstrated that median eminence noradrenaline afferents are inhibitory to GH secretion (Day and Willoughby 1980). This action may be mediated by facilitation of release from median eminence somatostatin terminals, for noradrenaline has been shown to stimulate the secretion of somatostatin from median eminence fragments incubated *in vitro* (Negro-Vilar *et al.* 1978). Systemic administration of adrenaline, which does not penetrate the blood brain barrier, also is known to inhibit GH secretion (Krulich *et al.* 1974), presumably by an action at the median eminence which lies outside the blood brain barrier.

In view of these data it is possible that median eminence noradrenaline afferents are important in mediating the suppression of GH which occurs in response to stressful stimuli in the rat. We have previously provided evidence that systemic administration of the catecholamine-specific neurotoxin 6-hydroxydopamine (6-OHDA) disrupts median eminence noradrenaline afferents without functionally impairing median eminence dopamine mechanisms (Day and Willoughby 1980). Systemic 6-OHDA affects only those nervous system structures which lie outside the blood brain barrier. These structures include the circumventricular organs (median eminence, area postrema, organum vasculosum of the lamina terminalis, subfornical organ, pineal gland, and subcommissural organ), as well

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as the peripheral autonomic nervous system. Using this approach the present study examined the effect of disruption of median eminence noradrenaline afferents upon the GH response to stress in the male rat.

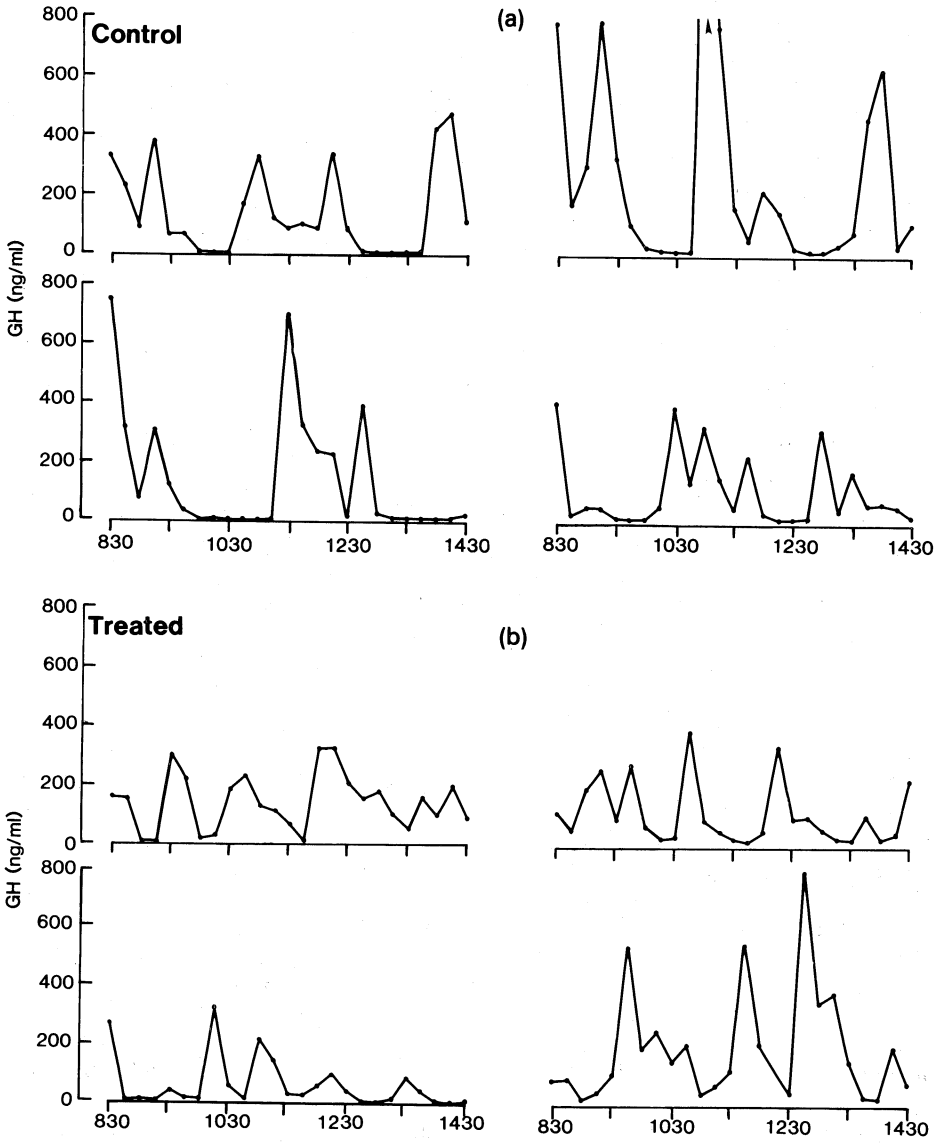

Fig. 1. Growth hormone secretory patterns for 6 h in four control rats (*a*) and in four 6-OHDA-treated rats (*b*). The characteristic 3-hourly pattern of the GH rhythm is evident in the control animals but the GH pattern now exhibits very frequent bursts in the 6-OHDA treated animals. Trough values are below assay sensitivity.

Materials and Methods

Male albino Porton rats (liveweight, 200–300 g) were permitted free access to food and water, and were maintained on a 12 : 12 light cycle (lights on 0730 h) at an ambient temperature of 23°C.

The animals received a chronic indwelling right atrial cannulae (Brown and Hedge 1972) and were housed in isolation boxes such that blood samples could be withdrawn via tubing leading to the outside of the box. Seven days after cannulation the rats received, via the indwelling atrial cannulae, injections (i.v.) of either 6-OHDA (50 mg base per kilogram liveweight) dissolved in physiological saline with 0·1 mg/ml ascorbic acid, or vehicle. On each of the two following days all animals were sampled every 15 min for 6 h beginning at 0830 h. On one of these days the animals were undisturbed for the full 6 h. On the other day, animals were subjected to the stress produced by an enforced swim for 30 min when sampling commenced. Animals were immersed in a deep water-bath at 27–30°C, and subsequently dried before being recaged.

Upon collection, blood samples were immediately centrifuged, the plasma (0·2 ml) was frozen for subsequent assay, and the red blood cells resuspended in 0·2 ml physiological saline and reinjected into the animal after the next sample. Plasma GH and prolactin concentrations were determined by radioimmunossay using a double antibody separation technique. Materials were provided by the N.I.A.D.D.K. and values are expressed in terms of the appropriate N.I.A.D.D.K. reference preparation. Minimum and maximum sensitivities of the GH assay were 6·25 and 800 ng/ml respectively, for prolactin 1·0 and 320 ng/ml. All samples taken from the one animal were assayed at the same time to avoid interassay variability (for GH 3%; for prolactin 5%). Intra-assay variability was 3% for both GH and prolactin assays. Statistical comparisons of data were made using two-tailed Student's *t*-tests, as well as analysis of variance for repeated measures.

Histofluorescence microscopy using the Faglu technique (Furness *et al.* 1978) was used to confirm loss of catecholamine fluorescence in the median eminence in animals treated with the same dose of 6-OHDA.

Results

Untreated control animals ($n = 6$) demonstrated pulsatile GH secretion with an approximate 3-hourly periodicity (Fig. 1*a*) as is well described (Martin 1976; Terry *et al.* 1976). Animals treated with intravenous 6-OHDA ($n = 7$) exhibited more frequent GH peaks and some had elevated trough levels (Fig. 1*b*) in agreement with our previous study (Day and Willoughby 1980). On defining secretory episodes as GH bursts commencing after troughs in which GH concentrations are below assay sensitivity, there was a shorter inter-peak period in 6-OHDA-treated animals: controls (mean±s.e.) 2·83±0·17 h; 6-OHDA-treated 1·75±0·17 h, $P<0·05$). The average GH concentration (ng/ml) after 6 h in treated rats, however, was not significantly increased above controls:

Treatment	*n*	Non-stressed rats	Stressed rats	*F*
Control	6	155±31·4	62·9±12·9	30·1 ($P<0·01$)
6-OHDA	7	168±30·8	91·8±16·9	28·6 ($P<0·01$

In the second part of the study, the enforced stress suppressed GH concentrations in all rats in both control and 6-OHDA-treated groups (see above tabulation), although complete suppression was present only for approximately 1 h. Profiles of average GH concentrations for control and stress experiments in both groups are shown in Fig. 2, and illustrate the similar responses in the two groups. GH concentrations in untreated controls reveal that there is an underlying partially synchronized 3 hourly rhythm of GH secretion, whereas in the 6-OHDA treated group, the frequent asynchronous GH peaks in individual animals produce a more stable mean GH profile. Although the overall suppression of 6 h average GH concentrations in control animals (56·4±8·1%) was more than the average suppression in 6-OHDA-treated rats (43·2±7·5%), the difference was not statistically significant ($P>0·6$, two-tailed *t*-test). In another assessment of whether GH was less effectively suppressed in 6-OHDA-treated animals, the average GH concentration at each sampling time in stressed animals was expressed as a percentage of the baseline concentration at that sampling time. These values were then compared for the two groups and were not significantly different: control 47±9·4% versus 6-OHDA-treated 42·0±7·0%.

Low-magnification histofluorescence examination of brains from rats treated in parallel with 6-OHDA revealed complete loss of fluorescence (Fig. 3). Swollen catecholamine fibres apparently approaching the median eminence were evident. Residual fluorescence was still present at high magnifications and probably reflects residual dopamine fluorescence.

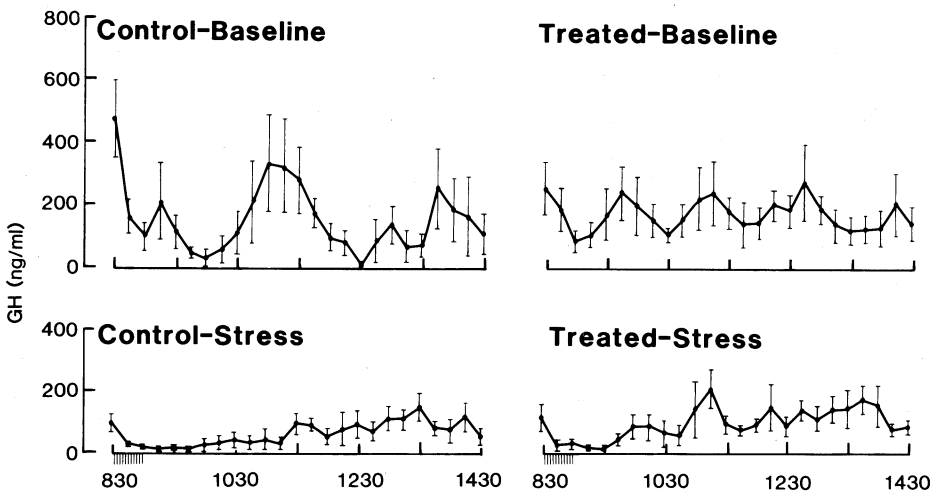

Fig. 2. Mean 6 h GH profiles in intact (control) and 6-OHDA-treated rats. Blood samples were taken for GH assay from the rats during non-stress conditions and following 30 min of enforced swimming in deep water-baths (indicated by the bar). 6-OHDA treatment disrupts the 3-hourly pattern of GH secretion, which is reflected in loss of the 3-hourly fluctuations in mean GH concentration seen in control animals. Exposure to stress suppresses GH secretion approximately equally in both groups.

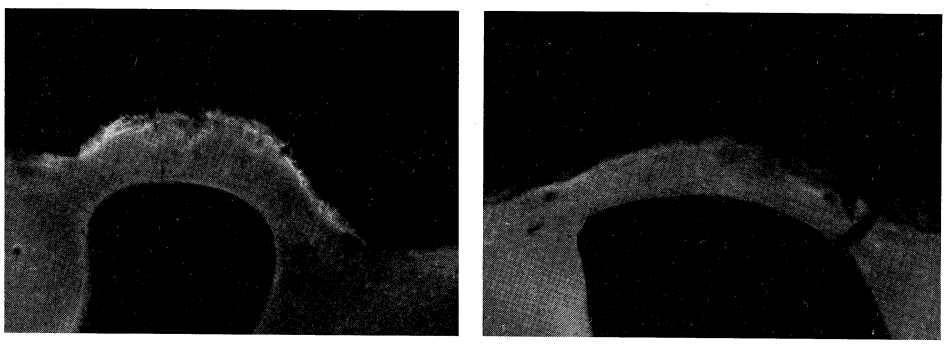

Fig. 3. Catecholamine fluorescence photomicrographs of the median eminence in control (*left*) and 6-OHDA-treated (*right*) animals. Diminished fluorescence is evident after treatment with 6-OHDA.

Serum prolactin concentrations were also measured in these animals. There was no disruption of the expected prolactin response to stress: control 300±143% increase; 6-OHDA-treated 472±172% increase.

Discussion

The results confirm our previous study that intravenous administration of 6-OHDA disrupts the pulsatile secretion of GH so that an increased frequency of GH peaks occurs (Day and Willoughby 1980). Noradrenergic terminals in the median eminence, which are affected by this treatment therefore appear to have an inhibitory effect on GH secretion,

and might theoretically mediate stress-induced GH suppression in this species. Even though sufficient catecholamine disruption had occurred to disturb normal rhythmic GH secretion, suppression of GH by stress was only slightly affected in treated animals (76·6–89·0% of controls, depending on the method of assessment) and failed to reach statistical significance. The physiological function of noradrenergic afferents to the median eminence in GH regulation, therefore, seems to be primarily that of regulating the rhythmicity of the GH pulsatile rhythm. Noradrenergic afferents may participate slightly in the stress response but do not appear to be essential for it.

Although systemic 6-OHDA also disrupts noradrenergic innervation of the other circumventricular organs, a significant function for these structures in GH regulation has not yet been established. It seems likely, in view of the central role of the median eminence in pituitary regulation, that the effects of 6-OHDA are due to disruption of median eminence noradrenaline afferents.

Because a histochemical fluorescence method has been used in this study, the extent of noradrenergic denervation cannot be quantified. Evidence that denervation is functionally effective, so far as GH regulation is concerned, is that the time course of the disturbance of GH regulation correlates with that of the histochemically observed depletion in fluorescence in the median eminence (Day and Willoughby 1980). Furthermore, in this model, there appears to be no functional impairment of dopaminergic neurones, as judged by normal basal levels of prolactin after 6-OHDA (Day and Willoughby 1980). It is known that systemically administered 6-OHDA causes degeneration of peripheral autonomic noradrenergic nerves, but does not impair adrenal medullary function. Moreover, maintenance of normal blood pressure immediately following such treatment depends on a compensatory increase in adrenal function (Kostrzewa and Jacobowitz 1974). As a consequence, if adrenaline and noradrenaline in the systemic circulation participate in stress suppression of GH by an action at the median eminence (outside the blood brain barrier), it might be expected that GH concentrations would be suppressed following systemic 6-OHDA treatment. Even though this is not the case, it may be possible for a further stress-induced rise in circulating adrenaline to effect stress-induced suppression of GH, so obscuring any change in GH regulation produced by 6-OHDA. To the present time, however, amines and peptides of peripheral origin have not been thought to participate in pituitary regulation in physiological circumstances.

This study also indicates that the Porton strain of rat is relatively resistant to stress-induced GH suppression. It is known that GH secretion in the Sprague Dawley rat may remain fully suppressed for 5 h after a stress identical to the one used here (Terry *et al.* 1976). We have evidence that the DA Agouti strain also is extremely stress-sensitive, for when simply caged in isolation these rats fail to thrive and do not secrete GH (M. F. Menadue and J. O. Willoughby, unpublished data). Although certain neuroendocrine responses to stress may be important adaptive mechanisms, the variation in GH stress responses across rat strains and across many mammalian species (Martin 1976) makes it unlikely that the GH response to stress is an important aspect of stress physiology.

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