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Regulation of Hypothalamic Somatostatin by Glucocorticoids

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Glucocorticoids (GCs) play a key role in the physiology of the hypothalamic–somatotroph axis, since these steroids enhance growth hormone (GH) gene transcription and increase GHRH receptor synthesis. However, GC excess inhibits normal growth in all species studied. This is mainly due to the impaired GH secretion observed during hypercortisolism, a situation in which GH responses to a number of stimuli, including GHRH, are blunted. The inhibitory effect of GCs on GH secretion seems to be dependent on enhanced hypothalamic SS secretion. Since SS release is stimulated by β-adrenergic agonism we tested the possibility that GC inhibition of GH secretion would depend on increased β-adrenoceptor activity in SS-producing neurons. The experimental design consisted in evaluating the GH response to GHRH in normal subjects after having induced hypercortisolism, with DEX, and blocking β-adrenoceptors with propranolol (PRO). Moreover, to investigate the specificity of this mechanism, GHRH-induced GH release was tested after inducing hypercortisolism and enhancing α_2-adrenergic or muscarinic cholinergic tone, by giving clonidine (CLO) or pyridostigmine (PD), respectively. As expected, nocturnal DEX administration inhibited the GH response to GHRH. In this situation of hypercortisolism, both PRO and CLO, but not PD, were able to reverse the inhibitory effect of DEX on GHRH-elicited release. However, the potentiating effect of these drugs on the GHRH-induced GH secretion was only observed for PRO. These data confirm that GC excess inhibits GH release by increasing hypothalamic SS secretion, and that the mechanism is mediated by GC-induced enhanced β-adrenergic responsiveness. Therefore, the defective GHRH secretion observed in chronic hypercortisolism must be a consequence of the continuous blockade that SS excess exerts on GHRH-producing neurons. Our postulate agrees with other data in the literature showing that GCs modulate the secretion of some hypothalamic peptides by changing the responsiveness of the producing neurons from α_2-adrenoceptors to that of β-adrenoceptors.

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INTRODUCTION

Hypothalamic SS plays the main role in growth hormone (GH) control [1]. In fact, although hypothalamic GH releasing hormone (GHRH) must reach the pituitary somatotropes in order for GH secretion to be elicited, the release of this peptide seems to be modulated by SS [2], which otherwise counteracts the GH-releasing effect of GHRH at the somatotroph level. According to this hypothesis, the hypothalamic–somatotroph rhythm (HSR) [3] which establishes the episodic pattern of physiological GH secretion must have its main oscillator in SS-producing neurons. SS secretion, by blocking both GHRH release and the secretagogue effect of the peptide, interrupts pituitary GH secretion. The rhythmic lack of SS release, most likely governed by suprahypothalamic structures, will allow GHRH to be secreted and therefore induce GH release to the blood. These concepts are shown schematically in Fig. 1.

On these bases, the factors involved in GH control would mainly act by affecting hypothalamic SS secretion. Therefore, in order to ascertain how GH is regulated, the putative effect of each factor studied on hypothalamic SS release must first be investigated [1]. Such an investigation would take into account that adrenergic inputs to SS-producing neurons play the main role in SS control, it now being clear that while α_2-adrenergic stimulation inhibits SS release in all

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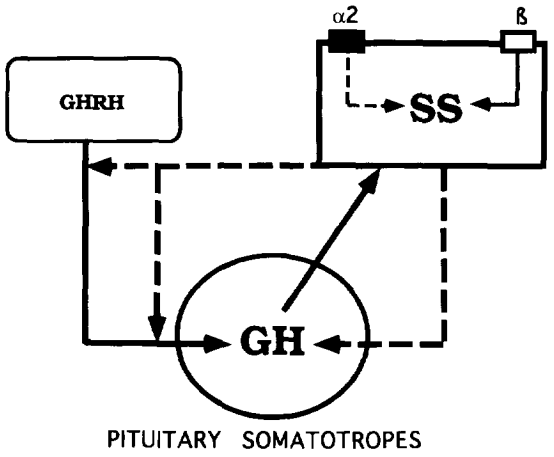

Fig. 1. Hypothalamic growth hormone control. SS inhibits GH secretion by counteracting the effect of GHRH at the somatotropes level and directly inhibiting the hypothalamic release of the peptide. In turn, SS secretion is mainly dependent on the balance between α_2-adrenergic (inhibitory) and β-adrenergic (stimulatory) activities at the SS-producing neurons. GH directly stimulates SS release. ⟶ stimulation. - - - → inhibition.

species in which it has been studied [4], β-adrenergic agonism stimulates the secretion of this inhibitory peptide.

GLUCOCORTICOIDS AND GROWTH HORMONE SECRETION

Glucocorticoids (GCs) are a key component for the physiological functioning of the hypothalamic–somatotroph axis [1]. These steroids enhance GH gene transcription [5] and increase GHRH receptor synthesis [6]; consequently, it seems to be logical that a synthetic GC such as dexamethasone (DEX) stimulates both GHRH-induced GH release [7] and spontaneous secretion of the hormone [8]. In line with this, an adequate GC replacement is needed to restore a normal GH secretion in patients with idiopathic ACTH deficiency [9]. However, it is well known that GC excess inhibits normal growth in all species studied [10]. Although this results from the conjunction of a number of peripheral alterations, chronic hypercortisolism is associated with impaired GH secretion [11]. Either the spontaneous secretion of the hormone or its release in response to a number of stimuli, including GHRH, are blunted in situations of GC excess. Therefore paradoxically GCs seem to play two opposite roles in GH control: facilitatory, exerted at the pituitary level; and inhibitory, most likely exerted on the hypothalamic structures. The former, a physiological effect, must be associated with the permissive role that these steroids play in the organism [1]; however, when trying to provide a physiological explanation for the inhibitory action that GCs exert on GH secretion, a number of questions arise. That is, why does this effect occur? Where and how does the inhibition take place? What is the physiological meaning of this effect?

In order to provide an adequate answer to these questions, we must first know how GH secretion occurs (for review see Ref. [1]). GH is released into peripheral blood episodically. This episodic pattern is necessary for the optimal induction of physiologic effects at the peripheral level. The rhythm, most likely established by suprahypothalamic structures, is basically modulated by the SS–GH relationships. GH release stimulates SS secretion [12], which in turn interrupts the secretory activity in somatotropes. Interestingly, the lack of GH does not appear to affect the rhythmic secretion of SS [4], while in the absence of this peptide a tonic GH secretion can be observed. According to this, as pointed out in the Introduction, SS plays the main role in GH control. The rhythmic interruption of SS release allows GHRH to be secreted; then GHRH induces a rapid, dose-dependent, release of GH somatotropes, but also the transcription of the GH gene. In turn, increased GH would represent a positive signal to SS-producing neurons (Fig. 1). Their response would optimize, by blocking GHRH secretion and action, the amount of GH released and consequently the pituitary stores in the hormone.

A number of reports suggested a primary GC-induced GHRH blockade as the mechanism directly responsible for the lack of GH seen in hypercortisolism [13, 14]. However, we hypothesized that the inhibitory effect of GCs on GH secretion had to be exerted by stimulating SS secretion [1, 15]. In our hypothesis, SS excess being the primary event, SS-dependent GHRH blockade would therefore take place [2]. In fact, in rats, the administration of pharmacological doses of DEX for 3 days increases the hypothalamic content of SS [16], whereas the administration of SS antiserum enhances the blunted GH response to GHRH observed in animals given DEX for 4 days [17].

The possibility that hypercortisolism led to increased SS secretion was tested according to our recent model indicative of how the GH responses to physiological or pharmacological challenges should be interpreted [1]. Moreover, since SS control is mainly exerted by inhibitory α_2-adrenergic pathways and stimulatory β-adrenergic pathways, our experimental model was based on the possibility that an increased SS tone would be dependent on enhanced β-adrenoceptor responsiveness (Fig. 1). The support for such a hypothesis proceeds from several findings demonstrating that, at the peripheral level, GCs increase β-agonist-stimulated adenyl cyclase activity and β-adrenoceptor number [18]. In fact, the sequence homologous to glucocorticoid-responsive elements has been identified in the 3′ flanking region of the β_2-adrenoceptor gene [19, 20].

Our study [15], in normal humans, basically consisted of the evaluation of the GH response to GHRH after inducing hypercortisolism with DEX, and then

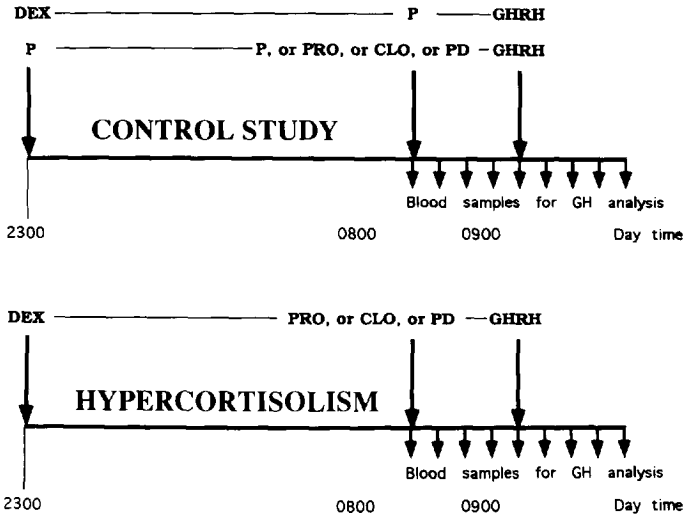

Fig. 2. This diagram schematically shows the experimental protocol followed. In a control study, the GH response to GHRH was analyzed after giving dexamethasone (DEX) or placebo (P) 10 h before. The GH response to GHRH was then tested under β-adrenergic blockade with propranolol (PRO), or α_2-adrenergic agonism with clonidine (CLO), or cholinergic agonism with pyridostigmine (PD). These studies were repeated under hypercortisolism elicited by giving DEX 10 h before the GHRH challenge.

blocking β-adrenoceptors with propranolol (PRO) (Fig. 2). With this experimental design we attempted to ascertain whether β-adrenoceptor blockade would be able to counteract the inhibitory effect of GCs on the GH response to GHRH. In such a case, a logical conclusion would be that the mechanism postulated for GCs at the peripheral level [18] would also be operative on the SS-producing neurons. In addition, and to better elucidate the specificity of this putative mechanism of action, we also tested the GHRH-induced GH release in the same subjects after inducing hypercortisolism and enhancing α_2-adrenergic or muscarinic cholinergic tone, by giving clonidine (CLO) or pyridostigmine (PD), respectively (Fig. 2). The rationale for this was based on (1) the direct SS-release inhibiting effect found for CLO by our group [21]; and (2) the fact that while cholinergic pathways play an important role in SS control [22], their effects seem to be mediated by catecholamines [23], as it occurs in the periphery. On these bases, it would be expected that CLO, acting at the post-synaptic level in the SS-producing neurons, would be able to counteract the inhibitory effect of DEX on the GH response to GHRH, while this would not occur with PD, acting proximal to adrenergic neurons.

Results from this study [15] confirmed the experimental hypothesis. Nocturnal DEX administration induced the existence of a refractory phase [3] in all the subjects at the time of GHRH tests, 9 h later. This indicated a high amount of SS inputs reaching the somatotropes. In this situation, and as previously described in other studies, GHRH challenge was not able to evoke a significant GH release. Both PRO and CLO, but not PD, were able to reverse the inhibitory effect of DEX on the GHRH-elicited GH response. However, while the PRO-enhanced GHRH-induced GH secretion remained unaffected by hypercortisolism, this was no longer observed with CLO. From these data, summarized in Table 1, we concluded that the inhibitory effect of GC excess on GH release

Table 1. Plasma GH peaks ($\mu g/l$; mean $\pm$ SEM) elicited by GHRH in the different experimental situations. In the control study the GH response to GHRH was significantly ($P < 0.01$) enhanced after pretreatment with propranolol (PRO), clonidine (CLO) or pyridostigmine (PD). Nocturnal dexamethasone administration significantly ($P < 0.05$) reduced the GH response to GHRH. This blunted GH response was totally or partially restored after β-adrenergic blockade, with PRO, or α_2-adrenergic agonism, with CLO, respectively, but unaffected by cholinergic agonism with PD

	P or DEX	PRO	CLO	PD
Control	20.3 ± 5.5*	43 ± 4.6**	55.6 ± 5.6**	51.2 ± 7**
Hypercortisolism	10.7 ± 3.9	39 ± 5.5**	25.9 ± 3.9*	12.9 ± 3.1

*$P < 0.05$ vs control study under hypercortisolism (DEX). **$P < 0.01$ vs placebo study (P, control).

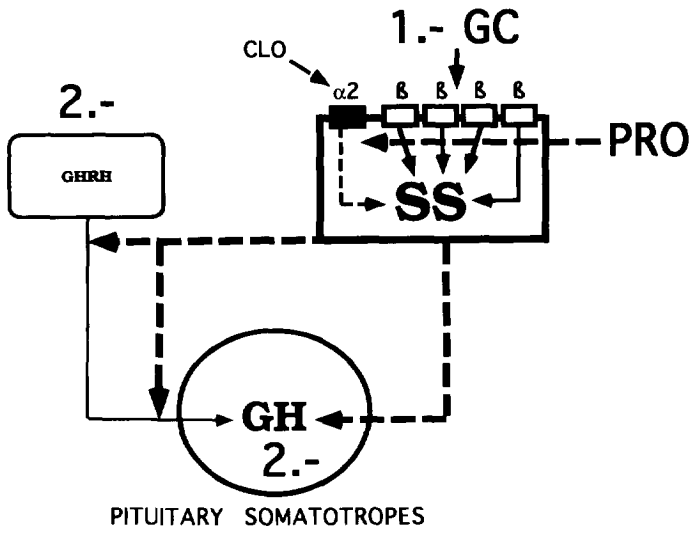

Fig. 3. GCs inhibit GH secretion by enhancing SS release. This effect seems to be due to increased β-adrenergic activity in SS neurons (1). the defective GHRH secretion observed in hypercortisolism must occur as a consequence of the tonic inhibition that SS exerts on GHRH neurons (2). GH secretion is then blunted (2). β-adrenergic blockade with PRO impedes SS to be secreted. CLO acting directly on α_2-adrenoceptors would allow a certain degree of SS inhibition, therefore leading to a GH response to GHRH challenge. ⟶ stimulation. - - - → inhibition.

is due to increased hypothalamic SS secretion which appears to be dependent on DEX-induced enhanced β-adrenergic responsiveness [15] (Fig. 3). Moreover, the study allowed us to confirm our postulate regarding the major role that central adrenergic pathways play in SS, and therefore in GH, control. In effect, the lack of action of the enhancement of cholingeric tone, with PD, under hypercortisolism, compared to that of CLO in the same experimental situation, would be related to the different site of action of these drugs [23]. PD acting proximal to adrenergic neurons would be unable to antagonize β-adrenergic-induced SS release, whereas CLO directly stimulating α_2-adrenoceptors in SS neurons would partially counteract the DEX-induced increased β-adrenergic activity (Fig. 3). Since CLO is a rather selective α_2-adrenergic agonist, it is likely that the opposing effects of endogenously enhanced β-adrenergic responsiveness and exogenously induced α_2-adrenergic stimulation could account for the GH responses to GHRH seen after giving DEX + CLO. That is, those subjects in which the effect of DEX on β-adrenoceptors had been low; or those in which this effect had partially disappeared at the time of CLO administration, exhibited the normal CLO-enhanced GHRH-induced GH release [21]. This is consistent with the fact that the cellular responses of many tissues to NE depend on the relative availability of its receptor subtypes (α_1, α_2, β).

Our postulate that glucocorticoids increase SS release by enhancing β-adrenoceptor responsiveness is also supported by a recent study by Huang *et al.* [24]. These authors described that GCs modulate irANP secretion in hypothalamic neurons by changing the responsiveness of the cells from α_2-adrenoceptors to that of β-adrenoceptors.

The possibility of enhanced SS tone being responsible for the inhibitory effect of GC on GH release seems to dispute data indicating that it is the lack of GHRH which accounts for the blunted GH secretion seen in chronic hypercortisolism. This argument is based on the finding that pyridostigmine administration is unable to restore GH release in Cushing's patients, whereas priming with GHRH partially reinstated the GH response to cholinergic agonism in these patients [25]. However, GHRH administration does not elicit GH release in the presence of elevated SS inputs to the pituitary, and PD is not able to block β-adrenergic dependent SS-enhanced release [15]. Therefore, although it is likely that a GHRH defective secretion exists during chronic hypercortisolism, it must be due to the tonic inhibitory effect that SS hypertone exerts on GHRH neurons (Fig. 3). A GHRH challenge after a few days β-adrenergic blockade in Cushing's patients would clarify this aspect.

Assuming that GCs stimulate hypothalamic SS secretion, the observation that an acute effect of these steroids is to consistently induce a clear GH release [26] appears to be quite surprising. Thus, the administration of DEX (4 mg, i.v.) 3 h prior to a GHRH challenge increases basal plasma GH values and potentiates the GH response to GHRH. How can we conciliate this finding with the SS-mediated inhibitory effect described above?

Interestingly, the GH-releasing effect of GC is consistently observed 3 h after the steroid administration; it is not potentiated by SS-inhibiting drugs [26, 27], but its apparition is delayed by the induction of SS

release with atropine. These data indicate that the secretion of GH induced by DEX is mediated by inhibited SS secretion. Moreover, a functionally intact hypothalamic–somatotroph axis is needed in order to observe the DEX-induced GH secretion [28]. Therefore, a direct effect of GC at the somatotropic level as the factor responsible for the acute GH response may be discarded.

Considering the above, and being aware of the existence of a HSR modulated by GH–SS relationships, these data lead us to speculate that DEX-induced GH release is also a consequence of the DEX-elicited SS secretion. That is, the disruption of the HSR by DEX administration would reset the rhythmicity of the system. Thus, in a first step DEX would increase SS release, therefore blocking GH secretion. The lack of GH would in turn interrupt SS secretion thus allowing GH to be secreted. There are no experimental data to support the hypothesis that such a mechanism would be responsible for the acute GH-releasing effect of GCs; however, the resynchronization of the HSR following its rupture by clonidine has been reported by our group [29]. Therefore, the fact that DEX administration acutely synchronizes the rhythm of GH secretion exactly 3 h afterwards [26] might be explained as described above. Testing the GH response to GHRH at short time intervals after giving DEX and having induced or not β-adrenergic blockade would demonstrate whether or not this hypothesis is correct.

In summary, the inhibitory effect that GCs play on GH secretion is clearly mediated by β-adrenergic-induced SS release. The lack of GHRH observed in chronic hypercortisolism is most likely dependent on the continuous blockade that SS hypertone exerts on GHRH-producing neurons. The acute, facilitatory role that GCs play in GH control is most likely mediated by the stimulating effect of these steroids on SS release as well, resulting in a resynchronization of the HSR. This is apart from the well known effects of these steroids on the number of GHRH receptors [6] and on GH gene expression [5].

These postulates agree with our experimental data indicating that SS plays the most important role in GH control and that SS secretion is mainly regulated by the balance between α_2- and β-adrenergic activity in SS-producing neurons [1].

The control of hypothalamic SS secretion by GC would be related to the regulatory role that these steroids play in the organism.

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