J. Steroid Biochem. Molec. Biol. Vol. 40, No. 1-3, pp. 165-173, 1991
Printed in Great Britain. All rights reserved

0960-0760/91 $3.00 + 0.00
Copyright © 1991 Pergamon Press plc

THE ROLE OF SEXUAL STEROIDS IN THE MODULATION OF GROWTH HORMONE (GH) SECRETION IN HUMANS

JESUS DEVESA,* NOEMI LOIS, VICTOR ARCE, MARIA J. DIAZ, LUIS LIMA and JESUS A. F. TRESGUERRES[1]

Department of Physiology, Faculty of Medicine, Santiago de Compostela and [1]Department of Physiology, Faculty of Medicine, Madrid, Spain

Summary—Sex steroids contribute to modulate GH secretion in man. However, both the exact locus and mechanism by which their actions are exerted still remain not clearly understood. We undertook a number of studies designed to ascertain: (1) whether or not sudden or chronic changes in circulating gonadal steroids may affect GH secretion in normal adults; and (2) the reason(s) for gender-related dimorphic pattern of GH release.

The pituitary reserve of GH, as evaluated by means of a GHRH challenge, was similar in women with anorexia nervosa and in normally menstruating women. Estrogenic receptor blockade with tamoxifen (TMX) did not significantly change GHRH-induced GH response in these normal women. Therefore, acute or chronic hypoestrogenism apparently had no important effects at level of somatotrophs.

In another group of normal women we tested the possibility that changes in circulating estrogens might induce changes in the hypothalamic–somatotroph rhythm (HSR). GHRH challenges were performed throughout a menstrual cycle, and again after having achieved functional ovarian blockade with a GnRH agonist treatment. Short-term ovarian blockade did not significantly affect the parameters of GH response to GHRH, although it was accompanied by an increase in the number of women in a refractory HSR phase at testing. This suggested a low potentiating effect on the basic pattern of somatostatin (SS) release occurring as a consequence of the decrease in circulating estrogens.

In normal men, neither the GH response to GHRH nor the HSR were affected by functional testicular blockade (after GnRH agonist treatment). However, the administration of testosterone enanthate (250 mg) to another group of men increased both the GHRH-induced GH release and the number of subjects in a spontaneous secretory HSR phase at testing; these were reversed by estrogenic receptor blockade with TMS.

In another group of normal men, the fraction of GH secreted in pulses (FGHP) during a nocturnal sampling period was significantly decreased by testicular blockade. Other parameters of GH secretion, such as the number of GH pulses and their mean amplitude (A), and the mean plasma GH concentration (MCGH), showed a slight, although not significant, decrease following the lack of androgens. The administration of testosterone enanthate (500 mg) reversed these parameters to values similar to those in the basal study. Interestingly, when tamoxifen was given after testosterone enanthate, A, MCGH and FGHP increased to values significantly higher than in any other experimental condition in that study.

In all, these data suggest that 17β-estradiol may participate in GH modulation by inhibiting the hypothalamic release of somatostatin, while testosterone stimulates it. The results obtained after estrogenic receptor blockade appear to indicate that the effect of testosterone in such a modulation is dependent on its aromatization to 17β-estradiol. The differential levels of this steroid in both sexes might account for the sexual dimorphic pattern of GH secretion. From other data in the literature, obtained in rats, and our preliminary data in children with constitutional delay of growth and puberty, it is tempting to speculate that the effect of 17β-estradiol may be exerted by modifying the functional activity of α-2 adrenergic pathways involved in the negative modulation of SS release.

INTRODUCTION

Pituitary GH secretion occurs episodically in all species in which it has been examined [1]. This is mainly dependent on the rhythmic alternation in the hypothalamic release of two peptides: GHRH and somatostatin (SS) acting as stimulatory and inhibitory hormones, respectively [2]. Each GH secretory episode would be initiated by a burst of GHRH release into the hypophyseal portal system, preceded by a reduction of

Proceedings of the VIIIth International Congress on Hormonal Steroids, The Hague, The Netherlands, 16–21 September 1990.

*To whom correspondence should be addressed.

inhibitory somatostatinergic input to the pituitary. In turn, GHRH and SS release and/or their effects on the somatotrophs are modulated by a complex network of neurotransmitters, metabolic signals and other hormones [3].

The pulsatile pattern of GH release appears to be a physiological determinant for the expression of their maximal biological effects at the level of peripheral target tissues [1]. In man, secretory bursts of GH occur 4–8 times over a 24-h period [4]. However, both age and gender can modify this standard pattern [1, 5, 6]; therefore, it appears that sexual steroids must play a physiological role in the control of GH release in humans.

The existence of a sexual dimorphism of GH secretion was first described in the rat [7], and afterwards it has been widely analysed in a number of reports (for reviews see Refs [1, 8, 9]). Most of these studies have been performed in the rat, a species in which the effect of gonadal steroids on GH secretion appears to be quite different to that in humans [10]. Otherwise, data in humans are often conflicting; both the exact locus and mechanism by which sex steroids contribute to modulate GH release in man still remain controversial [6]. For instance, a number of investigators have reported higher GH secretion in women than in men in response to classical stimuli [11, 12], whereas others have described a potentiating effect of androgens, but not estrogens on this secretion [13, 14].

These premises prompted us to seek further insight into the role that sex steroids play in GH control. We therefore studied the effect of different functional or pharmacological changes in circulating levels of these hormones on the GH release in adult volunteers of both sexes.

ESTROGENS AND GROWTH HORMONE SECRETION

A number of reports describe a positive effect of estrogens on GH release (for reviews see Refs [9, 15]). Physiologically, puberty is associated with increased integrated concentrations of GH, and the higher GH release seen in girls with central precocious puberty is decreased after blockade of ovarian activity by treatment with GnRH agonists. Moreover, estrogen therapy increases integrated GH concentrations in postmenopausal women, and mean plasma GH levels are higher in pregnant women or in women taking oral contraceptives than in normally menstruating women. From these data it seems to be clear that estrogens positively modulate GH secretion; however, no evidence exists indicating whether the pituitary or the hypothalamus (or both) are the locus for their action, or the exact role played by these steroids in GH control in man.

Our group attempted to investigate both physiological questions. In a first study [16], we analysed the GH release elicited by a direct pituitary stimulus, by administering GHRH (GRF 1–29, Serono, Spain; 1 μg/kg as i.v. bolus), in three different estrogenic situations: anorexia nervosa patients, normal control women and normal women in which a pharmacological blockade of estrogenic receptors was induced by giving tamoxifen. Women with anorexia nervosa ($n = 8$; aged 15–24 years) were studied in the relapse of their illness after having been chronically underweight for many years. Secondary amenorrhoea was present in all the patients. Normal age-matched controls ($n = 6$) were tested in the follicular phase of their menstrual cycle, before and after they were given tamoxifen (Nolvadex, ICI Farma, Spain; 10 mg orally every 8 h for 2 days plus 10 mg 3 h prior to GHRH administration). With this experimental design we tried to investigate whether or not sudden or chronic changes in estrogenic levels might affect the pituitary reserve of GH; an expression of the biosynthesis/release ratio.

Plasma 17β-estradiol (E_2) levels were significantly lower in anorectic women than in controls (Fig. 1). However, the mean GHRH-elicited GH peak and the amount of GH released, as expressed by the area under the GH curve (AUC), were similar in the three experimental groups, as Fig. 1 shows. This was consistent with other reports demonstrating no

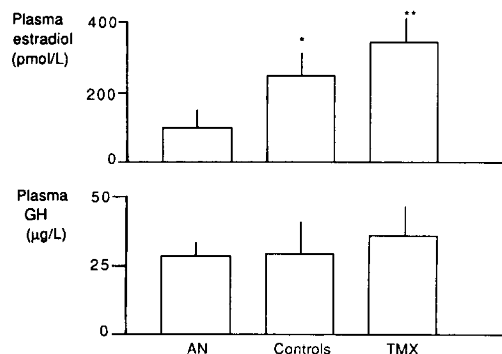

Fig. 1. Plasma 17β-estradiol levels (top) and GHRH-elicited GH peaks (bottom) in anorexia nervosa patients (AN), and in normal women before (controls) and after blockade of estrogenic receptors with tamoxifen (TMX). Values are mean $\pm$ SEM. *$P < 0.05$ vs AN. **$P < 0.05$ vs controls.

differences in GH response to GHRH administered throughout the menstrual cycle [17, 18], and appeared to exclude a main effect of estrogens on the somatotrophs. We therefore concluded that the putative potentiating effect of these steroids on GH secretion must have been exerted at the hypothalamus or higher brain regions [16].

Somatotrophs have binding sites for estrogens [19]; furthermore, in rats, E_2 stimulates the continuous release of GH from pituitaries autotransplanted under the kidney capsule [20]. Hence, the possibility existed that when analysing our results we did not take into account the endogenous hypothalamic–somatotroph rhythm (HSR); a key factor when interpreting GH responses to GHRH challenges as we thereafter demonstrated [6, 21]. Nutritional state and caloric intake influence GH secretion [10]. Chronic malnutrition is associated with elevated GH levels and subnormal IGF-I levels, a picture frequently observed in anorectic women [22]. Therefore, the lack of differences in the GHRH-induced GH release between the patients and normal controls in our study [16] might have been dependent on a more persistent spontaneous secretory phase [6, 21] in the former group, and not on the lack of effects of E_2 on somatotrophs.

To investigate further this last possibility, we designed another experimental protocol in which the GH responses to GHRH challenge were evaluated on the basis of our postulate [6, 21] according to the functional somatotroph status at the time of testing. In that study [23], GHRH tests were performed in ten normal women (aged 18–24 years) in the follicular (days 4–6) and luteal (days 20–22) phases of a normal menstrual cycle, and again after achieving functional ovarian blockade by means of a treatment with GnRH-agonist (Suprefact, Hoechst; 1500 μg/day, nasal spray in 6 doses, 40 days). The data obtained agree with the previous report of Evans *et al.* [17] and Gelato *et al.* [18] showing that in women there are no changes in the GH responsiveness to exogenous GHRH stimulation at any time during the menstrual cycle. As shown in Fig. 2, neither the mean GHRH-elicited GH peak nor the AUC after GHRH were significantly different when tests were performed in the follicular or luteal phases, despite that plasma E_2 levels were significantly higher in the latter (Fig. 2). Ovarian blockade 40 days after GnRH-agonist treatment was evident because E_2 plasma levels

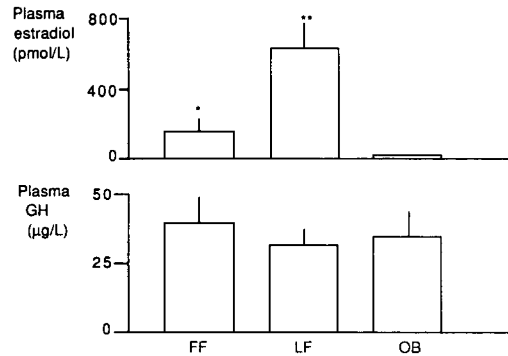

Fig. 2. Plasma 17β-estradiol levels (top), and GH peaks (bottom) in response to a GHRH challenge in normal women tested during the follicular (FF) and luteal (LF) phases of a menstrual cycle, and again after achieving functional ovarian blockade (OB) by administering a GnRH agonist (Suprefact). Values are mean $\pm$ SEM. *$P < 0.05$ vs OB; **$P < 0.05$ vs FF.

were at undetectable values (Fig. 2); however, GH responses to GHRH did not differ significantly from those elicited during the control cycle (Fig. 2). Interestingly enough, while the percentual distribution of HSR phases at the time of GHRH challenge was similar in follicular and luteal phases of the control cycle (80% of the women in spontaneous secretory phase and 20% in refractory period), the ovarian blockade led to a decrease in the number of women in secretory phase when GHRH was administered (50%), as Fig. 3 shows.

These results indirectly seem to discard the possibility of a potentiating activity of estrogens on GH secretion exerted at somatotroph level. In all the situations of total acute (normal women pretreated with tamoxifen), short-term (after ovarian blockade) and chronic (anorectic women) hypo-estrogenism that we analysed, the GH responses to exogenous GHRH remained unaltered, as compared to those observed in the normally menstruating women. However, the absence of ovarian activity was associated with a change in the ratio between spontaneous secretory and refractory HSR phases. Since we

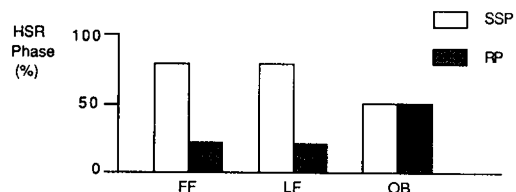

Fig. 3. Functional status of the hypothalamic–somatotroph rhythm (HSR) at the time of a GHRH challenge performed during the follicular (FF) and the luteal (LF) phases of a normal menstrual cycle, and when functional ovarian blockade was achieved (OB). SSP: percentage of women in spontaneous secretory phase. RP: percentage of women in a refractory phase.

demonstrated [6, 21] that HSR appears to be markedly constant for each individual when GHRH tests are performed at the same time of day, it is likely that the decrease of circulating estrogens was the factor responsible for the change in HSR pattern. Therefore, a main role for these steroids in the GH control would then be exerted at suprapituitary level, by modulating the rhythmic GHRH–somatostatin interplay. This hypothesis is compatible with the early study of Thompson *et al.* [24], and with the data of Ho *et al.* [25] demonstrating a strong correlation between free E_2 plasma levels and indices of total and pulsatile GH release.

ANDROGENS AND GROWTH HORMONE SECRETION

Since the early study of Martin *et al.* [13], in boys with constitutional delay of puberty, several reports have demonstrated an enhancing effect of testosterone on GH secretion in those situations in which gonadal activity is low or absent in men (for reviews see Refs [1, 9, 15]. As it occurs in girls, GH secretion in boys appears to be low during infancy and increases slightly until just before puberty [26], a period at which the secretion of the hormone is strongly increased [27]. Recent data [28] show that there exist two different GH secretory steps at this pubertal period in males, respectively characterised by a slight decrease in early puberty followed by a strong rise late in puberty coinciding with the pubertal growth spurt. However, these changes in the 24-h GH secretory pattern are not correlated with plasma testosterone changes [25], and this has led Edén *et al.* [9] to hypothesize that the GH increase during puberty is partly independent of gonadal steroids, as these authors demonstrated to occur in the rat [29].

An alternative explanation could be that testosterone does not act primarily on GH secretion, but secondarily to its aromatization to E_2.

We attempted to understand the mechanism by which androgens putatively affect GH release. Ten normal men (aged 19–25 years) underwent GHRH tests in basal conditions and after 40 days of treatment with a GnRH-agonist, prescribed similarly to as was described in women. The pituitary reserve of GH, as measured by GHRH-induced GH release, was not affected by testicular blockade. Despite the

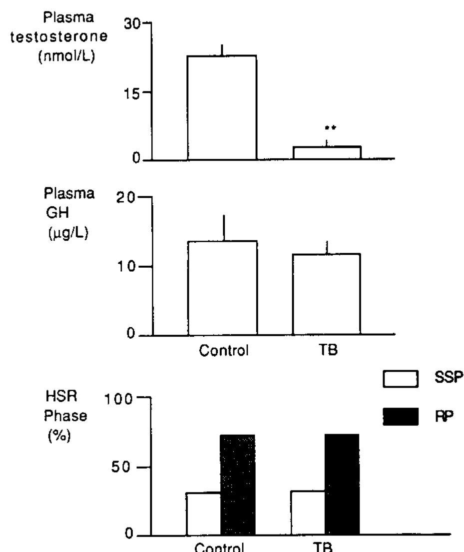

Fig. 4. The administration of a GnRH agonist produced functional testicular blockade (TB) in a group of normal men, as plasma testosterone changes (upper) indicated. However, neither the GH peak response to GHRH (middle), nor the functional somatotroph status (lower) at the time of GHRH test were significantly affected. Values are mean ± SEM. SSP: percentage of men in spontaneous secretory phase. RP: percentage of men in refractory period. $^{**}P < 0.01$ vs control.

strong changes in plasma testosterone levels, the mean GHRH-elicited GH peak and the AUC were similar in both GHRH challenges (Fig. 4). Most of the subjects (70%) were in a refractory HSR period [6, 21] at the time of GHRH control test, and the same was observed after testicular blockade (Fig. 4). Therefore, it appeared that the lack of androgens had no significant effect on the individual HSR.

In the same study [23], another group of normal men ($n = 7$; 19–24 years) were prescribed testosterone enanthate (Testovirón Depot, Schering, Spain; 250 mg i.m.), and 8 days later tamoxifen was given in a similar dose schedule to that in women. GHRH tests in this group were performed before testosterone administration, 8 days later and again after tamoxifen. All the men in this group began the control test in a refractory HSR period. Hence, GH responses were very low (Fig. 5). The administration of testosterone led to a significantly higher GHRH-induced GH release. This was probably a consequence of a lower number of subjects (70%) in refractory period in this test (Fig. 5), but not of a direct stimulatory effect on the pituitary biosynthesis of GH. Tamoxifen treatment did not affect the increased plasma testosterone values reached after testosterone enanthate administration (Fig. 5); but it

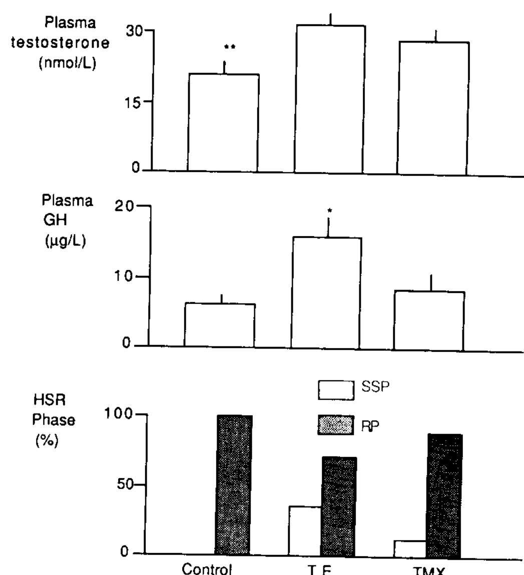

Fig. 5. Following the administration of a single dose (250 mg i.m.) of testosterone enanthate (TE), either plasma testosterone levels (upper) and GHRH-elicited GH peaks (middle), were significantly increased in a group of normal men. All the men were in a refractory HSR phase (RP) when tested in basal conditions (lower), but TE administration increased the percentage of cases in a spontaneous secretory phase (SSP) at the time of GHRH testing. Estrogenic receptor blockade with tamoxifen (TMX) did not change plasma testosterone levels, but reversed both GH peaks and the SSP to RP ratio to values similar to those in control tests. Values are mean ± SEM. $^{**}P < 0.05$ vs TE and TMX. $^{*}P < 0.05$ vs control and TMX.

reversed the HSR pattern to a percent (90% in refractory period) similar to that observed in the control test. In these conditions, GH responses were significantly lower (Fig. 5).

From these data, we concluded [23] that testosterone would act in GH control mainly at hypothalamic level, by a mechanism secondary to its aromatization to E_2. The fact that testosterone enhanced pulsatile GH secretion in peripubertal boys, while the non-aromatizable androgen oxandrolone did not [14], agreed with this hypothesis.

To test such a possibility, eight normal men (aged 21–26 years) were given testosterone enanthate (500 mg i.m.) 40 days after they had received a single injection of a GnRH agonist (Zoladex, ICI); eight days later a blockade of estrogenic receptors was performed with tamoxifen (10 mg orally every 8 h for 2 days; the last dose was administered 2 h prior to the GH analysis). The pattern of nocturnal GH release, from 2300 to 0800 h, was analysed in plasma samples withdrawn at 20 min intervals, in basal conditions (control study), 40 days after Zoladex administration, 8 days after testosterone enanthate was given, and again after tamoxifen. GH pulses and their mean amplitude were determined as previously described [30].

The results of this study are summarised in Table 1. Total (T) and free testosterone (fT) and E_2 plasma levels all significantly decreased after testicular blockade. In these conditions, while both the number of GH peaks and their mean amplitude slightly decreased, their mean width was unaltered; consequently, the fraction of GH secreted in pulses (FGHP) was significantly lower after testicular blockade. Testosterone enanthate administration increased plasma T and E_2 levels to values that for the former steroid were significantly higher than those in baseline conditions. This, however, was not accompanied by significant changes in GH pulsatility parameters as compared to control values. Surprisingly, following the blockade of estrogen receptors with tamoxifen, both the mean amplitude of GH pulses and the fraction of GH secreted in them reached values significantly higher than in any other experimental condition in this study. This was also observed for the mean GH concentration (MCGH), that otherwise had not been significantly changed either by testicular blockade or by testosterone enanthate administration.

Table 1. Mean (±SEM) plasma levels of 17β-estradiol (E_2), and total (T) and free testosterone (fT), and indices of total and pulsatile GH release during a sampling period lasting from 2300 to 0800 h in eight normal men throughout the different experimental conditions

	Control	GnRH-a	TE	TE + TMX	
T	17.6 ± 2.8	2.6 ± 0.2*	52 ± 9**	45 ± 8.2**	nmol/l
fT	79.1 ± 4.4	5.4 ± 0.5*	258 ± 22**	227 ± 12**	pmol/l
E_2	198 ± 18	78 ± 7*	173 ± 16	216 ± 29	pmol/l
N	2.3 ± 0.4	1.7 ± 0.2	1.8 ± 0.2	1.8 ± 0.2	
Amplitude	9.3 ± 1.5	7.5 ± 1	9.1 ± 1.1	12.6 ± 1.8*	µg/l
Width	101 ± 21	102 ± 12	125 ± 14	133 ± 12	min
MCGH	2.7 ± 0.3	2.3 ± 0.4	2.2 ± 0.1	3.2 ± 0.1*	µg·min/l
FGHP	83.7 ± 6.3	73.1 ± 5.8*	84 ± 4.7	90.2 ± 4.1**	%

Control: Basal values; GnRH-a: Testicular blockade, 40 days after Zoladex was given; TE: 8 days after testosterone enanthate was administered (500 mg i.m.); TE + TMX: blockade of E_2 receptors by administering tamoxifen. *N* = number of GH pulses; amplitude = GH peak minus the preceding nadir value of each GH pulse; width = duration of each GH pulse; MCGH = mean plasma GH concentration; FGHP = fraction of GH secreted in pulses. $^{*}P < 0.05$ vs the other experiments. $^{**}P < 0.05$ vs control.

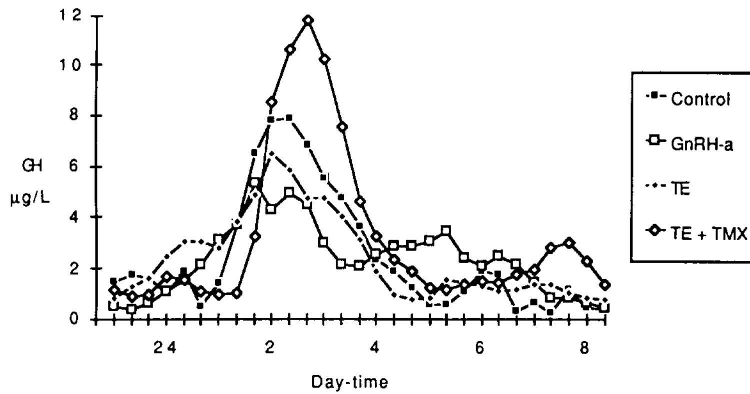

Fig. 6. Mean nocturnal plasma GH profiles in a group of normal men in the different experimental conditions. Control: basal values; GnRH-a: 40 days after administration of Zoladex; TE: 8 days after administration of a single dose (500 mg) of testosterone enanthate; TE + TMX: 2 days after a tamoxifen treatment was prescribed.

As it can be seen in Fig. 6 the basic endogenous hypothalamic–somatotroph rhythm remained constant throughout these different pharmacological manipulations.

CONCLUSIONS

From these data an opposite effect for testosterone and 17β-estradiol on the hypothalamic release of somatostatin appears to be suggested. While testosterone would stimulate somatostatin secretion, 17β-estradiol would inhibit it. Therefore, in humans, gonadal steroids would participate in GH control in a similar way to that postulated to occur in the rat [8, 9].

We found that gonadal blockade in men was associated with a decrease in the amount of GH secreted during peak episodes, without a significant change in the mean plasma GH concentration. This indicates that basal GH levels between peaks were higher at this time. The lack of testosterone, together with the presence of significant, although lowered, plasma levels of 17β-estradiol appears to be responsible for this effect. Following testosterone enanthate administration an increased amplitude of GH pulses must be expected [31]; this, however, did not occur. Given that the dose of testosterone enanthate given in our study was five-fold higher than in the work of Mauras *et al.* [31], a significant effect of the steroid on the pulse amplitude could have been partially counteracted by an increased free 17β-estradiol. In fact, despite that we only measured plasma levels of total 17β-estradiol, and that these were similar in basal conditions and after testosterone enanthate administration, free testosterone was three-fold increased by this treatment. Because testosterone both decreases SHBG synthesis and has a higher affinity for this steroid carrier than 17β-estradiol, it is likely that an increase in the androgen will produce higher free 17β-estradiol levels. The balance between both steroids would explain the lack of significant changes in the indices of GH pulsatility observed following testosterone treatment, as compared to those in basal conditions: neither the higher free testosterone nor the higher free 17β-estradiol will, respectively increase the pulse amplitude and GH levels between pulses.

Another possibility such as a lack of significant effects of testosterone on GH secretion in adult men appears to be discarded given the results obtained following 17β-estradiol receptor blockade. This clearly increased the amplitude of GH pulses and the total amount of GH secreted, while the amount of the hormone secreted during trough periods was decreased. Therefore, it is likely that testosterone has potentiated the rhythmic somatostatin release, hence facilitating enhanced GH responses to endogenous GHRH pulses.

An opposite effect of testosterone and 17β-estradiol on somatostatin release may better explain our previous data [6, 16, 21, 23]. The fact that the ovarian blockade diminished the percentage of women in spontaneous secretory phase at the time of GHRH testing [23] is compatible with a lack of inhibitory effects of 17β-estradiol on somatostatin secretion. Conversely, the administration of supraphysiological doses of testosterone to normal men [23], increased the percentage of subjects in spontaneous secretory phase, a phenomenon likely

due to higher testosterone-derived 17β-estradiol, given that it was reversed after the blockade of 17β-estradiol receptors. Also, the higher GH responses to exogenous GHRH challenge in women [21] appear to be dependent on a lower basal somatostatin release.

From our data it appears that the sexual dimorphic GH secretion in humans occurs as a consequence of the balance between the inhibitory effects of 17β-estradiol and the stimulatory effect of testosterone mainly acting on hypothalamic somatostatin release. However, a secondary role of both steroids on GHRH secretion cannot be excluded.

An important question still remains to be answered: which is the initial mechanism subserving this putatively opposite action of both steroids on somatostatin?

High concentrations of estrogen receptors exist in areas actively engaged in the synthesis of somatostatin, such as mediobasal hypothalamus and the preoptic area [32]. Furthermore, it has been recently described that 17β-estradiol may induce changes in hypothalamic levels of somatostatin mRNA. Estrogen replacement therapy quickly reversed the decrease in somatostatin mRNA in ovariectomized rats; however, in castrated male rats a similar effect lasted more time after testosterone treatment [33]. These data seem to clearly suggest that 17β-estradiol affects the expression of somatostatin gene in the hypothalamus, but also that the effect of testosterone might occur secondarily to its aromatization to 17β-estradiol. In fact, aromatase activity is present in hypothalamus and the preoptic area [32] where it appears to be specifically induced by testosterone.

Therefore, the differential levels reached by 17β-estradiol in both sexes might then be the key factor in the regulation of somatostatin. Both, the fact that the presence of ovaries can prevent the masculinizing effect of neonatal androgen exposure on GH storage and secretion in adult female rats [34], and our results after testosterone enanthate administration and estrogenic receptor blockade in men are compatible with this hypothesis.

There is no evidence demonstrating that the estrogen-responsive element exists in the gene for somatostatin [33]. Therefore, a direct effect of 17β-estradiol on somatostatin neurons appears to be unlikely.

Gonadal steroids influence catecholaminergic systems in areas involved in somatostatin control (see Ref. [1]). Thyroxine hydroxylase activity is down-regulated by 17β-estradiol in the hypothalamus, while the 2-hydroxylated metabolites of the steroid have the ability to inhibit catechol-*O*-methyl transferase activity [32]. Therefore, hypothalamic levels of 17β-estradiol may substantially affect the availability of catecholamines. Furthermore, estrogen administration increases hypothalamic binding sites for the α-2 agonist clonidine in rats [35]. Otherwise, orchidectomy enhances the GH response to GRF in male rats [36], an effect attributed to reduced somatostatin release. Conversely, the stimulating effect of clonidine on GH release is lost in castrated male rats and the effect is reestablished by testosterone replacement [36, 37]. We recently demonstrated [38, 39] that α-2 adrenergic pathways play, in humans, a major role in the neuroregulation of GH by inhibiting the hypothalamic release of somatostatin. Therefore, and despite that a similar role in rats still has not been demonstrated, it is likely that changes in α-2 responsiveness must also primarily affect somatostatin secretion in these animals.

Preliminary data from our group (unpublished results) indicate that the amount of GH released in response to a combined clonidine plus GHRH stimulation (Fig. 7) is significantly lower in children diagnosed from constitutional delay of growth and puberty ($n = 10$) than in age-matched short normal children ($n = 13$); this is consistent with a main role of gonadal steroids on α-2 adrenergic pathways as it has been postulated to occur in the rat [1, 36].

In all, these data indicate that sex steroids contribute to the modulation of GH secretion at hypothalamic level, mainly by affecting the functionality of α-2 adrenergic pathways involved in somatostatin control.

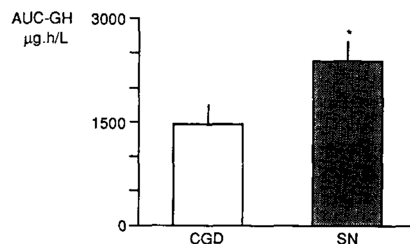

Fig. 7. Area under the GH curve (AUC) released (from 60 to 120 min) in response to a GHRH challenge given 60 min after clonidine pretreatment in a group of children with constitutional delay of growth and puberty (CGD), as compared to that elicited by the same combined stimulation in age-matched short normal children (SN). Values are mean ± SEM. **P* < 0.05. Clonidine: 0.150 mg/m^2, orally at time 0 min. GHRH: 1 µg/kg, i.v. bolus at time 60 min.

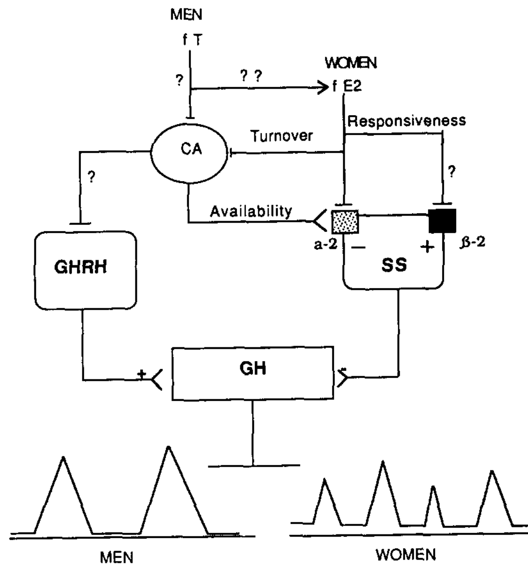

Fig. 8. Sexual dimorphic pattern of GH secretion may be the consequence of a main effect of free 17β-estradiol (fE_2) on somatostatinergic neurons. According to this hypothesis, gender-related differential levels of hypothalamic fE_2 would differentially affect catecholamines (CA) metabolism in areas involved in somatostatin (SS) control. The activity of α-2-adrenoceptors (a-2) that negatively modulate SS release would then be also affected. A similar, although opposite and weaker, effect would occur on GHRH neurons.

What remains to be established is (1) whether this action is exerted by modifying the turnover of catecholamines and/or the responsiveness of α-2 adrenoceptors [40] in somatostatinergic neurons; (2) whether these changes affect somatostatin synthesis at the DNA level and/or the release of this peptide; and (3) if the sexual dimorphic pattern of GH release is only dependent on gender-related hypothalamic free 17β-estradiol levels. A temptative model to explain these concepts is schematised in Fig. 8.

An additional question to be resolved is whether there is a neonatal imprinting effect of sex steroids on hypothalamic structures governing the underlying hypothalamic–somatotroph rhythm in humans, similarly to that occurring in the rat [34].

Acknowledgements—This work was supported by Grants FISS 87/1359 and DGICYT PM88-0208. The expert technical help of Ms Ana Iglesias is gratefully acknowledged. We acknowledge Ms Sinead Reddin her careful help when typing this manuscript.

REFERENCES

1. Jansson J. O., Edén S. and Isaksson O.: Sexual dimorphism in the control of growth hormone secretion. *Endocrine Rev.* **6** (1985) 128–150.
2. Tannenbaum G. S. and Ling N.: The interrelationship of growth hormone (GH)-releasing factor and somatostatin in generation of the ultradian rhythm of GH secretion. *Endocrinology* **115** (1985) 1952–1957.
3. Frohman L. A., Downs T. R., Katakami H. and Jansson J. O.: The interaction of growth hormone-releasing hormone and somatostatin in the regulation of growth hormone secretion. In *Growth Hormone—Basic and Clinical Aspects* (Edited by O. Isaksson, C. Binder, K. Hall and B. Hökfelt). Elsevier, Amsterdam (1987) pp. 63–77.
4. Quabbe H. J.: Growth Hormone. In *Neuroendocrinology* (Edited by S. L. Lightman and B. J. Everitt). Blackwell Scientific, Oxford (1986) pp. 409–449.
5. Müller E. E.: Neural control of somatotropic function. *Physiol. Rev.* **67** (1987) 962–1053.
6. Tresguerres J. A. F., Lima L., Arce V. and Devesa J.: Controversies in the GH response to GHRH in man. In *Recent Advances in Basic and Clinical Neuroendocrinology* (Edited by F. F. Casanueva and C. Diéguez). Elsevier, Amsterdam (1989) pp. 251–261.
7. Tannenbaum G. S. and Martin J. B.: Evidence for an endogenous ultradian rhythm governing growth hormone secretion in the rat. *Endocrinology* **98** (1976) 562–570.
8. Robinson I. C. A. F. and Clark R. G.: The secretory pattern of GH and its significance for growth in the rat. In *Growth Hormone—Basic and Clinical Aspects* (Edited by O. Isaksson, C. Binder, K. Hall and B. Hökfelt). Elsevier, Amsterdam (1987) pp. 109–127.
9. Edén S., Jansson J. O. and Oscarsson J.: Sexual dimorphism of growth hormone secretion. In *Growth Hormone—Basic and Clinical Aspects* (Edited by O. Isaksson, C. Binder, K. Hall and B. Hökfelt). Elsevier, Amsterdam (1987) pp. 129–151.
10. Thorner M. O. and Vance M. L.: Growth hormone, 1988. *J. Clin. Invest.* **82** (1988) 745–747.
11. Frantz A. G. and Rabkin M. T.: Effects of estrogen and sex difference on human growth hormone. *J. Clin. Endocr. Metab.* **25** (1965) 1470–1475.
12. Merimée T. J. and Fineberg S. E.: Studies on the sex based variation of human growth hormone secretion. *J. Clin. Endocr. Metab.* **33** (1971) 886–892.
13. Martin L. G., Clark J. W. and Connor T. B.: Growth hormone secretion enhanced by androgens. *J. Clin. Endocr. Metab.* **28** (1968) 425–431.
14. Link K., Blizzard R. M., Evans W. S., Kaiser D. L., Parker M. W. and Rogol A. D.: The effect of androgen on the pulsatile release and the 24-hour mean concentration of growth hormone in peripubertal males. *J. Clin. Endocr. Metab.* **62** (1986) 159–165.
15. Bourguignon J. P.: Linear growth as a function of age at onset of puberty and sex steroid dosage: Therapeutic implications. *Endocrine Rev.* **9** (1988) 467–490.
16. Casanueva F. F., Borrés C. G., Burguera B., Lima L., Muruais C., Tresguerres J. A. F. and Devesa J.: Growth hormone and prolactin secretion after growth hormone-releasing hormone administration, in anorexia nervosa patients, normal controls and tamoxifen-pretreated volunteers. *Clin. Endocr.* **27** (1987) 517–523.
17. Evans W. S., Borges J. C. C., Yance M. L., Kaiser D. L., Rogol A. D., Furlanetto R., Rivier J., Yale W. and Thorner M. O.: Effects of human pancreatic growth hormone-releasing factor-40 on serum growth hormone, prolactin, LH, FSH, and somatomedin C concentrations in normal women throughout the menstrual cycle. *J. Clin. Endocr. Metab.* **59** (1984) 1006–1010.
18. Gelato M. C., Pescovitz O. H., Cassorla F., Loriaux D. L. and Merriam G. R.: Dose-response relationships for the effects of growth hormone-releasing factor in young adult men and women. *J. Clin. Endocr. Metab.* **59** (1984) 197–201.
19. Herbert D. C. and Sheridan P. J.: Uptake and retention of sex steroids by the baboon pituitary gland—evidence

of sexual dimorphism with respect to dihydrotestosterone. *Biol. Reprod.* **28** (1983) 377–392.
20. Jansson J. O., Carlsson L., Seeman H.: Estradiol—but not testosterone—stimulates the secretion of growth hormone in rats with the pituitary gland autotransplanted to the kidney capsule. *Acta Endocr.* **103** (Suppl. 256) (1983) 212.
21. Devesa J., Lima L., Lois N., Fraga C., Lechuga M. J., Arce V. and Tresguerres J. A. F.: Reasons for the variability in growth hormone (GH) responses to GHRH challenge: the endogenous hypothalamic–somatotroph rhythm. *Clin. Endocr.* **30** (1989) 367–377.
22. Beumont P. J. Y. and Russel J.: Anorexia nervosa. In *Handbook of Psychiatry and Endocrinology* (Edited by P. J. Y. Beumont and A. Burrows). Elsevier, Amsterdam (1982) pp. 63–96.
23. Lima L., Arce V., Lois N., Fraga C., Lechuga M. J., Tresguerres J. A. F. and Devesa J.: Growth hormone (GH) responsiveness to GHRH in normal adults is not affected by short-term gonadal blockade. *Acta Endocr. (Copenh.)* **120** (1989) 31–36.
24. Thompson R. G., Rodríguez A., Kowarski A. and Blizzard R. M.: Growth hormone: metabolic clearance rates, integrated concentrations, and production rates in normal adults and the effect of prednisone. *J. Clin. Invest.* **51** (1972) 3193–3198.
25. Ho K. Y., Evans W. S., Blizzard R. M., Veldhuis J. D., Merriam G. R., Samojlik E., Furlanetto R., Rogol A. D., Kaiser D. L. and Thorner M. O.: Effects of sex and age on the 24-hour profile of growth hormone secretion in man: importance of endogenous estradiol concentrations. *J. Clin. Endocr. Metab.* **64** (1987) 51–58.
26. Glick S. M., Roth J., Yalow R. S. and Berson S. A.: The regulation of growth hormone secretion. *Recent Prog. Horm. Res.* **21** (1965) 241–283.
27. Rudman D., Kutner M. H., Rogers M., Lubin M. F., Fleming G. A. and Baine R. P.: Impaired growth hormone secretion in the adult population. *J. Clin. Invest.* **67** (1981) 1361–1369.
28. Martha P. M., Rogol A. D., Yeldhuis J. D., Kerrigan J. R., Goodman D. W. and Blizzard R. M.: Alterations in the pulsatile properties of circulating growth hormone concentrations during puberty in boys. *J. Clin. Endocr. Metab.* **69** (1989) 563–570.
29. Jansson J. O., Ekberg S., Isaksson O. G. P. and Edén S.: Influence of gonadal steroids on age- and sex-related secretory patterns of growth hormone in the rat. *Endocrinology* **114** (1984) 1287–1294.
30. Loche S., Puggioni R., Fanni T., Cella S. G., Müller E. E. and Pintor C.: Augmentation of growth hormone secretion in children with constitutional growth delay by short-term clonidine administration: a pulse amplitude-odulated phenomenon. *J. Clin. Endocr. Metab.* **68** (1989) 426–430.
31. Mauras N., Blizzard R., Link K., Johnson M. L., Rogol A. D. and Yeldhuis J.: Augmentation of growth hormone secretion during puberty: evidence for a pulse-amplitude modulated phenomenon. *J. Clin. Endocr. Metab.* **64** (1987) 596–601.
32. McEwen B. S.: Binding and metabolism of sex steroids by the hypothalamic–pituitary unit: physiological implications. *A. Rev. Physiol.* **42** (1980) 97–110.
33. Werner H., Koch Y., Baldino F. and Gozes I.: Steroid regulation of somatostatin mRNA in the rat hypothalamus. *J. Biol. Chem.* **263** (1988) 7666–7671.
34. Jansson J. O. and Frohman L. A.: Inhibitory effect of the ovaries on neonatal androgen imprinting of growth hormone secretion in female rats. *Endocrinology* **121** (1987) 1417–1423.
35. Fuxe K., Andersson K., Agnati L. F., Ferland L., Hokfelt T., Eneroth P., Gustafsson J. A. and Skett P.: Central catecholamine systems and neuroendocrine regulation: controllers of anterior pituitary secretion. In *Catecholamines: Basic and Clinical Frontiers.* Pergamon Press, New York (1979) pp. 1187–1203.
36. Kiem D. T., Stark E. and Fekete M. I. K.: Loss of the effects of clonidine on the Growth Hormone release and hypotensive action in long-term castrated rats: the possible role of testosterone on alpha-2-adrenergic mechanisms of clonidine. *Neuroendocrinology* **51** (1990) 226–232.
37. Jansson J. O., Eriksson E., Eden E. and Modigh K.: Effects of gonadectomy and testosterone replacement on growth hormone responses to alpha-2-adrenergic stimulation in male rat. *Psychoneuroendocrinology* **7** (1982) 245–248.
38. Devesa J., Arce V., Lois N., Tresguerres J. A. F. and Lima L.: Alpha-2 adrenergic agonism enhances the Growth Hormone (GH) response to Growth Hormone-releasing hormone through an inhibition of hypothalamic somatostatin release in normal man. *J. Clin. Endocr. Metab.* **71** (1990) 1581–1588.
39. Arce V., Lima L., Lois N., Diaz M. J., Tresguerres J. A. F. and Devesa J.: The role of central dopaminergic pathways in the neural control of Growth Hormone (GH) secretion in normal man: studies with metoclopramide. *Neuroendocrinology* **53** (1991) 143–149.
40. Johnson A. E., Nock B., McEwen B. S. and Feder H. H.: Alpha-1 and alpha-2 noradrenergic receptor binding in guinea pig brain: sex differences and effects of ovarian steroids. *Brain Res.* **442** (1988) 205–213.