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Review

Gonadotropin-releasing hormone and the control of gonadotrope function

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Abstract – Normal gametogenesis and steroidogenesis is highly dependent on the pulsatile release of hypothalamic GnRH that binds high-affinity receptors present at the surface of pituitary gonadotrophs thereby triggering the synthesis and release of the gonadotropins LH and FSH. The mammalian GnRH receptor displays the classical heptahelical structure of G protein-coupled receptors with, however, a unique feature, the lack of a C-terminal tail. Accordingly, it does not desensitise sensu stricto, and internalises very poorly. It is now well established that GnRH stimulation induces the activation of a complex network of transduction pathways involved in the control of gonadotropin release and subunit gene expression. Other authors and ourselves have demonstrated that the GnRH action is associated with an increased complexity regarding gene regulation/cell function. Indeed GnRH affects the GnRH receptor gene itself and a number of additional genes that include some involved in cell signalling and auto-/paracrine regulation. The fact that GnRH regulates the expression of its own receptor, together with a host of other genes typically involved in its signal transduction cascades implies alteration/auto-adaptation in gonadotropic responsiveness. Furthermore, some of these genes respond differentially depending on whether the GnRH stimulation is intermittent or permanent suggesting specific roles in the dual process of activation/desensitisation. Thus, it can be assumed that the importance of pulsatility of GnRH action is closely related to, or dependent on, the inability of the GnRH receptor to desensitise. Moreover, multiple post-receptor events are crucial for both the regulation/plasticity of gonadotropic function and the maintenance of cell integrity.

GnRH receptor / signalling / gene regulation / transcription factor / gonadotropins / secretion

1. INTRODUCTION

The hypothalamic gonadotropin-releasing hormone pGlu-His-Trp-Ser-Tyr-Gly-Leu-Arg-Pro-Gly-NH_2 known as mammalian GnRH or GnRH I plays a key regulatory role in the neurohormonal control of reproduction by stimulating the release of the pituitary gonadotropins LH and FSH. The pulsatile neuronal delivery of GnRH, itself centrally regulated in amplitude and frequency, is essential for maintaining the serum gonadotropin profiles required for normal steroidogenesis and gametogenesis.

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Whereas physiological fluctuations in GnRH orchestrate normal reproduction, high levels or prolonged stimulation lead to the suppression of gonadotropin secretion. This dual response has made the GnRH receptor a target for short- or long-term treatments with GnRH analogues in a wide range of human or animal applications such as reproduction/fertility, steroid/growth-related dysfunction or sex steroid-dependent cancer [1, 2]. The mechanisms by which GnRH could regulate the secretion of two gonadotropins, notably in some instances in a coordinate or differential manner, have been the subject of ongoing intensive investigation. Over the past 25 years, molecular biology, and more recently imaging and transgenic techniques have been used extensively, providing a substantial amount of novel information. In mammals, GnRH was shown to operate via an atypical G protein-coupled receptor and to regulate cellular function by affecting the activity of a growing number of genes, some of which would modulate its functional impact in relation with the stimulation mode.

2. GnRH AND PITUITARY GONADOTROPIN SECRETION

The fundamental function of GnRH, which led to the initial peptide isolation, was the induction of the release of pituitary gonadotropins. Nevertheless, the question of whether GnRH alone or in combination with other hormones/factors may also regulate the synthesis of LH and FSH was addressed very early. As described below, the biosynthesis and release of these hormones occur in the context of a complex secretory mechanism and the two processes are thus closely interconnected.

2.1. Structure and biosynthesis of pituitary gonadotropins

LH and FSH belong to a family of structurally related glycoprotein hormones that include the pituitary thyrotropin TSH and, in certain species such as primates and equids, the placental choriogonadotropin CG [3]. All these hormones are composed of two distinct, noncovalently associated glycosylated subunits, a common α- and a specific β-subunit. Cell-free translation studies and molecular cloning, have demonstrated that each subunit is synthesised as a precursor with a signal peptide preceding the authentic subunit sequence and these precursors are encoded by distinct mRNA transcribed from separate genes (Tab. I). LH and FSH, i.e. subunits α, LHβ and FSHβ are expressed within the same cells although mono- and bihormonal gonadotrophs coexist [4]. Precursor cleavage and first steps of N-glycosylation occur cotranslationally as the nascent proteins enter the endoplasmic reticulum, allowing early association into immature α/β heterodimers. The completion of a functional LH or FSH then takes place during migration along the Golgi apparatus resulting in the progressive acquisition of a specific mature glycosylation and conformation, and an ultimate achievement within the secretory granules [4, 5]. The synthesis of gonadotropins thus follows the general pathway of secreted proteins in eukaryotes with, however, some distinct features concerning essentially heterodimerisation, sugar type N-glycosylation and sorting in separate granules. Although this biosynthetic system has the potential for a number of control points this review will concentrate essentially on the gene expression level.

2.2. GnRH control of gonadotropin secretion

2.2.1. Importance of the GnRH I system in mammals

While GnRH was initially isolated and characterised by its ability to induce the release of pituitary gonadotropins, an array of evidence initially argued in favour of an indispensable role for GnRH in promoting gonadotropin gene expression. Indeed, it has been known for a long time that a genetic defect in GnRH I secretion in

Table I. Major characteristics of the human and rat gonadotropin subunit genes, mRNA transcripts and precursors.

	Chromosome assignment	Transcription unit (kb)	Number of Exons	mRNA (kb)[a]	Signal peptide (aa)	Subunit (aa)	S–S bonds	CHO[b]
hu αGSU	6 (q12-q21)	9	4	0.8	24	92	5	2
rat αGSU	5 (q13-q24)	8	4	0.8	24	96	5	2
hu LHβ	19 (q13.32)	1.4	3	~ 0.7	20	121	6	1
rat LHβ	1 (q22)	1.1	3	~ 0.7	20	121	6	1
hu CGβ[c]	19 (q13.32)	(cluster)	3	1	20	145	6	2
hu FSHβ	11 (p13)	3.9	3	1.7	19	110	6	2
rat FSHβ	3 (q33)	3.1	3	1.7	19	110	6	2

[a] Includes the poly(A+) sequence.
[b] CHO: asparagine-linked carbohydrate chains.
[c] No CGβ gene in the rat.
hu, human; aa, amino acids.

rodents [6] and humans [7, 8] results in a substantial decrease in serum gonadotropins associated with hypogonadism, impuberism and infertility. The same phenotype was observed more recently in patients with loss of function mutations in the GnRH type I receptor gene [9]. Such mutation-induced dysfunctions are consistent with a similar substantial depression in serum LH and FSH observed in response to experimental disruption of the GnRH/GnRH receptor system, which result from endogenous GnRH immunoneutralisation, surgical hypothalamus-pituitary disconnection or an antagonist-mediated GnRH receptor blockade [10, 11]. In all cases, a low expression of all three gonadotropin subunit genes α, LHβ and FSHβ was confirmed via measurement of pituitary mRNA content. In spite of the possible expression of more than one GnRH variant in a given species, and notably of GnRH II (pGlu-His-Trp-Ser-His-Gly-Trp-Tyr-Pro-Gly-NH_2) in humans [12], the above data demonstrated the crucial importance of the GnRH I system in the control of gonadotropin expression and consequently, of the pituitary gonadotropic function. Accordingly, only reference to GnRH I will be made further in this review, under the usual generic term GnRH.

2.2.2. GnRH is crucial for regulated expression of gonadotropin subunit genes

A number of investigations have demonstrated that GnRH stimulates in vitro the synthesis of gonadotropin subunits and increases α, LHβ and FSHβ subunit mRNA levels as well as the transcriptional activity of corresponding gene promoters. Taking into account its crucial importance on gonadotropin release, the effect of GnRH pulse frequency and/or amplitude on gonadotropin subunit gene expression was evaluated. Several in vitro and in vivo models such as anterior pituitary cell cultures or animals with surgical or pharmacological hypothalamus-pituitary disconnection were used to demonstrate that the individual gonadotropin genes responded differentially to the frequency of GnRH pulses. Indeed, low frequencies in the range one stimulus every two or four hours appear to preferentially increase FSHβ mRNA levels whereas higher frequencies preferentially stimulate LHβ and α subunit mRNA [13, 14]. In vivo, permanent exposure to GnRH leads to a more or less rapid depletion of both FSHβ and LHβ mRNA in a manner that suggests a rapid transcription arrest followed by RNA degradation, while the α-subunit mRNA

remains constant or increases only slightly for days [15–17]. The latter data are consistent with current clinical studies showing that long-term administration of GnRH superagonists induces depletion of LH and FSH but increases free α-subunit levels in the serum of treated patients [18].

Collectively these data suggest the presence of some specific mechanisms that differentially regulate the expression of three genes within a single cell. These may involve GnRH receptor signalling, mRNA stability and/or subunit gene promoter properties. Alternatively, an indirect modulation by factors such as steroids or members of the transforming growth factor-β superfamily i.e. activins, bone morphogenetic proteins (BMPs) and inhibins, has also been suggested. Indeed, the latter three types of substances as well as follistatin, a polypeptide which binds and functionally incapacitates activins and BMPs, are specific regulators of FSH and, in the absence of a specific FSH-releasing factor, may explain instances in which FSH and LH secretion diverge. The fact that these factors are produced in the anterior pituitary support the potential for a paracrine/autocrine regulation. The mechanism of action of such factors in conjunction with GnRH is, however, unclear. Moreover, increasing the degree of complexity of the regulatory system, GnRH appears to function through interaction with a particular unusual receptor.

3. STRUCTURE AND FUNCTIONAL PROPERTIES OF THE GnRH RECEPTOR

3.1. The peculiar structure of mammalian GnRH receptor

It was suggested by the mid 1980s [19] that the GnRH receptor belonged to the family of G protein-coupled receptors (GPCR). However, it was only after 1992 that the cloning of cDNA from various species and the deduced amino acid sequences confirmed that the GnRH receptor possessed the typical structural features of this superfamily. These include the seven membrane spanning α-helices, which contribute to the conformation of the ligand-binding pocket, connected to three intracellular and three extracellular loops functionally crucial for ligand binding and signal transduction, respectively [20]. The most striking structural feature of the mammalian GnRH receptor is the lack of a cytoplasmic C-terminal tail. This terminal extension present in all classical GPCR is the target for GPCR kinases and the consequent phosphorylation-mediated β-arrestin binding that results in the endocytic processing of the receptor-ligand complex via clathrin-coated pits [21, 22]. Interestingly, the non-mammalian GnRH receptors that were discovered later do exhibit a C-terminal tail structurally and functionally similar to that of other members of the GPCR family [20, 23]. Therefore, in contrast to the latter receptors, the mammalian GnRH receptor does not desensitise and internalises poorly [24–26]).

Such pecular properties, which suggest genetic/functional evolutionary adaptation in relation with the pulsatile character of GnRH secretion, imply that the so-called "desensitisation" i.e. the loss of a releasing response to GnRH, does not result from proper receptor inactivation/recycling as is common to all GPCR but rather from post-receptor mechanisms. In this respect, another important implication for cell function is the possibility that GnRH, depending on the frequency and the time of action may exert a selective activation or inactivation on specific intracellular processes.

3.2. GnRH receptor signalling

It has long been established that the GnRH receptor preponderantly coupled with Gαq/Gα11 activates phospholipase C (PLC, β isoform) resulting in the formation of diacylglycerols and inositol-trisphosphate (IP3). These latter two second messengers are responsible for the activation of several protein kinase C (PKC) isoforms and the mobilisation of intracellular Ca^{2+}, respectively. This process together with the

massive entry of Ca^{2+} that occurs through voltage-dependent or -independent channels [27] is primarily involved in acute gonadotropin release. GnRH can also induce the activation of the mitogen associated protein kinase (MAPK) cascade through PKC to stimulate the expression of gonadotropin subunit genes [28, 29]. Elevation of intracellular Ca^{2+} also activates the NO synthase (NOS I) cascade (NOS I/NO/soluble guanylate cyclase) that results in the rapid production of cGMP [30, 31], providing another example of an indirectly activated signalling pathway. GnRH can also induce phospholipases D and A2, as well as a delayed production of cAMP however, the mechanisms involved remain obscure. Cyclic AMP has been shown to be capable of inducing increased expression of LH subunit genes and the release of newly synthesised LH [32, 33] as well as an increased expression of the GnRH receptor [34] and NOS I [35, 36] genes.

Sustained activation of the PKC is followed by proteasome mediated degradation of certain isoforms, a process that may be involved, at least in part, in the desensitisation of GnRH-induced cellular responses [37]. A similar GnRH-induced degradation of IP3 receptors has also been described [38]. In addition to such metabolic events, studies have shown that GnRH may also alter the degree of its effect on gonadotropin secretion through its multigenic regulatory action within the gonadotrophs.

4. ACTIVATION OF THE GnRH RECEPTOR MODULATES ACTIVITY OF A LARGE NUMBER OF GENES: POTENTIAL FOR SUBTLE, FINE-TUNE CONTROL OF GONADOTROPIN SECRETION

4.1. The multigenic impact of GnRH stimulation

Previously a few genes, in the range of 10-20, have been shown to be regulated by GnRH using various conventional investigations. More recently, microarray analyses have increased the number of these genes to over 200 [39]. To date, however, much more is known from the first approach. Interestingly, excluding the three gonadotropin subunit genes, GnRH regulates genes essentially associated with the signalling network such as the GnRH receptor itself [17, 40], NOS I [41], or PKA and PKC subunit isoforms [42, 43] as well as transcription factors such as cFos, cJun or Egr-1. Regulation of the GnRH receptor gene followed a similar extreme dependency on the mode of GnRH stimulation previously revealed for the gonadodotropin β-subunit genes [17, 40]. For both the GnRH receptor and the NOS I genes, evidence exists that the expression is up-regulated at proestrus of the ovarian cycle [31, 44, 45] suggesting a functional adaptation of the signalling machinery at this important physiological stage. GnRH receptor and NOS I promoters have been isolated and studies have characterised their tissue-specific and regulated expression in vitro using a transient transfection assay [36, 46–51] and/or in vivo using transgenesis [52–54]. In each case and regardless of their degree of characterisation, promoters appear to require a specific combination of transcription factors to achieve both tissue-specific and regulated gene expression as illustrated for the GnRH receptor gene in Figure 1. It is noteworthy that combinations of transcription factors share several elements in common with those of the gonadotropin subunit genes. In these combinations, the orphan nuclear receptor, steroidogenic factor 1 (SF-1), might be considered as the most critical since it has been shown to be involved in the tissue-specific expression of the mouse, rat and human GnRH receptor gene as well as in the expression of the alpha, LHβ and FSHβ subunit genes (Tab. II).

Like gonadotropin subunits, the availability of tissue-specific promoters for the GnRH receptor and NOS I genes shows that GnRH itself operates through stimulation of transcriptional activity. Domains involved

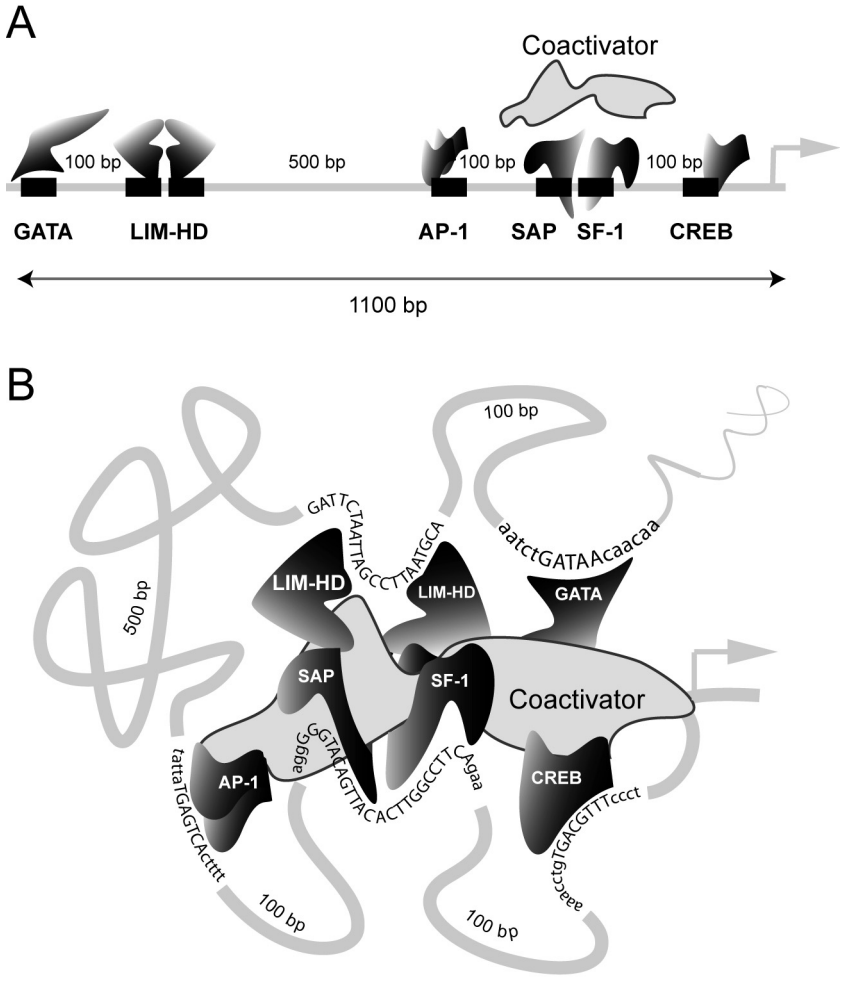

Figure 1. Gonadotrope-specific elements within the promoter of the rat GnRH receptor gene. (**A**) Linear representation of the elements identified in the proximal domain (AP-1, SAP, SF-1 and CREB) and in the distal enhancer (GATA, LIM-HD). The proximal elements act cooperatively, most probably by recruiting a coactivator as yet unidentified. The distal enhancer is functionally and, at least for a part, physically linked to a couple of motifs in the proximal domain that bind SAP and SF-1. (**B**) Speculative representation of the promoter taking into account the functional and physical interactions that were recently established with regards to SF-1 and the LIM-HD factors of the distal enhancer ([49] and Granger et al., manuscript in preparation). Similar interactions most likely occur between SF-1 and GATA as well as between SAP, LIM-HD and GATA factors. The coactivator may facilitate interactions between distal and proximal domains of the promoter. The motifs identified by linker-scanning mutagenesis for each factor are indicated with their approximate interval distances in base-pairs (bp). GATA; GATA-related factor, LIM-HD; LIM homeodomain proteins, AP-1; activating protein 1, SAP; SF-1 associated factor (unidentified), SF-1; steroidogenic factor 1, CREB; cAMP response element binding protein.

Table II. Transcriptional factors shared in the combinatorial codes of GnRH regulated genes in the pituitary gonadotrophs.

	Pitx1-RE	GATA	LIM-HD-RE[a]	SF-1/GSE	CRE	Sp1	AP-1
αGSU							
human	–80/–65	–161/–141	–329/–320	–220/–211	–146/–111		
murine	–398/–385	–	–344/–300	–220/–202	–144/–126		
LHβ							
rat	–99/–96			–58/–51		–366/–354	
				–127/–119		–450/–434	
bovine	–100/–95			–59/–52			
				–128/–121			
FSHβ							
rat	–54/–49		–				
porcine			–219/–209				
	–		–259/–253				
			–295/–284				
			–838/809				
			–1442/–1423				
			–5057/–5030				
murine			–	–341/–333[b]			–72/–69[c]
	–			–239/–231[b]			
GnRH rec							
human				–142/–135[d]	–110/–103[d, e]		
rat	–	–983/–962[d]	–871/–859[d]	–245/–237[d]	–110/–104[d]		–352/–346[d]
murine	–370/–326[d]	–	–	–243/–235[d]	–109/–102[d]		–336/–330[d]
NOS I							
rat			–62/–55[f]		–4/+4[f]	–66/–57[f]	
murine				–206/–298[g]			

The presence of only clearly demonstrated functional sites is indicated in bp. In the absence of specific indications, numbering is expressed with reference to initiation of transcription. Pitx1-RE, pituitary homeobox 1-responsive element; LIM-HD-RE, LIM homeodomain-responsive element; SF-1, Steroidogenic factor 1; GSE, gonadotrope specific element; Sp1, Specific protein 1; AP-1, Activating protein 1.
[a] Corresponds to PGBE (pituitary glycoprotein hormone basal element).
[b] Function in interaction with nuclear factor Y.
[c] Half-site.
[d] Numbering relative to ATG translation start site where A is at position +1.
[e] Unpublished data from our laboratory.
[f] Numbering relative to exon 1a transcription start site (major promoter resides in exon 1a).
[g] Numbering relative to exon 2 translation start site (major promoter resides in exon 2).

in the GnRH response have been localised and in some cases identified [36, 47, 50, 55, 56]. In the rat, mouse and human, the regulation of the GnRH receptor gene involves PKC/MAPK and the induction of early genes cFos and cJun that interact with activating protein-1 (AP-1) motifs. With regards to the mouse promoter, additional elements that bind octamer binding protein Oct-1 and nuclear factor-Y (NF-Y) are required to fulfil homologous up-regulation by GnRH in gonadotrope-derived cell lines [57]. In contrast to the GnRH receptor, regulation of the rat NOS I gene involves mediation via the cAMP pathway and activation of factors that interact with a bipartite element composed of a cAMP regulatory element (CRE) bound to a CRE Binding (CREB)-related factor and an additional domain situated a few base pairs upstream [36].

4.2. The multigenic influence of GnRH as an integrate mechanism for regulated gonadotropin secretion

4.2.1. LH

As mentioned above, GnRH can induce cFos and cJun that form a heterodimeric complex of transcription factors, which increase the activity of AP-1-possessing promoter genes. Egr-1 (early growth response protein 1) is another transcription factor encoding gene that is rapidly induced by GnRH in the gonadotrophs, and this occurs through PKC/MAPK [58, 59]. Egr-1 is indispensable for the tissue-specific expression of the LHβ subunit gene in concert with SF-1, Sp1 and the pituitary homeobox 1 Pitx1 [60, 61] and its increased expression enhances LHβ production. Interestingly, a delayed induction of the Egr-1 co-repressor Nab1 has been described in the clonal murine gonadotrope αT3-1 cell line under prolonged stimulation with GnRH that could be responsible for the arrest of LHβ transcription [59]. Induction of Egr-1 and Nab1 may thus provide an attractive model to account for an initial activation followed by inactivation of the LHβ gene, and could thus explain the dual regulatory action on this gene of a single stimulator, GnRH.

4.2.2. FSH

Compared to LHβ, the nature of the cis regulatory elements involved in the GnRH activation of the FSHβ gene is poorly understood. Several AP-1 domains could be involved via PKC [62, 63]. As illustrated in Figure 2, a time-course study by Burger et al. [14] using as a model the castrated testosterone-treated male rat showed that transcriptional activation of the FSHβ gene was progressive but high and durable at low GnRH pulse frequencies. However, under a relatively rapid stimulation, it declined after a swift but transient (1.5 h) increase. Since the expression of the FSHβ gene is also activine-dependent [64] and gonadotrophs express the activin regulator, follistatin [65], the effects of pulsatile *vs.* permanent presence of GnRH on the expression of the follistatin gene were examined. As shown in Figure 2, while GnRH applied at slow frequency was rather inefficient, rapid pulse frequencies increased follistatin mRNA thus supporting the concept of an activin/follistatin autocrine/paracrine loop [14, 66, 67]. No such effects have been observed on activin βB mRNA levels although they significantly decreased after prolonged high frequency stimulation. These data thus could account for the higher expression of the

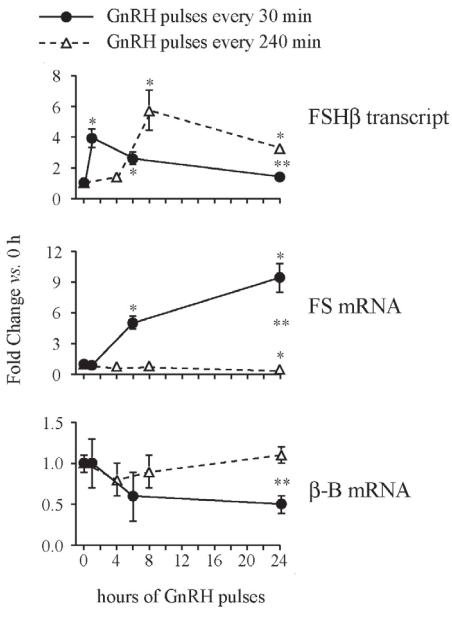

Figure 2. Influence of the GnRH pulse frequency on FSHβ, follistatin and activin βB gene expression. Castrated, testosterone replaced male rats were *iv* pulsed with 25 ng GnRH every 30 (fast) and 240 (slow) min for 1–24 h ($n = 4$–8 rats/observation). Levels of primary FSHβ transcript and follistatin (FS) and activin βB mRNA were measured by quantitative RT-PCR using appropriate primers and data are expressed as fold change vs. controls (0 h). * Significant differences ($P < 0.05$) vs. untreated castrated-testosterone controls (0 h). ** Significant differences between GnRH pulse regimen at 24 h. From Burger et al. [14], with permission. Copyright 2002, The Endocrine Society.

FSHβ gene in response to GnRH at low frequencies as described earlier [13] and suggest that the low FSHβ expression observed at high frequencies could result from the induction of follistatin working to counteract the stimulatory effects of activin. These actions represent another mechanism, by which the pulsatile profile of GnRH secretion could differentially regulate FSH and LH secretion.

5. CONCLUSION

In conclusion, while the functional activity of pituitary gonadotrophs is highly dependent on the GnRH pulse frequency, this property can not rely on the classic homologous desensitisation/endocytic recycling of GPCR receptors. Instead, the GnRH action should be viewed as an interaction of this neurohormone with a strongly atypical receptor lacking the C-terminal tail and thus incapable of rapid desensitisation in contrast to the vast majority of GPCR. Consequently "desensitisation" (estimated through LH and FSH release) does not reflect general cell refractoriness, but rather individual responses limited to a group of genes/proteins, while expecting other typical regulations to be maintained or even amplified. Indeed under a similar paradigm i.e. a given typical GnRH stimulation frequency, some GnRH responsive genes are observed to be activated while others are not (or become inactivated), providing the first arguments in favour of this concept and substantiates a participation of the multigenic action of GnRH in the modulation of its own action. Recent studies have demonstrated that, with the exception of rare primates, mammals exclusively expressed the GnRH receptor type I while in such species the identification of genomic remnant traces of type II GnRH receptor suggested the silencing of this gene during evolution [68]. The acquisition of a single gene encoding a GnRH receptor deprived of a C-terminal tail, together with the episodic nature of the GnRH release may represent an evolutionary adaptation to improve integral neuroendocrine control of reproduction. It is most probable that further studies will allow the identification of some additional alternative strategies in the differential modulation of the gonadotropic secretory function.

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