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The tail of the gonadotrophin-releasing hormone receptor: desensitization at, and distal to, G protein-coupled receptors

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Abstract

In recent years a general scheme for the rapid desensitization and cycling of G protein-coupled receptors (GPCRs) has emerged. In this scheme agonist-induced phosphorylation (most often in the receptors' C-terminal tail) causes association with β-arrestin which not only reduces the efficiency of G-protein activation, but also targets these desensitized receptors for internalization, after which they may be either proteolytically degraded or resensitized and recycled back to the cell surface. Although sustained stimulation of pituitary gonadotrophs with gonadotrophin-releasing hormone (GnRH) is known to cause a pronounced desensitization of GnRH-stimulated gonadotrophin secretion, the discovery that mammalian GnRH receptors do not possess C-terminal tails raised the question of whether receptor desensitization is involved. This review outlines data demonstrating that tail-less mammalian GnRH receptors can be considered as natural desensitization and internalization deficient 'mutants'. This is in stark contrast to non-mammalian GnRH receptors which do possess tails and conform to the general scheme. In the absence of receptor desensitization, post receptor mechanisms take on increasing importance for desensitization of GnRH action via mammalian GnRH receptors. The down regulation of $Ins(1,4,5)P_3$ receptors and consequent desensitization of GnRH effects on cytosolic Ca^{2+} are discussed as a novel mechanism for such desensitization.

Keywords: Gonadotrophin-releasing hormone; G protein-coupled receptor; Desensitization; Internalization; Ca^{2+}

1. Desensitization and cycling of GPCRs

G protein-coupled receptors (GCPRs), the largest known class of signaling proteins, are characterized structurally by the presence of seven trans-membrane domains, linked by a series of intracellular and extracellular loops. They mediate responses to a vast array of extracellular stimuli ranging from photons, amines, and lipids to peptides and proteins, and are currently major targets for therapeutic interventions. Following agonist binding, GPCRs act as guanine nucleotide exchange factors for their cognate heterotrimeric G-proteins, stimulating the exchange of GDP for GTP. This causes dissociation into βγ and (GTP bound) α-subunits which regulate effector proteins including ion channels, adenylyl cyclase and phospholipase C (PLC). The intrinsic GTPase of the α-subunit then hydrolyses GTP to GDP, and the α-subunit recombines with βγ-subunits to terminate signaling. The intrinsic GTPase activity of heterotrimeric G-protein α-subunits can be enhanced, either by the effector molecule (e.g. PLC, Berstein et al., 1992) or by recently described GTPase activating proteins, termed regulators of G-protein signaling (RGS, Koelle 1997).

The initial response to ligand activation of a GPCR is followed by a wide range of adaptive responses which ultimately alter the availability and/or activity of signaling proteins. This typically alters the amplitude and/or nature of the response during sustained stimulation or, if the ligand is removed, to a subsequent stimulation.

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Such regulation may be either homologous (i.e. regulation of responses through the same receptor) or heterologous (i.e. regulation of responses through different receptors). The best explored example of such adaptation is that of homologous desensitization which is very often caused by sustained or repetitive GPCR activation and is defined as a waning of response in the face of constant (sustained or repeated) stimulation. Such desensitization often reflects partial or complete uncoupling of the receptors from their effector proteins, which may occur within seconds-minutes of agonist occupancy (rapid homologous receptor desensitization). Following this, receptors are sequestered from the plasma membrane and are internalized, within minutes to hours, to be either recycled to the cell surface or proteolytically degraded in lysosomes (Lefkowitz et al., 1990; Dohlman et al., 1991). Thus, uncoupling of cell-surface GPCRs from their effector molecules, and removal of GPCRs from the cell surface will promote desensitization, whereas recycling of receptors back to the cell-surface will promote resensitization (Fig. 1).

Extensive studies of the β2-adrenoreceptor (which couples to adenylyl cyclase via $G_s\alpha$) have revealed the importance of agonist-induced receptor phosphorylation for rapid homologous desensitization (Lefkowitz et al., 1990; Dohlman et al., 1991; Lattion et al., 1994).

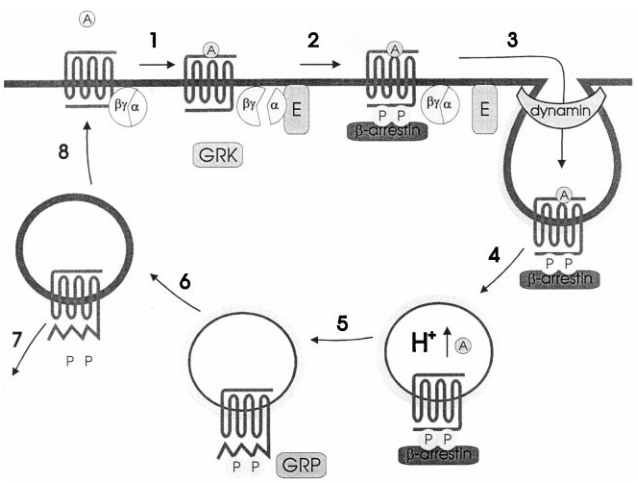

Fig. 1. General scheme for desensitization and cycling of GPCRs. Binding of the agonist (A) not only causes activation of the effector (E) but also facilitates phosphorylation of the receptor (often by GRKs on specific sites within the C-terminal tail) and this enables binding of β-arrestin (1-2). β-arrestin prevents effector activation and targets the desensitized receptor for internalization via clathrin-coated vesicles which are pinched off from the plasma membrane by dynamin (2-3). The receptor is then dephosphorylated by a (poorly characterized) GPCR phosphatase (GRP), a process which may be facilitated by acidification of the vesicle lumen and consequent alteration of receptor conformation (4-6). The receptor can then be targeted to lysosomes for degradation (7) or, after dephosphorylation (resensitization), recycled to the cell surface (8). Note that GPCRs can also be internalized by bulk endocytosis and that by mechanisms independent of receptor phosphorylation.

This phosphorylation is mediated by the second messenger-activated protein kinase A (PKA), at low levels of agonist occupancy and by a GPCR kinase (GRK) at high levels of agonist occupancy. Such phosphorylation may, itself, attenuate interaction of the receptor with its G-protein, but phosphorylation of sites within the C-terminal tail of these receptors by GRK2 also increases their affinity for β-arrestin. The binding of this accessory protein, uncouples the receptor from its G-protein, thereby inhibiting activation of adenylyl cyclase (Fig. 1). Interestingly, recent studies have revealed that this phosphorylation acts as a switch, targeting the receptor to a different G-protein ($G_i\alpha$) and that β-arrestin acts as an adapter, targeting desensitized β2-adrenoreceptors for internalization via clathrin-coated vesicles (Ferguson et al., 1997). This internalization serves not only to target receptors to lysosomes for proteolysis but also allows receptor dephosphorylation and the recycling of resensitized receptors back to the cell surface (Krueger et al., 1997). This general model (Fig. 1) has received widespread support as a common, if not universal, means of GPCR regulation.

2. GnRH receptors and effectors

GnRH is a hypothalamic decapeptide which acts via GPCRs on gonadotrophs to stimulate the exocytotic secretion of luteinizing hormone and follicle-stimulating hormone. Agonist occupancy of GnRH receptors activates one or more isoforms of PLC, which hydrolyze membrane phosphoinositides. Hydrolysis of the minor membrane phospholipid, phosphatidylinositol 4,5-bisphosphate ($PtdIns(4,5)P_2$) yields both diacylglycerol and $Ins(1,4,5)P_3$ which activate most isoforms of protein kinase C (PKC) and mobilize Ca^{2+} from intracellular stores, respectively (Kakar et al., 1992; Tsutsumi et al., 1992; Berridge 1993; Stojilkovic and Catt, 1995; Conn 1995). GnRH also increases Ca^{2+} entry into gonadotrophs, predominantly via voltage-operated Ca^{2+} channels (VOCCs) and the increase in cytosolic Ca^{2+} ($[Ca^{2+}]_i$) caused by GnRH is primarily responsible for the increase in exocytotic hormone release (Hansen et al., 1987; McArdle and Poch, 1992; Stojilkovic and Catt, 1995; Conn 1995; Hille et al., 1995; Tse et al., 1997). GnRH activates PLC via α-subunits of the Gq and 11 families (Hsieh and Martin, 1992; Shah and Milligan, 1994) and because RGS3, co-transfected with GnRH receptors into COS mouse fibroblast cells, inhibits the effect of GnRH on $Ins(1,4,5)P_3$ levels (Neill et al., 1997a) these G-proteins, activated by GnRH, are apparently also regulated by, at least one RGS protein (Koelle, 1997).

Physiologically, GnRH is secreted in a pulsatile fashion, but sustained exposure to GnRH is known to reduce GnRH-stimulated gonadotrophin secretion and

this form of homologous desensitization underlies the suppression of the reproductive system which is exploited in the major clinical applications of GnRH analogues (Barbieri, 1992). This phenomenon, which was originally considered to be entirely pharmacological, is now known to occur within the time-frame of endogenous GnRH pulses and may therefore also be physiologically relevant (Weiss et al., 1995). Sustained stimulation with GnRH also causes GnRH receptor internalization and down-regulation. Although these effects have the potential to cause desensitization, they can be uncoupled from the changes in gonadotroph responsiveness, demonstrating the involvement of additional mechanisms (Dekoning et al., 1978; Jinnah and Conn, 1985, 1986; McArdle et al., 1987, 1988). Given the model of GPCR regulation described above and the evidence that many PLC coupled receptors undergo rapid homologous desensitization (Kwatra et al., 1993; Wojcikiewicz et al., 1993; Tobin et al., 1992; Tobin, 1998), it had been assumed that agonist-induced receptor phosphorylation and β-arrestin binding would underlie agonist-induced GnRH receptor desensitization and internalization. However, cloning of the first mammalian GnRH receptors (Tsutsumi et al., 1992; Kakar et al., 1992; Stojilkovic et al., 1994) revealed that they lack any C-terminal tail and have a comparatively short third intracellular loop (the role of the C-terminal tail in phosphorylation and desensitization appears, in some receptors, to have been taken over by this region). These unexpected structural features, particularly the lack of the C-terminal tail, which is unique amongst known PLC-activating GPCRs, raised the question of whether GnRH receptors are able to desensitize.

The functional desensitization of PLC-activating GPCRs is most often revealed experimentally as a failure to maintain the initial rate of ligand-stimulated total [^{3}H]inositol phosphate ([^{3}H]IP$_x$) accumulation against a Li^+ block of inositol monophosphatase (thereby providing an index of total PLC activity). Such desensitization is often also revealed by a transient agonist-stimulated elevation of Ins(1,4,5)P$_3$ mass (although this has the potential to reflect changes in Ins(1,4,5)P$_3$ metabolism as well as generation). However, neither of these hallmarks were observed when the endogenous mouse GnRH receptors of the gonadotroph-derived αT3-1 cell line (Windle et al., 1990) were stimulated with GnRH. In these cells, the initial rate of GnRH-stimulated [^{3}H]IP$_x$ accumulation is maintained for at least 90 min (Davidson et al., 1994) and GnRH increases Ins(1,4,5)P$_3$ mass to a plateau which is maintained for at least 5 min (Anderson et al., 1995; McArdle et al., 1996; Merelli et al., 1992; Willars et al., 1998a). These data indicate that the GnRH receptor does not undergo rapid homologous desensitization in αT3-1 cells and imply that this is because it has no C-terminal tail and therefore lacks phosphorylation sites necessary for agonist-dependent desensitization. Moreover, the lack of GnRH receptor desensitization, along with earlier work showing that desensitization of GnRH action (e.g. gonadotrophin secretion) can be uncoupled from receptor internalization and desensitization (above), implies that such desensitization must reflect adaptive responses distal to the receptor. The remainder of this article deals with the most recent data for and against these interpretations. Down-stream mechanisms pertinent to desensitization of GnRH effects on gene expression have been reviewed recently (Kaiser et al., 1997) and desensitization of GnRH effects on other signaling systems (Mitchell et al., 1998; Ulloa-Aguirre et al., 1998) has not been explored in detail.

3. Do mammalian GnRH receptors undergo rapid homologous desensitization?

Although rapid receptor desensitization (through phosphorylation) is a generally accepted mechanism for GPCR regulation, it has proven technically difficult to demonstrate unambiguously that effects at the receptor level underlie desensitization of PLC-activating GPCRs (Wojcikiewicz et al., 1993) because factors other than receptor desensitization can influence the kinetics of [^{3}H]IP$_x$ and Ins(1,4,5)P$_3$ responses. Positive feedback effects of Ca^{2+} on PLC could conceivably lead to maintenance of high initial [^{3}H]IP$_x$ accumulation rates, in spite of receptor desensitization, whereas depletion of the preferred substrate for PLC (PtdIns(4,5)P$_2$) could lead to a decrease in [^{3}H]IP$_x$ accumulation rates even in the absence of receptor desensitization. Similarly, high receptor number could conceivably mask receptor desensitization because even with the majority of receptors uncoupled from their effectors, receptor reserve could still ensure retention of sufficient active receptors to support a maximal response. Moreover, receptor desensitization must depend upon the repertoire of pertinent proteins (e.g. GRKs) expressed in any given cell and is therefore context-dependent. With such caveats it was inevitable that the relevance of data from mouse GnRH receptors in αT3-1 cells to other systems would be contested. Indeed, GnRH receptors have long been known to aggregate over, and internalize via, clathrin-coated vesicles and sustained exposure to GnRH certainly does cause GnRH-receptor down-regulation in rat gonadotrophs (Conn et al., 1987). Moreover, αT3-1 cells and rat gonadotrophs express GRKs (Neill et al., 1996) and over-expression of GRKs 2, 3 or 6 reduces GnRH-stimulated Ins(1,4,5)P$_3$ increases in COS cells transiently transfected with rat GnRH receptors (Neill et al., 1998), and the maximal initial rate of GnRH-stimulated [^{3}H]IP$_x$ accumulation was not maintained beyond 60 s in these cells (Neill et al., 1997b). These data are entirely compatible with the general scheme in Fig. 1 and imply that the lack of rapid homologous desensitization to GnRH in αT3-1 cells is a limitation of the model (e.g.

of receptor number or cellular context) rather than the mouse GnRH receptor per se.

To address these issues, we have investigated desensitization of other endogenous GPCRs and have shown that the effects of pituitary adenylate cyclase activating polypeptide 1-27 (PACAP27) on cAMP accumulation (mediated by the type I PACAP receptor) do rapidly desensitize in αT3-1 cells (McArdle and Forrest-Owen, 1998). Desensitization of endogenous PLC-activating GPCRs in these cells proved more difficult to investigate because although they express PLC-coupled receptors for endothelin 1 (ET1A, McArdle et al., 1992), oxytocin (Evans et al., 1997), ATP (P2U, Chen et al., 1996) and methacholine (muscarinic, Willars et al., 1998a), they mediate $[^3H]IP_x$ responses which are only 5–20% of those caused by GnRH (e.g. responses too low for accurate quantification of rapid homologous desensitization). Using Ca^{2+} responses (fura 2 imaging) as an indirect measure of receptor desensitization, we have obtained evidence that each of these receptors undergoes rapid homologous desensitization in αT3-1 cells (Forrest-Owen et al., 1998). To assess this more directly, we have transfected αT3-1 cells with human M_3 muscarinic receptors, as an example of PLC-activating receptors which are known to rapidly desensitize in other cells (Tobin and Nahorski, 1993). The responses to activation of these receptors showed the expected characteristics of rapidly desensitizing GPCRs (Willars et al., 1998a). Thus, the initial rate of methacholine-stimulated $[^3H]IP_x$ accumulation was not maintained beyond 1 min and methacholine caused a transient increase in $Ins(1,4,5)P_3$ mass, as compared to the responses to GnRH, both of which were sustained (Fig. 2, Willars et al., 1998a). To exclude the possibility that the lack of desensitization of responses to GnRH might be the result of high receptor number (receptor reserve) or access to an atypically large (or rapidly refilled) pool of $PtdIns(4,5)P_2$ we determined responses to GnRH after reduction of GnRH receptor number, by irreversible blockade using a photo-affinity antagonist. In addition we reduced the receptor accessible $PtdIns(4,5)P_2$ pool size, by pretreatment of cells expressing the recombinant muscarinic M_3 receptor with methacholine in the presence of Li^+ (Forrest-Owen et al., 1998). Although both treatments reduced the magnitude of responses to GnRH, neither altered the temporal profiles of $[^3H]IP_x$ or $Ins(1,4,5)P_3$ responses. Thus, although GnRH receptor number and $PtdIns(4,5)P_2$ pool size are major determinants of the magnitude of these responses to GnRH, they do not mask functional receptor desensitization in αT3-1 cells (Forrest-Owen et al., 1998).

These data show that αT3-1 cells are capable of desensitizing other GPCRs, indicating that the lack of desensitization of the GnRH receptor is a functional characteristic of the receptor itself. A complementary approach is to ask whether GnRH receptors are able to

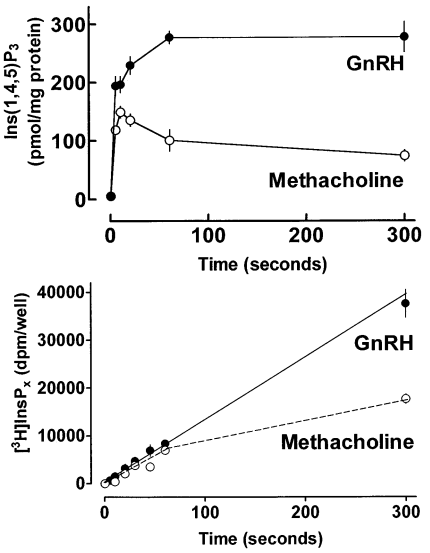

Fig. 2. Effects of GnRH and methacholine on $[^3H]IP_x$ accumulation and $Ins(1,4,5)P_3$ levels in stable M_3 receptor transfected αT3-1 cells. Cells were stimulated at time 0 with 10^{-6} M GnRH or 10^{-3} M methacholine (upper panel) or with 10^{-7} M GnRH or 10^{-3} M methacholine in the presence of 10 mM LiCl (lower panel). Note that the $Ins(1,4,5)P_3$ response to methacholine is transient (upper panel) because in addition to $Ins(1,4,5)P_3$ metabolism, their is a reduction in PLC activity, as indicated by the failure to maintain the initial rate of $[^3H]IP_x$ accumulation (lower panel). In contrast, GnRH increases $Ins(1,4,5)P_3$ to a maintained plateau without any measurable reduction in the rate of $[^3H]IP_x$ accumulation. Adapted from Willars et al., 1998a with permission.

desensitize in other cells (known to support desensitization of other PLC-activating GPCRs) but initial experiments using this approach gave equivocal results. Thus, as indicated by measurement of $[^3H]IP_x$ accumulation rates, human or mouse GnRH receptors transfected into GH_3 cells did not desensitize (Davidson et al., 1994), whereas rat GnRH receptors transfected into COS cells did desensitize (Neill et al., 1997a). However, these studies were both performed using transiently transfected receptors where receptor number was not known (for the sub-set of cells transfected) and the relevance of the caveats outlined above for these models cannot be known. More recent studies using stably transfected cell lines (e.g. rat and mouse GnRH receptors expressed in HEK-293 cells (Heding et al., 1998a and Hislop, Davidson and McArdle, unpublished)) have revealed no rapid homologous desensitization of mammalian GnRH receptors (see Section 4).

4. Is the lack of desensitization of mammalian GnRH receptors a result of the lack of required sites for phosphorylation in the C-terminal tail?

The obvious test for whether the lack of mammalian GnRH receptor desensitization is a result of the absence of a C-terminal tail would be to make chimeric

receptors containing the GnRH receptor and the C-terminal tail of another GPCR. However, interpretation may be difficult as there is no guarantee that the added tail will adopt an appropriate structure and perform its appropriate function in this environment. It therefore created considerable interest when the first non-mammalian GnRH receptor was cloned and found to possess a cytoplasmic C-terminal tail. To date, sequences of 10 GnRH receptors are known (Sealfon et al., 1997; Troskie et al., 1997) and all mammalian forms (rat, mouse, sheep, cow, pig, human) are tail-less, whereas all non-mammalian forms (catfish, frog, chicken, goldfish) have C-terminal tails containing multiple serine or threonine residues (potential sites for phosphorylation by PKA, PKC or GRKs). When rat GnRH receptors were stably transfected into HEK-293 cells, these did not rapidly desensitize (e.g. $[^3H]IP_x$ accumulation was linear for at least 5 min) whereas TRH receptors, catfish GnRH receptors and rat GnRH receptors with C-terminal tails from TRH receptors or catfish GnRH receptors (investigated under comparable conditions), all desensitized within minutes of stimulation (Heding et al., 1998a,b). In vivo phosphorylation studies, performed by immunoprecipitation of normal and/or Hepitope tagged receptors have revealed that in these cells, GnRH does not induce phosphorylation of rat GnRH receptors, but rapidly increases the phosphorylation of catfish GnRH receptors, and of rat GnRH receptor chimeras with C-terminal tails of TRH receptors or catfish GnRH receptors (Willars et al., 1998b).

The data above strongly suggest that mammalian GnRH receptors fail to desensitize because they lack required phosphorylation sites within the C-terminal tail and therefore do not undergo rapid agonist-induced phosphorylation. Given the established role of phosphorylation-dependent β-arrestin binding in targeting of GPCRs for internalization via clathrin-coated vesicles (Ferguson et al., 1997) this also raises the question of the relevance of C-terminal tails for GnRH receptor internalization. Pawson et al., (1998) have recently shown that agonist-stimulated chicken GnRH receptors internalize at least 15 times faster (in COS cells) than human GnRH receptors. Moreover, progressive truncations of the tail of the chicken GnRH receptor caused decreases in the internalization rate constant proportional to the number of tail residues removed (Pawson and Davidson, in preparation). Similarly, internalization rates were found to be much lower for rat GnRH receptors than for TRH and catfish GnRH receptors or rat GnRH receptors with TRH or catfish tails (Heding et al., 1998a,b). Thus, the mammalian GnRH receptor can be considered as a naturally occurring internalization-deficient 'mutant'. Nevertheless, agonist-induced internalization of mammalian GnRH receptors does occur (albeit at a relatively low rate) and such internalization is apparently via clathrin-coated vesicles and contributes to the GnRH receptor down-regulation (Conn et al., 1987). The means by which GnRH stimulates mammalian GnRH receptor internalization, therefore remains to be established but may be related to that underlying the phosphorylation-independent internalization of C-terminal truncated opioid receptors (Murray et al., 1998).

5. What mechanisms of desensitization operate distal to the GnRH receptor?

It has long been known that GnRH-stimulated gonadotrophin secretion desensitizes during sustained stimulation of gonadotrophs with GnRH. The multiple mechanisms contributing towards this effect include depletion of intracellular gonadotrophin pools, internalization of GnRH receptors and GnRH receptor down-regulation. However, because these can be uncoupled from desensitization of gonadotrophin secretion (Jinnah and Conn 1985, 1986; McArdle et al., 1987; Conn et al., 1987) and mammalian GnRH receptors do not desensitize, post-receptor mechanisms must be involved. Indeed, GnRH action on gonadotrophs provides a unique model for investigation of such down-stream mechanisms in the absence of receptor desensitization. Our studies, focusing on desensitization of the GnRH-mediated elevation of $[Ca^{2+}]_i$, have revealed that pretreatment of αT3-1 cells for 60 min with GnRH causes a pronounced reduction of subsequent GnRH-induced increase in $[Ca^{2+}]_i$ (Fig. 3 and McArdle et al., 1995). Both the spike phase of the response (which reflects mobilization from intracellular $Ins(1,4,5)P_3$-sensitive Ca^{2+} stores) and sustained phase of the response (which is dependent upon Ca^{2+} entry across the plasma membrane) were attenuated by GnRH pretreatment. Half-times for the onset of, and recovery from, desensitization of the spike-type response were estimated as approximately 15 min and 6 h, respectively (Fig. 3 and McArdle et al., 1995). VOCC desensitization provides the most likely explanation for desensitization of the plateau response, as GnRH has been shown to desensitize VOCCs in rat gonadotrophs (Stojilkovic et al., 1989) and GnRH pretreatment also reduced the effect of KCl on $[Ca^{2+}]_i$ in αT3-1 cells (McArdle et al., 1995). However, this clearly can not explain attenuation of the $Ins(1,4,5)P_3$-mediated mobilization of Ca^{2+} from the intracellular stores.

Pretreatment for 60 min with GnRH causes a modest reduction in GnRH-stimulated $Ins(1,4,5)P_3$ elevation which most likely results from the concomitant 40% reduction in cell surface GnRH receptor number, rather than receptor desensitization (McArdle et al., 1995). Comparison of dose-response relationships reveals that in control cells an increase in $Ins(1,4,5)P_3$ mass equivalent to only 5–10% of the maximal GnRH effect elicits maximal $[Ca^{2+}]_i$ increase in a Ca^{2+}-free medium (i.e.

release form intracellular stores only) (McArdle et al., 1996). Thus, in the absence of additional changes, the Ins(1,4,5)P_3 response of cells that had been pretreated with GnRH would have been sufficient to elicit maximal mobilization of Ca^{2+} from intracellular stores. The fact that it did not, could not be explained by depletion of intracellular hormone-mobilizable Ca^{2+} pools, because GnRH and ionomycin mobilized Ca^{2+} from functionally indistinguishable intracellular pools and a robust ionomycin response was maintained after GnRH pretreatment. Instead, this form of desensitization apparently reflects a reduction in the efficiency with which Ins(1,4,5)P_3 mobilizes Ca^{2+} from an extant intracellular store (McArdle et al., 1996).

Sustained stimulation of several PLC-activating GPCRs has been shown to down-regulate Ins(1,4,5)P_3 receptors, most probably as a consequence of Ca^{2+}-induced proteolysis (Wojcikiewicz and Nahorski, 1991). We therefore investigated this as a possible mechanism for attenuation of GnRH-stimulated mobilization of Ca^{2+} by GnRH pretreatment. αT3-1 cells express predominantly type I Ins(1,4,5)P_3 receptors and radioligand binding revealed the presence of high affinity (K_d approximately 10 nM) binding sites for Ins(1,4,5)P_3. Pretreatment with GnRH caused parallel dose-and time-dependent reductions in Ins(1,4,5)P_3 receptor number (defined by radioligand binding) and type I Ins(1,4,5)P_3 receptor protein (determined by Western blotting). Moreover, the time-courses of these changes (half-times < 30 min) were similar to those for the onset

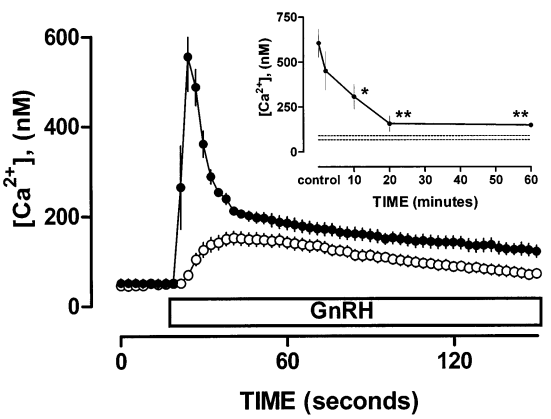

Fig. 3. Desensitization of GnRH-stimulated Ca^{2+} mobilization. Main panel-αT3-1 cells were pretreated for 60 min with 0 (filled circles) or 10^{-7} M (open circles) GnRH, then washed and used for fura-2 measurement of the effect of 10^{-7} M GnRH (bar) on cytosolic Ca^{2+} concentration. Note that the GnRH pretreatment reduced both plateau and spike phases of the response and that the latter effect could not be explained by desensitization of Ins(1,4,5)P_3 responses or depletion of the hormone-mobilizable Ca^{2+} pool. Inset-cells were pretreated for the indicated times with 10^{-7} M GnRH then washed and used for measurement of the spike response to 10^{-7} M GnRH as in the main panel. The dotted lines show basal Ca^{2+} levels (SEM limits) under the same conditions. From McArdle et al., 1996 with permission.

of desensitization of the spike-type Ca^{2+} mobilization by GnRH (McArdle et al., 1997).

In other systems Ins(1,4,5)P_3 receptor down-regulation is usually relatively slow (half-times of > 4 h) and the unusual rapidity of this response to GnRH may occur because the GnRH receptor does not rapidly desensitize and therefore causes a sustained elevation of Ins(1,4,5)P_3. To test the relevance of Ins(1,4,5)P_3 receptor regulation to the observed desensitization, we have made use of thimerosal (a thiol modifying reagent) which can increase the affinity of Ins(1,4,5)P_3 receptors for Ins(1,4,5)P_3 (Thrower et al., 1996). In Ca^{2+} imaging experiments, thimerosal had no effect alone and did not alter the response of control cells to GnRH, but partially reversed the desensitization of the spike-type Ca^{2+} mobilization response by GnRH pretreatment (McArdle and Willars, unpublished). This supports the interpretation that the Ins(1,4,5)P_3-mobilizable Ca^{2+} pool is intact and that Ins(1,4,5)P_3 receptor occupancy is the limiting factor in the GnRH pretreated cells, presumably because of Ins(1,4,5)P_3 receptor down-regulation. Moreover, desensitization as a result of Ins(1,4,5)P_3 receptor down-regulation would be expected to be heterologous and pretreatment with GnRH also abolishes the spike phase of the response to PACAP27 (McArdle et al., 1996). Thus, Ins(1,4,5)P_3 receptor down-regulation apparently provides a novel mechanism for desensitization of GnRH effects on $[Ca^{2+}]_i$ in the intermediate time-frame (several minutes-hours). Since exocytosis is regulated by $[Ca^{2+}]_i$ this mechanism may contribute toward desensitization of GnRH-stimulated gonadotrophin secretion although evidence exists that the tight causal relationship between Ca^{2+} elevation and exocytosis (Tse et al., 1997) is lost in desensitized gonadotrophs (Waters and Conn, 1992).

6. Summary and directions

Mammalian GnRH receptors are unique. They are the smallest known GPCRs, the only PLC-coupled GPCRs known to lack C-terminal tails and the only PLC-activating GPCRs known not to undergo agonist-induced phosphorylation and desensitization. These features are in stark contrast to those of the non-mammalian GnRH receptors, and indeed to the vast majority of GPCRs including those of yeast. The implication is that these receptors have undergone a period of dramatically accelerated molecular evolution in the relatively recent past (e.g. after the separation of birds, mammals and amphibians from ancestral terrestrial vertebrates), during which they have simply discarded a structural feature (the C-terminal tail), and associated functions, which have been retained by > 99% of known GPCRs. Although it is not at all clear why these desensitization and internalization deficient 'mutants' should have evolved, it has two obvious consequences. First, $G_q\alpha$ and $G_{11}\alpha$ act

primarily as simple integrators of the activity of their associated guanine nucleotide exchange factor (GnRH receptor) and GTPase regulators (PLC and RGS) in gonadotrophs. Since the established mechanisms for acute regulation of the exchange factor are not pertinent (for mammalian GnRH receptors) it will be fascinating to know whether regulation by RGS proteins, for example, takes on increasing importance. Second, post-receptor mechanisms must underlie desensitization of GnRH-stimulated gonadotrophin secretion and these may well include down-regulation of $Ins(1,4,5)P_3$ receptors. In the absence of receptor desensitization such down-stream adaptive responses may become increasingly important as indicated by the unusual extent and rapidity of the response to GnRH.

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