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Targeting mediators of Wnt signalling pathways by GnRH in gonadotropes

Samantha Gardner, Emmanouil Stavrou, Patricia E Rischitor, Elena Faccenda and Adam J Pawson

Abstract

The binding of GnRH to its receptor on pituitary gonadotropes leads to the targeting of a diverse array of signalling mediators. These mediators drive multiple signal transduction pathways, which in turn regulate a variety of cellular processes, including the biosynthesis and secretion of the gonadotropins LH and FSH. Advances in our understanding of the mechanisms and signalling pathways that are recruited to regulate gonadotrope function are continually being made. This review will focus on the recent demonstration that key mediators of the canonical Wnt signalling pathway are targeted by GnRH in gonadotropes, and that these may play essential roles in regulating the expression of many of the key players in gonadotrope biology, including the GnRH receptor and the gonadotropins.

Introduction

GnRH occupancy of GnRH receptors leads to the activation of multiple signal transduction pathways (Naor 1997, 2009, Naor et al. 2000, Millar et al. 2004, 2008, Caunt et al. 2006, Dobkin-Bekman et al. 2006). In gonadotropes, GnRH activates phospholipase-Cβ via coupling to Gq/11, resulting in the hydrolysis of membrane-bound phosphatidylinositol 4,5-bisphosphate into inositol 1,4,5-triphosphate and diacylglycerol, which respectively mobilise intracellular Ca2+ and activate protein kinase C (PKC). The diverse mechanisms and intracellular signalling pathways that have been reported to contribute to the regulation of gonadotrope function in response to GnRH stimulation have been reviewed extensively (Millar et al. 2004, 2008, Pawson & McNeilly 2005, Naor 2009), and will not be discussed further here. Instead, this review will focus on the proposed roles of Wnt signalling mediators in regulating gonadotrope function in response to GnRH.

A ‘rough guide’ to Wnt signalling

Wnt signalling plays an important role in embryonic development influencing cell proliferation, survival and differentiation (Huelsken & Behrens 2002, van Es et al. 2003, Moon et al. 2004, Nelson & Nusse 2004). Aberrant Wnt signalling can lead to a range of diseases, most notably cancer (Polakis 2000). The canonical Wnt/β-catenin pathway is perhaps the best described Wnt signalling pathway. The key effector of this pathway is β-catenin. In the absence of Wnt ligand (19 family members in humans) stimulation of the Frizzled (FZD) family of receptors (10 family members in humans), cellular β-catenin levels are kept very low. This is because β-catenin is held in a destruction complex, which includes amongst others adenomatous polyposis coli, axin, glycogen synthase kinase 3 (GSK3) and casein kinase 1 (CK1). GSK3 hyperphosphorylates β-catenin, thereby targeting it for ubiquitination and subsequent degradation via the proteasomal degradation pathway. When Wnts bind to and activate the FZD receptors, GSK3 activity is inhibited, allowing the levels of β-catenin to stabilise, and β-catenin translocates to the nucleus where it acts as a co-factor to T cell factor (TCF)/lymphoid enhancer factor transcription factors to promote the transcription of Wnt target genes, many of which are key in developmental processes (Huelsken & Behrens 2002, van Es et al. 2003, Moon et al. 2004).
Less well characterised are the so-called non-canonical Wnt pathways, several of which have been proposed over the past 20 years, including the Wnt/Ca\textsuperscript{2+} pathway and Wnt/c-Jun N-terminal kinase (JNK) pathway (Kuhl et al. 2000, van Es et al. 2003, Veeman et al. 2003, Kohn & Moon 2005). These pathways are also activated by Wnt ligands binding to FZD receptors, and are thought to influence processes such as cell polarity, cytoskeletal reorganisation and cell movement, and utilise a diverse array of signalling mediators and transcription factors to drive these events, including PKC, Ca\textsuperscript{2+}/calmodulin (CaM), calcineurin (CaN), Ca\textsuperscript{2+}/CaM-dependent kinase II, nuclear factor of activated T cells (NFAT), dishevelled, RhoA, Rac, Cdc42 and JNK (Kuhl et al. 2000, van Es et al. 2003, Veeman et al. 2003, Kohn & Moon 2005, Katoh & Katoh 2007).

Although there are two isoforms (\(\alpha\) and \(\beta\)) of GSK3, it is GSK3\(\beta\) that phosphorylates \(\beta\)-catenin, and targets it for ubiquitination and proteolytic degradation in the inactive canonical Wnt/\(\beta\)-catenin signalling pathway (Frame & Cohen 2001). Following activation of Wnt/\(\beta\)-catenin signalling, GSK3\(\beta\) is inhibited by a poorly defined mechanism, thereby allowing the stabilisation and accumulation of \(\beta\)-catenin levels. GSK3 was originally identified as a key mediator of insulin signalling, and is now thought to be involved in many other signalling pathways with a diverse array of proposed substrates, including Tau, CREB, NFkB, MUC1, cyclin D1, MYC, NFAT and JUN (Frame & Cohen 2001, Grimes & Jope 2001). Multiple kinases have been implicated in the Ser\textsuperscript{9} and Ser\textsuperscript{21} phospho-inhibition of GSK3\(\beta\) and GSK3\(\alpha\) respectively, including the phosphatidylinositol 3-kinase (PI3K)\text{--}Akt/protein kinase B (PKB) signalling axis (such as that occurring in classical insulin signalling), p90RSK, PKC, protein kinase A (PKA), p70 S6 kinase and extracellular signal-regulated protein kinase (ERK) (Stambolic & Woodgett 1994, Frame & Cohen 2001, Grimes & Jope 2001). Multiple kinases have been implicated in the Ser\textsuperscript{9} and Ser\textsuperscript{21} phospho-inhibition of GSK3\(\beta\) within the Wnt/\(\beta\)-catenin pathway (Frame & Cohen 2001). However, a study using a mouse knock-in of a Ser\textsuperscript{9}Ala GSK3\(\beta\) mutation reported normal embryonic development, suggesting that the Ser\textsuperscript{9} phospho-inhibition of GSK3\(\beta\) is not implicated in canonical Wnt signalling (McManus et al. 2005). Both isoforms of GSK3 are probably important for Wnt signalling because deletion of one has no effect, as long as the other is present, possibly suggesting isoform redundancy (Doble & Woodgett 2003). Furthermore, in the mouse knock-in analysis where the Ser\textsuperscript{9/21} residues of GSK3\(\beta/\alpha\) respectively were mutated to alanine residues, normal \(\beta\)-catenin accumulation was observed in response to Wnt3a stimulation, again confirming that Ser\textsuperscript{9} phosphorylation is not the mechanism for inhibiting GSK3\(\beta\) in Wnt/\(\beta\)-catenin signalling (McManus et al. 2005). In addition, mutational studies of the ‘insulin pool’ of GSK3 suggest that insulin-induced Ser\textsuperscript{9/21} phospho-inhibition is not involved in the activation of the Wnt signalling pathway, and that Wnt signalling does not alter the glycogen synthase output of insulin signalling (Ding et al. 2000, Ng et al. 2009). The ‘insulin’ and Wnt pools of GSK3\(\beta\) may therefore be considered to be functionally distinct.

A number of theories have arisen regarding the mechanism involved in GSK3\(\beta\) inhibition in the Wnt/\(\beta\)-catenin pathway. Some involve the GSK3\(\beta\)-binding protein called frequently rearranged in advanced T-cell lymphoma (FRAT), which can block GSK3-induced phosphorylation of \(\beta\)-catenin without affecting glycogen synthase activity in the insulin signalling pathway (Thomas et al. 1999). Other theories involve the essential Wnt pathway protein dishevelled (Huelsken & Behrens 2002, van Es et al. 2003, Moon et al. 2004). It has also been demonstrated that Wnt signalling inhibits GSK3 through a PKC-mediated mechanism (Cook et al. 1996), suggesting that PKC may have a role in inhibiting GSK3 within the canonical Wnt/\(\beta\)-catenin pathway, in addition to the phospho-inhibition at Ser\textsuperscript{9/21} such as in classical insulin signalling. CK1 can enhance GSK3 activity by acting as a priming kinase. However, CK1 also has an essential role in positively transducing the canonical Wnt signal, suggesting its involvement in GSK3\(\beta\) inhibition (Doble & Woodgett 2003). Alternatively, different CK1 isoforms may positively and negatively regulate GSK3\(\beta\) activity. Interestingly, GSK3\(\beta\) not only has been implicated in canonical Wnt/\(\beta\)-catenin signalling, but may also play a negative regulatory role in the non-canonical Wnt/Ca\textsuperscript{2+} pathway, in which it is thought to function as a nuclear export kinase, thus terminating NFAT transcriptional activity (Crabtree & Olson 2002, van Es et al. 2003, Katoh & Katoh 2007).

The FZD family of receptors are seven-transmembrane-spanning receptors that resemble G protein coupled receptor (GPCRs), and are now listed by the International Union of Pharmacology as a novel and separate family of GPCRs, the ‘Class FZD’ (Schulte & Bryja 2007). One of the first demonstrations that FZDs signal via G-protein coupling came from the early studies by Malbon et al. (Liu et al. 1999, 2001). It was demonstrated that \(\beta\)\(\beta\)-adrenergic receptor/FZD1 chimera bearing the cytoplasmic domains of rat FZD1 was able to stimulate \(\beta\)-catenin stabilisation and \(\beta\)-catenin/TCF transcriptional activity in response to isoprenaline stimulation, and that this was inhibited when certain \(\alpha\)-protein \(\alpha\)-subunits (\(\alpha_{gz}\) and \(\alpha_{go}\)) were depleted by antisense RNA, or by pertussis toxin pretreatment (Liu et al. 1999, 2001). Importantly, these and subsequent studies appeared to suggest that non-FZD GPCRs could target \(\beta\)-catenin activity in response to stimulation by their cognate ligands (Malbon 2005).
Targeting Wnt signalling mediators by non-FZD GPCRs

A number of non-FZD GPCRs have been shown to target β-catenin/TCF activity in response to their cognate ligands, including the prostanoid receptors (Fujino & Regan 2001, Fujino et al. 2002), M1 muscarinic acetylcholine receptor (Farias et al. 2004), lysophosphatidic acid (LPA) receptor (Yang et al. 2005) and thromboxane A2/TPa receptor (Yan & Tai 2006). The first demonstration of non-FZD targeting of β-catenin/TCF-dependent signalling was through the FPa prostanoid receptor (Fujino & Regan 2001). Stimulation of FPa-expressing cells with prostaglandin F2α (PGF2α) led to reorganisation of β-catenin, a decrease in phosphorylation of cytoplasmic β-catenin and activation of β-catenin/TCF-dependent transcription. In addition to these findings, it was demonstrated that activation of the EP2 and EP4 prostanoid receptors by PGE2 promoted β-catenin/TCF-dependent transcription (Fujino et al. 2002). In addition, the EP2 and EP4 receptors were demonstrated to target Ser9 GSK3β phospho-inhibition by a PKA-dependent mechanism (Fujino et al. 2002). The M1 muscarinic acetylcholine receptor was demonstrated to inhibit GSK3β activity, stabilise β-catenin levels and induce the expression of the Wnt target genes en-1 and cycD1 resulting in the protection of neurons from amyloid-β-peptide neurotoxicity in a rodent model of Alzheimer’s disease (Farias et al. 2004). The LPA receptor was demonstrated to target β-catenin to induce colon cancer cell proliferation (Yang et al. 2005). LPA was shown to promote the nuclear translocation of β-catenin, activation of β-catenin/TCF-dependent transcription, phospho-inhibition of GSK3β and activation of β-catenin/TCF target genes. Furthermore, all these LPA-induced events were apparently dependent on conventional PKC activity (Yang et al. 2005). The thromboxane A2/TPa receptor was demonstrated to activate β-catenin/TCF-dependent transcription apparently through the phospho-inhibition of GSK3β (Yan & Tai 2006; Fig. 1).

The GnRH receptor targets GSK3β phospho-inhibition and β-catenin/TCF transcriptional activity

The above studies suggested that further cross-talk between signalling mediators of the Wnt/β-catenin pathway and those activated by GPCRs is likely. Indeed, several recent studies have demonstrated that GnRH, acting at the GnRH receptor, can target mediators of the Wnt/β-catenin signalling pathway in both a heterologous HEK293 model cell line and the L1βT2 gonadotrope cell line (Gardner et al. 2007, Salisbury et al. 2007, 2008, 2009, Gardner & Pawson 2009).

The initial studies reported GnRH-mediated nuclear accumulation of β-catenin, activation of β-catenin/TCF-dependent transcription (using a TCF-dependent luciferase reporter plasmid) and the up-regulation of several β-catenin/TCF target genes including Jun, Fra1 and Myc (Gardner et al. 2007). In addition, it was demonstrated that GnRH targets Ser9 GSK3β phospho-inhibition (Gardner et al. 2007). As discussed above, the accumulation of β-catenin in response to GnRH may be independent of the Ser9 phospho-inhibition of GSK3β since the Wnt pool of GSK3β is probably functionally

Figure 1 Targeting Wnt signalling mediators by GnRH in gonadotropes. GnRH activates PLC-β via coupling to Gq11, resulting in the hydrolysis of membrane-bound phosphatidylinositol 4,5-bisphosphate into inositol 1,4,5-triphosphate (InsP3) and diacylglycerol (DAG), which respectively mobilise intracellular Ca2+ and activate protein kinase C (PKC). GnRH targets Ser9 phospho-inhibition of GSK3β through a PKC-mediated mechanism, which may in part be responsible for the stabilisation and accumulation of β-catenin levels. β-catenin translocates to the nucleus where it acts as a co-factor to TCF, to increase the transcription of TCF target genes, including Fra1, Jun and Myc. β-catenin also interacts with SF1, which together with other DNA-binding proteins (including EGR1, Jun and ATF3) increases transcription of Lhb, and possibly of Cga, Fshr and Gntrh. In addition, β-catenin may act as a co-factor to regulate members of the FoxO family and the androgen receptor (AR). GSK3β may target the activities of other transcription factors in gonadotropes, including CREB and NFAT, which may therefore be subject to an additional level of regulation by GnRH, since GnRH inhibits GSK3β activity. See text for details.
distinct from the pool of GSK3β that GnRH targets. The mechanism whereby GnRH targets β-catenin accumulation and Ser9 GSK3β phospho-inhibition was shown to be mediated via Gq/11 coupling, and is most likely PKC dependent (Gardner et al. 2007). Furthermore, GnRH does not signal through PI3K-Akt/PKB to target GSK3β phospho-inhibition, as is the case in classical insulin signalling, since pharmacological inhibition of PI3K failed to block β-catenin/TCF transcriptional activity in response to GnRH (Gardner et al. 2007).

Apart from its role as a TCF co-factor in the canonical Wnt signalling pathway, β-catenin can function as a co-factor to a number of other transcription factors relevant to gonadotrope biology, including SF1 and JUN (Angel et al. 1988, Desclozeaux et al. 2002, Shah et al. 2002, Gummow et al. 2003, Mizusaki et al. 2003, Parakh et al. 2006). Thus, the ability of GnRH to stimulate the expression of β-catenin/TCF target genes suggests that SF1- and JUN-responsive genes may also be targets of GnRH-dependent β-catenin/TCF transcriptional activity. This is because functional cross-talk between SF1, JUN and β-catenin/TCF signalling has been demonstrated in several studies (Shah et al. 2002, Gummow et al. 2003, Mizusaki et al. 2003, Veeman et al. 2003, Le Floch et al. 2005). Furthermore, β-catenin acts as a co-factor of SF1 through a direct interaction (Gummow et al. 2003, Mizusaki et al. 2003, Parakh et al. 2006), while JUN can interact co-operatively with TCF in the β-catenin/TCF complex at JUN promoter sites (Nateri et al. 2005).

**Targeting β-catenin/TCF transcriptional activity in gonadotropes**

The ability of GnRH to stimulate the expression of β-catenin/TCF target genes, and the finding that both SF1- and JUN-responsive genes may also be targets of GnRH-dependent β-catenin/TCF transcriptional activity, has important implications for gonadotrope function, including the expression of Lhb, Cga, Fshb and Gnhr genes. An important study highlighting the role of β-catenin as a member of a transcription factor complex that drives maximal activity of the Lhb subunit promoter in response to GnRH was published (Salisbury et al. 2007). This study by Nilson et al. demonstrates the co-localisation of β-catenin with SF1 and EGR1 on the promoter of the Lhb subunit gene in response to GnRH, and suggests that endogenous SF1 and β-catenin can physically associate in LβT2 cells (Salisbury et al. 2007). A role for GnRH targeting of β-catenin/TCF activity to regulate the expression of JUN-responsive genes including Cga, Fshb and Gnhr, which additionally require SF1, is yet to be demonstrated. What is clear though is that β-catenin has an important role to play, and that it regulates gonadotrope responsiveness to GnRH.

**Concluding remarks and future direction**

The ability of GnRH to impinge on the activity of Wnt signalling mediators has several implications for further understanding key processes in gonadotrope biology. For example, CREB has a well-known role in regulating Gnrhr expression by targeting CRE sites within the Gnrhr promoter in response to GnRH stimulation (Cheng & Leung 2001, Maya-Nunez & Conn 2001). GSK3 has been proposed as one of a number of kinases that regulate CREB activity (Frame & Cohen 2001, Grimes & Jope 2001, Doble & Woodgett 2003). Thus, by promoting the Ser9 phospho-inhibition of GSK3β, GnRH signal transduction may provide an additional level of complexity in the regulation of CREB activity and its ability to function optimally at the Gnrhr promoter. GSK3 can also target the activity of the Ca2+-sensitive transcription factor NFAT by functioning as a nuclear export kinase, thereby terminating NFAT transcriptional activity (Crabtree & Olson 2002). GnRH has been shown to mediate the derepression of the Fshb gene in the αT3-1 gonadotrope cell line through the activation of the Ca2+/CaN pathway leading to NFAT-driven expression of Nur77 (Lim et al. 2007). Furthermore, a recent study has reported that GnRH-mediated Ca2+/NFAT signalling does not act as the GnRH pulse frequency decoder in LβT2 cells (Armstrong et al. 2009). It will be interesting to determine if there is a role for GnRH-mediated Ser9 phospho-inhibition of GSK3β in regulating NFAT nuclear residency and how this impacts on gonadotrope function with regard to these studies. As a multi-functional kinase with a diverse array of proposed targets, it is likely that many more roles for GSK3 in the gonadotropes will emerge.

Additional roles for β-catenin are also likely, including the regulation of androgen receptor (AR) activity. As an AR co-factor, β-catenin functions to both inhibit and stimulate AR target gene expression in a variety of cell types and tissues (Chesire & Isaacs 2003, Mulholland et al. 2005, Terry et al. 2006, Robinson et al. 2008). Expression of Cga (α-polypeptide glycoprotein hormones), Lhb and Fshb subunit gene is widely reported to involve a component of androgen regulation in gonadotropes; however, possible roles for β-catenin in modulating AR activity at this level (in response to GnRH) have not been reported yet (Curtin et al. 2001, Jorgensen & Nilson 2001a,b, Curtin et al. 2004, Spady et al. 2004, Thackray et al. 2006, Burger et al. 2007, Thackray & Mellon 2008). Furthermore, roles for FOXO transcription factors, which employ β-catenin as a co-factor, have been proposed (Essers et al. 2005, Malbon 2005, Hoogeboom et al. 2008, Jin et al. 2008, Hoogeboom & Burgering 2009, Stavrou et al. 2009). Although clearly speculative, elucidating potential roles for GSK3β and β-catenin would
enhance our understanding of the complexity of the regulation of gonadotropin subunit expression and gonadotrope function.

In conclusion, this review has highlighted both published and putative roles for mediators of the Wnt signalling pathways in targeting important biological processes in gonadotrope biology. We suggest that new avenues of research will continue to emerge in order to advance our understanding of the targeting of Wnt signalling mediators by GnRH to in turn regulate gonadotrope function. Indeed, such studies are already well underway.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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