Molecular and Cellular Endocrinology 615 (2026) 112735

Contents lists available at ScienceDirect

Molecular and Cellular Endocrinology

journal homepage: www.elsevier.com/locate/mce

The implementation of small molecule agonists and antagonists to elucidate gonadotropin receptor structure, function and physiology☆

James A. Dias [a], Claire L. Newton [b,c], Alfredo Ulloa-Aguirre [d,*]

[a] Department of Biomedical Sciences, State University of New York at Albany, Albany, NY, USA
[b] Centre for Neuroendocrinology, Department of Immunology, Faculty of Health Sciences, University of Pretoria, Private Bag X323, Gezina, Pretoria, 0031, South Africa
[c] Deanery of Biomedical Sciences, University of Edinburgh, Edinburgh, EH8 9JZ, UK
[d] Red de Apoyo a la Investigación, Universidad Nacional Autónoma de México-Instituto Nacional de Ciencias Médicas y Nutrición Salvador Zubirán, Mexico City, Mexico

ARTICLE INFO

Keywords:
Gonadotropins
Gonadotropin receptors
GPCRs
Small molecule agonists
Small molecule antagonists
Allosteric modulators

ABSTRACT

Pursuant to patient desires of alternatives to injectable gonadotropins, a plethora of attempts have identified, characterized, and demonstrated efficacy of small molecules that activate (agonists) gonadotropin receptors. Discoveries have also been made of small molecule gonadotropin receptor inhibitors (antagonists), which have potential as useful alternatives to steroid hormone-based contraception. Implementation of these small molecules in advanced testing systems not necessarily used in screening, which identified lead compounds, has yielded a bounty of wonders. It is likely that a richer understanding of the role of signaling platforms and conformation-dependent molecular assemblies are likely to emerge. Several small molecule agonists have been observed to function as conformational boosters that can rescue receptor trafficking defects, or initiate internalization of receptors without bound hormone. Still others have revealed insights into the role of molecular platforms in persistent signaling. Unexpectedly, such antagonists, like molecular scalpels, can ablate certain signaling pathways and not others leading to discovery of biased signaling in gonadotropin receptors. That seminal observation has led to studies of nuanced signaling and, consequently, nuanced gene expression. Gonadotropin receptor structure-based design for better specificity and potency of agonists and antagonists has been provided by new cryo-EM structures of the gonadotropin receptors, demonstrating proof of concept. Structural determination of downstream supramolecular assemblies will be necessary to validate and fully understand these complicated receptors and how their interaction with other proteins and when occupied by hormone and allosteric modulators, nuances their actions and, ultimately, fertility.

1. Introduction

Components of the hypothalamic-pituitary-gonadal axis control reproductive function. These components communicate with each other through distinct endocrine signals, which include the pituitary gonadotropins, follicle-stimulating hormone (follitropin; FSH), and luteinizing hormone (lutropin; LH). FSH and LH are released into the circulation by gonadotropes of the anterior pituitary in response to the actions of hypothalamic gonadotropin-releasing hormone (GnRH). Together with thyroid-stimulating hormone (thyrotropin; TSH) synthesized by the thyrotropes of the pituitary, and choriogonadotropin (CG; produced by the placental syncytiotrophoblasts), they comprise the glycoprotein hormone family (GPH) (Ulloa-Aguirre et al., 2017). The GPHs are large heterodimeric proteins that comprise a common α subunit in combination with a hormone-specific β subunit. The principal sites of action of the gonadotropins are the gonads. In women, FSH binds to FSH receptors (FSHRs) located on the surface of granulosa cells of developing ovarian follicles, where it promotes estrogen synthesis and the transition from pre-antral to antral follicles (Richards and Pangas, 2010), whereas LH binds to LHCG receptors (LHCGRs) to stimulate androgen production by the theca cells, as well as ovulation. CG also binds to LHCGR, present in luteinized granulosa cells that comprise the corpus luteum, stimulating progesterone production, which maintains early pregnancy. In men, FSH activates FSHRs resident on Sertoli cells lining the

☆ Given his/her/their role as (James Dias, (Editor) had no involvement in the peer-review of this article and has no access to information regarding its peer-review. Full responsibility for the editorial process for this article was delegated to another journal editor.

* Corresponding author.
E-mail address: aulloaa@unam.mx (A. Ulloa-Aguirre).

https://doi.org/10.1016/j.mce.2026.112735
Received 9 December 2025; Received in revised form 12 January 2026; Accepted 14 January 2026
Available online 20 January 2026

seminiferous tubules, where it plays an important role in supporting spermatogenesis (Huhtaniemi, 2015; Oduwole et al., 2021; Ulloa-Aguirre et al., 2018), whereas LH binds LHCGRs on Leydig cells present in the interstitial or stromal testis to primarily stimulate testosterone production, critically important for high-quality spermatogenesis (Huhtaniemi, 2015; Oduwole et al., 2021; Ulloa-Aguirre et al., 2018).

As mentioned above, gonadotropins specifically bind their cognate receptors located in the gonads. The gonadotropin receptors, as well as the thyroid-stimulating hormone receptor (TSHR), are G protein-coupled receptors (GPCRs) that comprise a glycoprotein hormone receptor (GPHR) cluster within the δ-group of the highly conserved and expansive Rhodopsin-like GPCR subfamily (Fredriksson et al., 2003). All GPCRs are characterized by a transmembrane (TM) domain (TMD) that consists of seven TM helices joined by intra- and extracellular loops. In addition to the TMD, the GPHRs exhibit a large extracellular domain (ECD) containing several leucine-rich repeats (LRRs), where recognition and specific binding of the corresponding glycoprotein hormones occur (Ulloa-Aguirre et al., 2018; Jiang et al., 1995, 2014a; Smits et al., 2003). This extracellular ectodomain is structurally linked to the TMD of the receptor by a hinge region (extended hinge loop) (Agrawal and Dighe, 2009; Mueller et al., 2009), which is involved in the activation of the receptor through transduction of ligand binding at the ECD to activation of the TMD (Duan et al., 2023a; Goodman et al., 1997; Jiang et al., 2012, 2014b). These receptors terminate in an intracellular COOH-terminus (or C-tail), which contains several motifs involved in internalization and other receptor functions (Ayoub et al., 2015; Banerjee and Mahale, 2015; Cassier et al., 2017; Ulloa-Aguirre and Zarinan, 2016). In addition to activation of the well-known canonical Gs/adenylyl cyclase/cAMP/-protein kinase A (PKA) signaling cascade, gonadotropin receptor activation has been linked to other Gs-independent signaling pathways. Indeed, there is a complexity of signaling cascades, grouped as signaling modules, activated by the agonist-occupied gonadotropin receptors. Furthermore, biased signaling in response to ligands and/or receptor modifications are now well-documented mechanisms whereby gonadotropin receptors activate, in a selective manner, signaling modules (Ulloa-Aguirre et al., 2011, 2025a).

Considering their key roles in reproductive function, exogenous gonadotropins are commonly administered therapeutically in assisted reproduction and for the treatment of reproductive dysfunction. Several reviews have covered progress made in development of alternatives to injectable gonadotropins which spawned a plethora of attempts to identify, characterize and demonstrate efficacy of non-peptidic small molecules that activate gonadotropin receptors (Lazzaretti et al., 2023; Nataraja et al., 2018; Anderson et al., 2018). Small molecules, so called because they have on average, a molecular weight of less than five hundred Daltons, must be able to pass the gut-blood barrier to be useful orally. Many different compounds comprising a range of chemical scaffolds and with varying receptor activities have been identified. Interestingly, the majority of these are “allosteric” compounds, that is, they interact with the receptors at sites distinct from the (orthosteric) native hormone-binding sites within the ECD (Anderson et al., 2018). Thus, by definition, an allosteric site cannot be an orthosteric site. It is not yet known if the glycoprotein hormone receptor allosteric agonists and antagonists bind to common or distinct sites. Agonists which bind to TSHR, LHR and FSHR bind to a common site as revealed by cryoEM structures as will be discussed below (van Koppen et al., 2013; Yu et al., 2014).

Allosteric GPCR ligands typically fall into three categories: allosteric agonists, which have activity in the absence of the native ligand and which can be full or partial eliciting equivalent or lesser responses in comparison to native ligand, respectively; and positive or negative allosteric modulators (PAMs and NAMs), which either potentiate or attenuate agonist-mediated responses, respectively, through modulation of orthosteric agonist binding affinity or the ability of the agonist-occupied receptor to interact with intracellular signal transducers. Furthermore, some small molecules are fully active in the absence of gonadotropin but also augment FSH actions (van Koppen et al., 2013), while still others, like some substituted benzamide agonists, are not fully active without the presence of FSH (Yu et al., 2014). Others, such as certain thiazolidinone compounds, can be fully active agonists (Sriraman et al., 2014) or turned into inhibitors with a single substitution (Nataraja et al., 2018; Sposini et al., 2020).

These endeavors sadly, to date, have yielded no small molecule gonadotropin receptor-targeted therapies, approved by the Federal Drug Administration (USA) for induction of ovarian follicle growth and ovulation or, conversely, for contraception. However, when made available to the scientific community, use of these compounds has facilitated a greater understanding of the structure and function of gonadotropin receptors that, in turn, may feed forward into identification of new targets for therapies. In this regard, some gonadotropin receptor antagonists, like molecular scalpels, have been found to ablate only certain receptor functions leading to discovery of biased signaling in gonadotropin receptors (Dias et al., 2010a, 2014; Ayoub et al., 2016). Similarly, some agonists have been demonstrated to exhibit biased activation of gonadotropin receptor signaling modalities (Hanyroup et al., 2021; Ulloa-Aguirre et al., 2025b). Such proof of biased agonism has rationalized studies of nuanced intracellular signaling and gene expression (Ulloa-Aguirre et al., 2025a; Zarinan et al., 2020; Hernandez-Ramírez et al., 2022). Some agonists have also been used as conformational boosters that can rescue trafficking defects in gonadotropin receptors harboring genetic variants, and even unveil constitutive activity of other misfolded variant receptors such as has been noted for the GnRH and vasopressin receptors (Ulloa-Aguirre et al., 2018, 2025c; Conn and Ulloa-Aguirre, 2010; Janovick et al., 2009; Ulloa-Aguirre and Conn, 2016; Hanyroup et al., 2021; Newton et al., 2011). Still others have revealed insights into the role of molecular platforms in persistent signaling of gonadotropin receptors (Sposini and Hanyaloglu, 2017).

Determination of gonadotropin receptor structures has given hope for gonadotropin receptor structure-based ligand design for better specificity and potency (Duan et al., 2021, 2023b). Continued implementation of these, and future gonadotropin receptor small molecules in experiments designed to understand the role of platforms and conformation-dependent molecular assemblies that impact fertility is likely to lead to new knowledge. Progress in these areas will be dependent on the access to existing and newly developed agonists and antagonists of gonadotropin receptors and of fuller understanding of the cellular processes that have been identified that are downstream of the initial binding event and are time- and space-dependent (Casarini et al., 2020). Ultimately, structural determination of these downstream supramolecular assemblies will be necessary to validate and fully understand these complicated receptors and how their interaction with other proteins nuances their actions and, consequently, fertility.

To unpack the foregoing, each of the steps of gonadotropin action are presented in a frame of how small molecule allosteric modulators of gonadotropin receptors have enhanced our understanding or have been utilized to explore gonadotropin and gonadotropin receptor structure and function.

2. Hormone-receptor interaction

An important aspect of gonadotropin receptor activity rests in their ability to bind FSH, LH and/or hCG. Allosteric inhibitors of FSHR and LHCGR have been described that either inhibit (van Straten et al., 2005) or negatively regulate (Bonger et al., 2009; Wortmann et al., 2019) gonadotropin action at FSHR or LHCGR respectively. Typically, agonists and antagonists were believed to bind either to a gonadotropin binding site in the extracellular domain (orthosteric) or TMDs (allosteric). In the absence of crystal or cryo-EM structures, these suppositions were based on the observations that some small molecule antagonists of FSHR, for example, have been shown to inhibit ^{125}I-human (h)FSH binding to receptor in a non-competitive manner (Arey et al., 2002) as well as showing competitive inhibition (van Straten et al., 2005; Wrobel et al.,

2002). Data demonstrating modulation of hormone binding by allosteric inhibitors, collected using radioactive ligand binding equilibrium assays, first indicated that long range interactions of the TMD could modulate gonadotropin binding at the ECD (Jiang et al., 2014b).

Early attempts to purify detergent-solubilized full length bovine FSHR required high levels of glycerol to prevent the inevitable total loss of FSH binding at 4 °C (Dias et al., 1981). Cloning of the gonadotropin receptor genes made it possible to determine the highaffinity binding site of the receptors, which not surprisingly turned out to be the ECD. Thus, it was clear early on that the ECD of the gonadotropin receptors are necessary and sufficient for formation of a high affinity interaction and stable hormone-receptor complex. Indeed, there was no requirement for the TM domains to stabilize ^{125}I-hFSH binding to the purified FSHR ECD when expressed in insect cells and secreted as a soluble protein (Jiang et al., 2012; Ryu et al., 1998; Fan and Hendrickson, 2005; Kene et al., 2005; Schmidt et al., 2001). This was a conundrum, but it suggested that in the absence of glycerol, when the receptor is detergent extracted from the membrane, it assumed a conformation that was not permissible for ^{125}I-hFSH binding. However, the ^{125}I-hormone-receptor complex preformed in membranes prior to detergent extraction was found to be stable (Dias and Reichert, 1982).

Indeed, a recent report that described the cryo-EM structure of FSHR (Duan et al., 2023b) employed a preformed hormone-receptor complex. In addition, the investigators took advantage of an FSHR small molecule agonist (compound 21f; cpd21f (Loozen and Timmers, 2010)) which, independent of FSH, stimulates FSHR, and was used to further stabilize the "active" FSHR structure (Fig. 1). cpd21f is described in patents but a detailed biochemical study of its effects on ^{125}I-FSH binding (other than a graph of the relative potency of cpd21 versus FSH) was not available in the patents nor the publications cited herein. This "active" FSHR protein used for cryo-EM analyses also embodied a constitutively active receptor mutation (S273I), and a tethered Gs-protein stabilized by a monoclonal antibody Nb35. It is likely that the small molecule helped to stabilize the transmembrane domains, contributing to the global resolution of 2.83 Å. The resultant agonist-bound structure provides new opportunities for design of more specific and potent FSHR agonists and antagonists and represents a major achievement in the field.

An unliganded (no FSH bound/"inactive") FSHR protein structure was also determined using FSHR purified in the presence of a Bayer antagonist, Compound 24 (cpd24) (Loozen and Timmers, 2010; Wortmann et al., 2008). Here again, neither the patent nor publication describing this compound provided a detailed biochemical study of ^{125}I-FSH binding in the presence of cpd24. Unfortunately, the resolution of the structure was only 6.01 Å, density of the small molecule was not visible in the structure, and only a polyalanine model was built. Nevertheless, a major positional difference of the ECD relative to the TMD was observed when the two different FSHR structures (active vs. "inactive") were compared. This led to the idea that a major conformational shift in the ECD relative to the TMD occurs when FSH binds to FSHR (Duan et al., 2023b).

A similar positional shift of the LHCGR ECD was seen with the cryoEM structure of two similar LHCGR experimental models (Duan et al., 2021): A constitutively active LHCGR S277I, with LH bound and with an LH small molecule agonist (Org43553) and a tethered Gs-protein with a resolution of 3.2 Å; and native LHCGR with no hormone bound, no tethered G-protein and with small molecule antagonist compound 26 (Wortmann et al., 2019) included in the purification process (again, not visualized in the structure) with a resolution of 3.8 Å (Duan et al., 2021, 2023b).

Based on these structural studies it was proposed that a weak collisional complex is formed with the C-terminal portion of the extended hinge loop of the receptors that interacts with the "far-right tip of the hormone". This interaction is proposed to "push" the ECD upwards to allow full engagement of the ligand with a highly conserved ten-residue section of the C-terminus of the hinge region (P10) (Bruser et al., 2016). There is additional early data which may lend support to this notion,

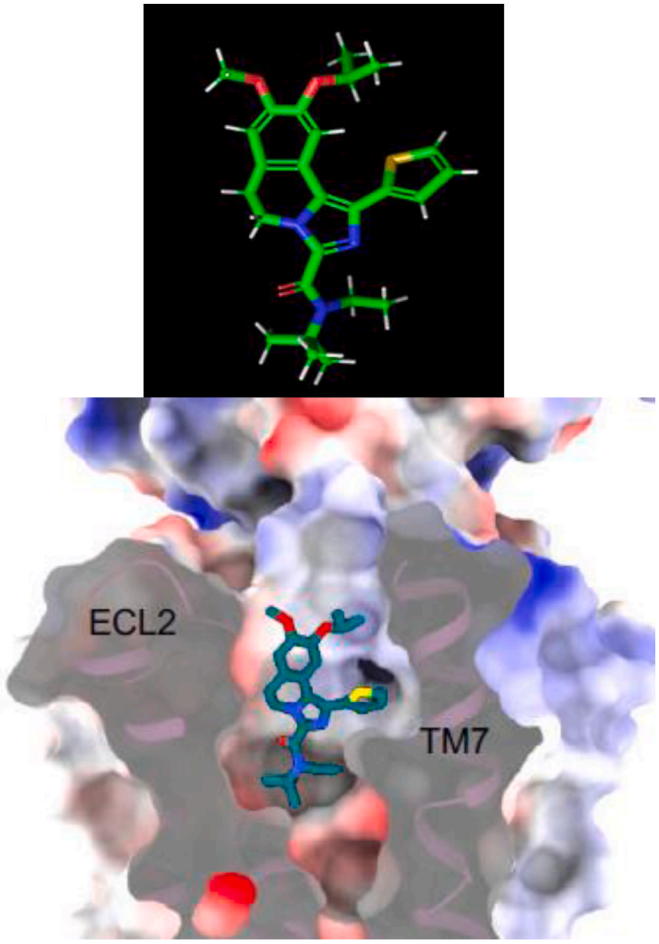


Fig. 1. a. Organon Cpd-21f agonist of FSHR used to stabilize the active FSHR structure for cryoEM. **b.**The binding site completely overlaps with LHCGR binding site of small molecule ligand agonist ORG43553. Conserved mechanism of action at the gonadotropin receptors involving M585 ($M^{6.48}$; amino acid numbering according to Ballesteros and Weinstein (Ballesteros and Weinstein, 1995)); H615 ($H^{7.42}$) in FSHR played an essential role determining FSHR ligand selectivity, providing new insights for designing more specific small molecule agonists of FSHR such as ORG21444-0 [figure taken from Duan et al. (Duan et al., 2023b) with permission from http://creativecommons.org/licenses/by/4.0/].

garnered using not small molecules but small synthetic peptides. Apropos of this, synthetic peptides corresponding to segments of the entire FSHR ECD were prepared and evaluated for their activity regarding FSHR stimulation or inhibition of ^{125}I-hFSH binding. A single peptide FSHR-221-252, which comprises most of the leucine-rich repeat 10 and the end of leucine-rich repeat 9, was identified that inhibited ^{125}I-hFSH binding to FSHR (Mahale et al., 2001). Peptide FSHR-221-252 represents an FSHR locality that borders the hinge region of the FSHR ECD in the cryoEM structure (Fig. 2). The peptide's mechanism of inhibitory action has yet to be determined, but is presumed to induce an allosteric effect. Indeed, it comprises the penultimate C-terminal end of the FSHR ECD, which has been proposed to form a weak collisional complex with FSH (and with hCG in the case of the LHCGR) (Fig. 2). Of interest, polyclonal antibodies against the peptide hFSHR-221-252 did not inhibit ^{125}I-hFSH binding to membrane-bound receptor but did inhibit ^{125}I-hFSH binding to detergent solubilized receptor suggesting that the epitope is metastable or inaccessible in the membrane (Mahale et al., 2001). Whether this is due to oligomerization of the FSHR in cell membranes, or attributable to other factors is not known. In any case, the use of small synthetic peptides is an additional approach to elucidate or generate proposals about the initial interaction of hormones with the

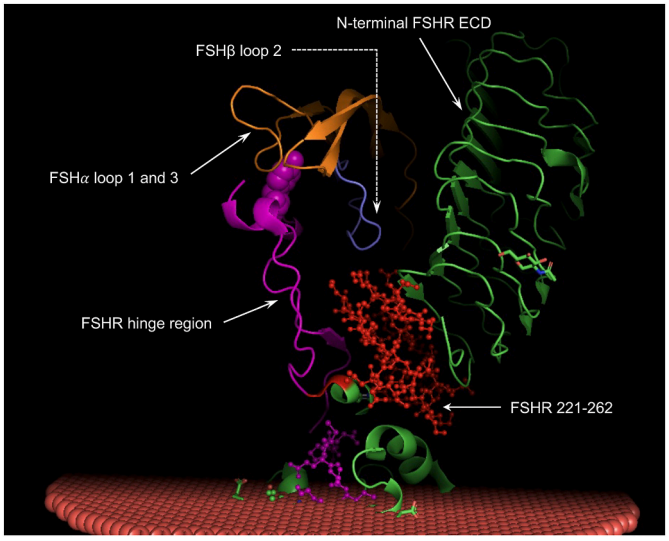


Fig. 2. An image of the active structure of FSHR showing the 221–262 sequence (red ball and stick) which is situated between the rest of the N-terminal FSHR ECD (green) and the FSHR hinge region with the sulfated tyrosine (Y335) in space filling mode (magenta ribbon). FSH**α** loop 1 and 3 is in orange and FSH**β** loop 2 is in blue. The cryo EM structures of the two FSHR experimental models evidence a major spatial difference of the 221–262 peptide sequence between the hormone occupied, Gs stabilized, and Organon compound 21f activated receptor compared to the compound 24 inhibited receptor (Duan et al., 2023b). Taken together with the data discussed hererin, it suggests that the mechanism by which peptide FSHR 221–252 may inhibit FSH binding to FSHR is by virtue of its ability to interact with the FSHR ECD itself. In theory this would restrain the spatial relationship of the ECD to the TM domains preventing a positional shift involving the sulfo-tyrosine 335 and FSH itself triggering hinge region shift and activation as confirmed in the cryo EM structure (Duan et al., 2023b). If this can be confirmed structurally, this may provide a targeted contraceptive therapy against the 221–252 region. Representation prepared with PYMOL, using the PDB structure 812G.

receptor. At the very least, those data suggested that design of stable peptides may provide an FSHR-based contraceptive effect.

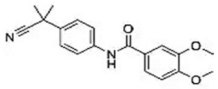
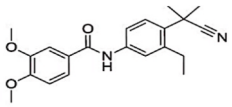
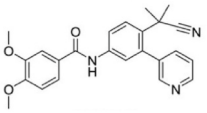
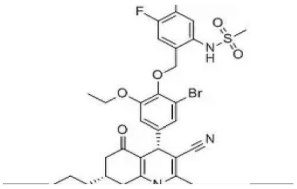
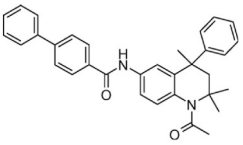
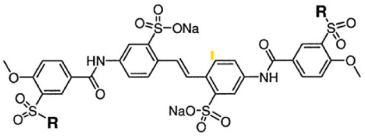
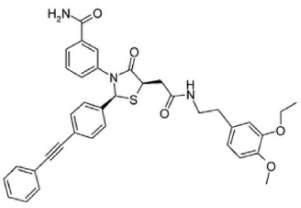
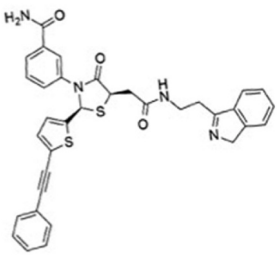
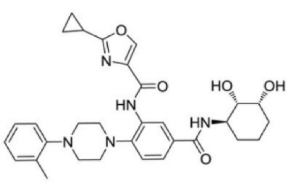
Since neither FSH nor LH can be modelled into either the unliganded FSHR nor unliganded LHCGR cryo-EM structures, respectively, without the carbohydrate or protein on the beta subunit clashing with the membrane, it isn't clear how hormone binding might occur with the "inactive" cryo-EM structure, but would seem entirely reasonable with the "active" structures. Therefore, it seems feasible to consider that the unliganded FSHR cryoEM structure may not accurately represent the native unliganded FSHR, which is active (binds FSH) but is not activated (binds FSH and induces signal transduction). There are certain data which may clarify, and are therefore desirable: First, the effects of cpd-24 on ^{125}I-FSH binding to membrane bound FSHR as well as solubilized FSHR; second, the stability of detergent solubilized FSHR (e.g. does FSH bind unliganded FSHR prepared for cryo-EM?); third, biochemical evidence regarding the effect of cpd-24 on FSH binding, its effect on activation of FSHR, and/or on FSH induced steroidogenesis; fourth, visualization of cpd-24 in the structure to assure the receptor can be classified as inactivated (and to clarify whether small molecule antagonists bind to the allosteric agonist binding pocket) (Duan et al., 2023b). Pertinently, despite no need for the TM domains to stabilize ^{125}I-hFSH binding to the purified FSHR ECD expressed in insect cells and secreted as a soluble protein (Schmidt et al., 2001), a small molecule FSHR allosteric modulator from Addex Therapeutics (San Francisco CA, USA) (benzamide based FSHR antagonist NAM ADX61623)(Table 1) actually increased ^{125}I-hFSH binding when incubated with HEK293 cells expressing native FSHR, despite it inhibiting FSH-induced cAMP production (Dias et al., 2011). At face value, since the ECDs of the two cryo-EM "inactive" structures (FSHR and LHCGR) do not appear to be able to bind FSH or LH/hCG and since the Addex FSHR small molecule does not inhibit FSH binding but rather increases FSH binding, these data suggest that the unliganded cryo-EM FSHR structure has not captured the unliganded native form which can bind FSH, or its FSH- and Addex small molecule antagonist bound form (which notably, as discussed below, is permissive for estradiol production only). An experimental structure of FSH-bound FSHR in the presence of Addex small molecule inhibitors of FSHR activation for cryo-EM studies may resolve some of these enigmas and provide a platform for further development of high affinity FSHR inhibitors.

Like the Addex small molecule FSHR antagonist ADX61623 (discussed above), the FSHR agonist Org214444-0 (Table 1) also induced an increase in ^{125}I-hFSH binding to both exogenously expressed FSHR on hinese hamster ovary (CHO) cell membrane preparations as well as to membrane preparations from human granulosa cells. That these effects were observed in membrane preparations confirm that they are not due to cellular trafficking of the receptor and, further indicate that perturbation of the TM domains by small molecule binding could induce long-range interactions that modulate gonadotropin binding within the ECD (van Koppen et al., 2013). It is, however, interesting to note that other FSHR small molecule agonists (such as the thiazolidinone derivative, T1) do not affect FSH binding (Arey et al., 2008; Maclean et al., 2004; Yanofsky et al., 2006). The mechanism underlying the increase of FSH binding remains unclear and will likely remain so until cryo-EM structures of these small molecules bound to FSHR monomers as well as dimers, are determined.

Early on then, small molecule effects of increasing binding of FSH to FSHR indicated a potential conformational change or stabilization which was consistent with an increase in hormone affinity of the receptor (van Koppen et al., 2013; Dias et al., 2010a) as well as an increase in maximum binding capacity (van Koppen et al., 2013). However, subsequent experiments with additional FSHR small molecules testing effects on binding, coupled with crystallographic data of the complete ECD, did not reach the same conclusion that affinity was affected but rather that an increase in binding sites occurred, leading to a hypothesis that not all available receptors can bind FSH, until a perturbation occurs, revealing cryptic binding sites (Jiang et al., 2014b). It is interesting that both agonist and antagonist can increase FSH binding to its receptor, while the conformation stabilized by each ligand is likely unique to either promote or inhibit signaling events. Clearly, unraveling the molecular signaling mechanism of agonist vs antagonist would help to develop unique therapeutics with specific properties (Nataraja et al., 2018).

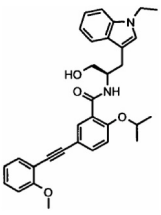
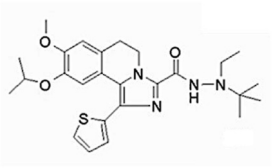
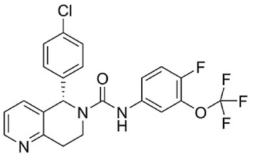
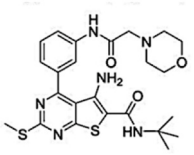
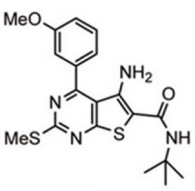
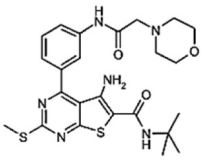
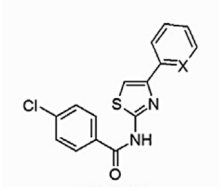
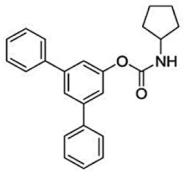
In summary, implementation of gonadotropin receptor-specific small molecule allosteric modulators which stabilize gonadotropin receptor proteins once removed from their membrane environment, have facilitated receptor structure determination using cryo-EM. Small molecule agonists have facilitated an understanding, at the atomic level, of the activated form of the receptors, which will serve to develop additional agonists through modelling with the new structures, to potentially develop new small molecules perhaps with better pharmacokinetic and/or nuanced pharmacodynamic properties. Observations of allosteric modulation of the FSHR using peptides derived from its extracellular domain suggest that further cryo-EM structure determinations that includethese peptides may yield additional therapeutics not based on small peptidomimetic molecules. Such peptide allosteric modulators provide a new class of modulators of GPCRs (Mannes et al., 2022). Indeed, this notion may apply to small peptides such as those derived from the FSHR extracellular domain that were reported to have agonist effects, amplifying FSH action *in vivo* (Prabhudesai et al., 2021) or antagonist effects (Mahale et al., 2001). There is a need for additional cryo-EM structures of FSHR, with ligand bound and with allosteric inhibitors (inactive), such as the Addex compounds. Such structures may point to key conformational changes that may provide targets for better biased small molecule intervention and contraceptive development.

Table 1
Chemical structures of small molecules discussed in the review, including their origins and some characteristics.

Organization	SMOL ID	Derivative Type	Chemical structure	Target	Effect[a]
Addex Ther.	ADX61623	Benzamide		FSHR	Antagonist IC_{50} 0.72uM HTRF assay HEK293-FSHR cAMP
Addex Ther.	ADX68693	Benzamide		FSHR	Antagonist IC_{50} 104 nM HTRF assay HEK293-FSHR
Addex Ther.	ADX68692	Benzamide		FSHR	Antagonist IC_{50} 140 nM HTRF assay HEK293-FSHR
Organon FSHR cryo-EM	214444–0	Hexahydro-quinoline		FSHR	Agonist EC_{50} 1.2 nM HEK293-FSHR CRE-luc
Organon	Antagonist 10	6-Amino-4-phenyltetrahydro-quinoline		FSHR	Antagonist IC_{50} 10 nM CHO-FSHR cAMP
Wyeth	Compound 1	(bis)sulfonic acid, (bis)benzamides		FSHR	Antagonist IC_{50} 7.0 uM CHO-FSHR cAMP
Wyeth/EMD Serono/ Tocophe Rx	Cmpd 1,5 T1	Thiazolidinone		FSHR	Agonist EC_{50} 80 nM CHO-FSHR cAMP
Wyeth/EMD Serono/ Tocophe Rx	Aka Cmpd 3 T2	Thiazolidinone		FSHR	Antagonist IC_{50} 1.7 uM Rat GC E_2
Tocophe Rx	Cmpd 9k	Benzamide		FSHR	Agonist EC_{50} 5 nM Rat GC cAMP

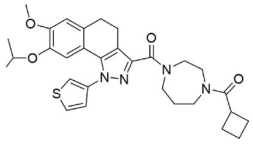
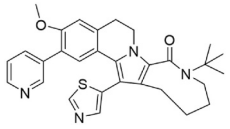
(*continued on next page*)

Table 1 (*continued*)

Organization	SMOL ID	Derivative Type	Chemical structure	Target	Effect[a]
Bayer Schering FSHR cryo-EM	Cmpd 24	1,2-Diarylacetylene Derivatives of Acyltryptophanols		FSHR	Antagonist IC_{50} 20 nM HTRF assay HEK293-FSHR
Organon FSHR cryo-EM	Compound 21f	(dihydro) imidazoiso (5, 1-a) quinolines		FSHR	Agonist EC_{50} 0.9 nM CHO-FSHR CRE-luc
Bayer LHR cryo-EM	Compound 26	Tetrahydro-1,6-naphthyridine		LHR	Antagonist IC_{50} 81 nM HEK293-LHR cAMP
Organonc ryo-EM LHR	ORG43553	thienopyrimidine		LHR	Agonist EC_{50} 34 nM HEK293-LHR cAMP
Organon	ORG41841	thienopyrimidine		LHR	Agonist EC_{50} 220 nM HEK293-LHR cAMP
Organon	ORG42599	thienopyrimidine		LHR	Agonist EC_{50} 7.5 nM HEK293-LHR cAMP
Schering	LUF5419	Thiazole		LHR	Agonist EC_{50} 23 uM Allosteric modulation of [^{3}H] Org43553 binding by LUF5419 Allosteric enhancer and stabilizer of ORG43553
Schering	LUF5771	Thiazole		LHR	Antagonist of ORG43553 2.3uM Weak agonist EC_{50} 1.6 uM B_{max} 1/3 hCG CHOK1-LHR

(*continued on next page*)

Table 1 (*continued*)

Organization	SMOL ID	Derivative Type	Chemical structure	Target	Effect[a]
Organon CanWell Pharma	CAN1404	Dihydro-benzoindazole		FSHR	Agonist EC_{50} 0.14 nM HTRF assay CHO-FSHR cAMP
CanWell Pharma	CAN1405	Dihydro-benzoindazole		FSHR	Agonist EC_{50} 0.035 nM CHO-FSHR cAMP

[a] Abbreviations: HTRF: homogenous time-resolved fluorescence; HEK293: Human embryonic-kidney 293 cells; CRE-Luc: cyclic AMP response element-luciferase; CHO: Chinese-hamster ovary cells; GC: granulosa cells; E_2: 17β-estradiol. Where indicated by "cryo-EM", the small molecules were implemented in an attempt to stabilize the receptors' conformations.

3. Receptor signaling

In contrast to stimulation by full agonists (balanced ligands), biased signaling (also referred to as functional selectivity) results in differential or imbalanced response(s), which are due to preferential activation of particular signaling pathway(s) at different levels along the downstream signaling cascades regulated by the activated receptor, eventually leading to distinct gene expression profiles (Ulloa-Aguirre et al., 2011, 2018; Zarinan et al., 2024; Zhan et al., 2024). The concept of biased signaling at the gonadotropin receptors, and in general at several GPCRs, is important as it represents a potential means for designing new therapeutic strategies focused on selective modulation of signaling and gene expression at different levels in the gonads, potentially useful in infertility treatment and contraception. Different ligands have been shown to promote biased signaling at the gonadotropin receptors. These include glycosylation variants of FSH (Ulloa-Aguirre et al., 2025a; Timossi et al., 2000) and, relevant to this review, small molecule ligands with agonist and antagonist activities, which have been invaluable in interrogating gonadotropin receptor signaling (Ulloa-Aguirre et al., 2025b).

Screening for first hits of extensive libraries may initially identify small molecules that activate or block canonical signaling, such as the Gs/cAMP/PKA pathway in the case of gonadotropin receptors. However, as introduced above, subsequent detailed biochemical studies implementing various small molecules have revealed nuances of effects on gonadotropin receptor function. For example, substitution of the Addex benzamide antagonist ADX61623 (Dias et al., 2011) yielded two additional FSHR antagonists (Dias et al., 2014). One substitution yielded a molecule ADX68692 which blocked both FSH-induced progesterone and estradiol biosynthesis and was more effective in blocking oocyte production in rats than ADX61623, which selectively blocked progesterone synthesis (Dias et al., 2014).

Despite having pharmacokinetic (PK) properties superior to ADX68692, and similar ability to block cAMP production, the second substituted molecule, ADX68693, had no effect on oocyte production in rats (Dias et al., 2014). This was the first evidence of biased antagonism of downstream steroidogenic pathways following FSH receptor activation and demonstrated an impact on physiological function. This remarkable observation led to the proposal that blocking estradiol production was necessary and sufficient for an orally bioavailable potential contraceptive to be viable, a reasonable hypothesis. These data also suggest that allosteric modulation of FSHR may differentially affect FSHR interactions with other potentially modifying, cell membrane resident, endosomal or cytosolic proteins that affect proliferation rather than differentiation pathways-the latter being generally driven by cAMP production. Transcriptomic studies employing these allosteric antagonists have shown that small modifications in small molecule negative allosteric modulators structure (e.g. ADX68692 and ADX68693) are sufficient to impact temporal gene expression and biological effects in granulosa cells exposed to FSH, likely related to cAMP-independent effects of FSH since both antagonists block cAMP (Hernandez-Ramírez et al., 2022).

Since most of the work to this point had been done in rats, human granulosa cells were used to confirm the dichotomy of these two compounds (unpublished). In those experiments, the biased antagonism observed when comparing ADX68692 and ADX68693 with ADX61623 was recapitulated. Thus, where ADX68692 blocked FSH-induced cAMP, progesterone and estradiol synthesis in human granulosa cells, ADX68693 blocked only cAMP and progesterone production, while sparing estradiol production. These observations suggested that FSH-FSHR biased signaling may involve other players and that their interaction with FSHR is perturbed by allosteric modulation of the receptor.

The theory that FSH-FSHR signaling may involve other signaling partners is consistent with studies concerning insulin-like growth factor-1 receptor (IGF-1R) in granulosa cells (Hayes et al., 2024). Inhibition of IGF-1R activity or expression using pharmacological, genetic, or biochemical approaches prevented the FSH-induced expression of steroidogenic genes and especially aromatase (CYP19), which is essential for estradiol production (Zhou et al., 2013). Direct activation of cAMP/PKA-mediated signaling with forskolin or dibutyryl cAMP alone barely induced CYP19 mRNA (consistent with earlier studies that demonstrated that, unlike FSH, constitutively active PKA does not fully activate aromatase gene expression (Escamilla-Hernandez et al., 2008), but significant gene expression was achieved when IGF-1 was added. However, blocking IGF-1R inhibited CYP19 expression by forskolin or the cAMP analog and IGF-1 alone did not induce CYP19 gene expression. These findings suggested that inhibition of IGF-1R blocked FSH-induction of other downstream signaling modalities (which subsequently were found to be linked to protein kinase B (Akt) phosphorylation) and demonstrate that, instead of a parallel synergistic interaction, FSH and IGF-I act in tandem. Notably, IGF-1R conditional knockout mice, where IGF-1R is only deleted in granulosa cells, are infertile, and FSH does not induce Akt phosphorylation or estradiol production (Baumgarten et al., 2017).

Based on the aforementioned studies from the Stocco lab (Hayes et al., 2024), IGF-1/IGF-1R and FSHR cross-talk appears to activate Akt signaling, which is important for estradiol production. Other mechanisms may also be involved in FSH-induced phosphoinositide 3-kinase (PI3K)/Akt pathway activation. For example, activation of extracellular signal-related kinases 1 and 2 (ERK1/2) via a β-arrestin-mediated pathway seemed a reasonable additional potential mechanism for activation of Akt signaling. However, inhibition of those kinases (directly or via inhibition of the mitogen-activated protein kinase MEK, which activates ERK1/2) did not block Akt phosphorylation, but, interestingly, did prevent FSH and FSH/IGF-I stimulation of Cyp19 expression as did inhibition of PI3K (Zhou et al., 2013; Donaubauer et al., 2016a).

Zeleznik and colleagues (Zeleznik et al., 2003) also suggested that the released βγ subunits of the Gs-protein might directly activate PI3K. Another mechanism proposed was activation of a protein phosphatase that dephosphorylates insulin receptor substrate 1 (IRS-1), which in a complex with IGF-1R would allow it to be phosphorylated by IGF-1R and then activate PI3K (Law et al., 2017), a scenario that is dependent on PKA activation and on IGF-1R being constitutively activated by IGF-1 produced by granulosa cells.

Although Akt clearly has an important role, its activation is only one part of the equation since Akt alone is not sufficient for CYP19 expression. Indeed, constitutively active Akt does not induce aromatase expression (Zeleznik et al., 2003) and, as discussed above, CYP19 expression can be blocked by inhibitors of ERK1/2, with no effect on Akt phosphorylation. Furthermore, the mechanisms discussed for Akt phosphorylation require FSH-induced activation of cAMP/PKA signaling. Since this is blocked in the presence of the small molecules ADX61623 and ADX68693 but estradiol synthesis proceeds with ADX68693, additional/yet unappreciated (cAMP-independent) inputs seem necessary for inducing aromatase expression and estradiol synthesis from androgen precursor.

One suggestion is that forkhead box protein O1 (FOXO1) and β-catenin, may be involved in the regulation of Cyp19 expression downstream of Akt (Parakh et al., 2006). It also appears that ERK1/2 (potentially via β-arrestin mediated activation, as discussed later) may have a role since, as mentioned above, its inhibition prevents Cyp19 expression without inhibiting Akt phosphorylation. Indeed, phosphorylation of Y-Box-binding protein-1 (YB-1) on Ser102 via the ERK/p90 ribosomal S6 kinase (RSK2) signaling pathway is necessary for FSH-mediated expression of target genes including CYP19A1 which are required for maturation of follicles to a preovulatory phenotype (Donaubauer and Hunzicker-Dunn, 2016). In granulosa cells, it has been shown that MEK, which phosphorylates ERK1/2, was active in the absence of FSH (downstream of the epidermal growth factor receptor (EGFR]), and that FSH decreases the activity of a phosphatase (DUSP6; mitogen activated protein kinase phosphatase [MKP] dual specificity phosphatase) which keeps ERK1/2 inactive, thus allowing accumulation of phosphorylated ERK1/2 (Donaubauer et al., 2016b). However, this pathway involves PKA-induced inhibition of DUSP6. Thus, based on these data, in the absence of cAMP (e.g. in the case of the Addex small molecule antagonists), it would be expected that ERK would be constitutively dephosphorylated and therefore inactive. Therefore, although the effects of the FSHR negative allosteric inhibitory small molecules of Addex Therapeutics point to a possibility that FSHR activates additional downstream pathways that induce aromatase expression, such a mechanism has remained elusive and a topic for further study.

Although much of the previous discussion focused on biased signaling discovered with negative allosteric modulators, the availability of new FSHR agonist small molecules have also revealed additional interesting and relevant observations. An FSHR agonist CAN1404, [a dihydrobenzoindazole analog originally described by Organon in the published patent WO2011/012674 (Timmers and Loozen, 2011)] elicited a much lower (16 % of the FSH response) inositol phosphate (IP) accumulation response (Gα16-mediated IP3 stimulation) at the WT FSHR, despite robust stimulation of cAMP-mediated signaling (as measured by CRE-luciferase reporter gene activity) (Hanyroup et al., 2021). The authors proposed that the simplest explanation is that CAN1404 is a biased agonist that induces a conformational change in the FSHR rendering it unable to couple with the Gα16 reporter designed to be promiscuous with many GPCRs (Liu et al., 2003a). Preliminary data has also indicated that CAN1405, another small molecule allosteric agonist exhibits biased agonism, strongly stimulating cAMP but not ERK1/2 phosphorylation (Ulloa-Aguirre et al., 2025b), suggesting that CAN1405 provokes a particular conformation of the FSHR that allows it to induce to cAMP production but not ERK1/2 activation as an agonist (i.e. it behaves as a biased allosteric agonist). This comports with earlier work where FSH treatment of an FSHR variant, I423T (which exhibits both partial failure to traffic to the cell membrane as well impaired activation by agonist), exhibited a 66 % decrease in cAMP compared to WT FSHR but only a 27 % decrease in ERK1/2 phosphorylation (Zarinan et al., 2021), suggesting differential modulation of these two pathways. These two studies are reminiscent of the inhibition of FSHR by ADX61623 and ADX68693, discussed above, where ADX68693 suppressed FSH-mediated cAMP but still induced Cyp19a expression and estradiol production. Taken together, these studies reprise the notion introduced above that the FSHR exhibits biased agonism that may be fine-tuned with FSHR small molecules. Such an approach may enable the stimulation of folliculogenesis with reduced estradiol production and a lower risk of ovarian hyperstimulation syndrome, with the ultimate goal of developing targeted therapeutics for safer ovarian stimulation. Conversely, two new FSHR dihydrobenzoindazole substituted agonists (TOP5300 and TOP5668) have been reported to induce greater levels of estradiol production than FSH in granulosa cells obtained from IVF patients undergoing oocyte retrieval (Nataraja et al., 2020). Recently, TOP5300 was also demonstrated to induce estradiol production in human granulosa cells from patients with advanced reproductive age and low levels of FSHR (as measured using monoclonal antibody against the FSHR ECD), or PCOS patients who had reasonable levels of receptor but poor response to FSH. This suggests that a physiological rescue or enhancement of function not possible with exogenous hormone treatment may be possible using small molecule agonists (Guner et al., 2023). No detectable increase in membrane receptor levels was observed with TOP5300 suggesting that the increased responsiveness to TOP5300 was due to a mechanism that did not involve trafficking of receptors. It is worth noting that TOP5300 has mixed FSHR/LHCGR activity (Nataraja et al., 2020). Therefore, it may be that through LHR, TOP5300 increases the production of androgen precursor, thereby increasing estrogen production.

In summary, small molecule FSHR NAMs have revealed that the estradiol biosynthetic steroidogenic pathway is capable of biased control despite common antagonism at the cAMP level. Those observations may correlate with differential effects of FSH glycoforms on the transcriptome of granulosa cells (discussed later) (Zarinan et al., 2024). The complexity and coordination of estradiol biosynthesis and granulosa cell proliferation is an ongoing area of research. This research is underpinned by a wealth of information which has led to the understanding that IGF-1/IGF-1R is necessary, but not sufficient, for estradiol production and that FSH is still required (Fig. 3). With regards to the biased antagonism exhibited by the Addex small molecule antagonists, perhaps a phosphorylation barcode of the ADX68693-FSH-FSHR unique from the ADX68692-FSH-occupied receptor, or induction of unique structural conformations, may influence physical interaction with other membrane proteins.

4. cAMP “independent” and persistent signaling: the role of platforms

The initial burst of cAMP following FSH activation of the FSHR is brief, in part shortened by the action of phosphodiesterases within the cell, and the attenuation of signaling by phosphorylation of the receptor intracellular loops by G-protein receptor kinases (GRK) and the subsequent binding by β-arrestins (Wess et al., 2023).

Previous data, discussed above, with FSHR small molecule NAMs begs the question: In the face of suppression of FSH-induced cAMP by ADX68693 or ADX61623, sufficient to block progesterone production, how is aromatase gene expression induced? Observations of the effects of these FSHR small molecules have stimulated the thinking that there may be FSH-stimulated cAMP-independent (and potentially more persistent) signaling pathways that induce aromatase expression which cannot be suppressed by ADX68693 or ADX61623, but can be suppressed by the related analog ADX68692, which blocks both progesterone and estradiol production.

As discussed above, many of the signaling pathways that have been

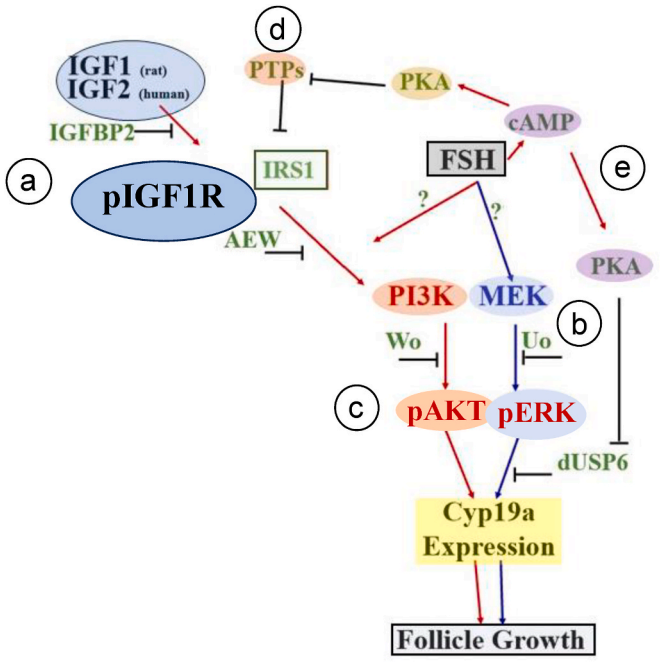


Fig. 3. The complexity and coordination of estradiol biosynthesis and granulosa cell proliferation: **a.** Inhibition of insulin-like growth factor receptor (IGF-IR) activity or expression (e.g. *via* IGF binding protein 2 (IGFBP2) or by AEW-541 inhibitor) prevented FSH-induced expression of steroidogenic genes (including the Cyp19 gene that encodes the aromatase enzyme) and estradiol production, and blocked phosphorylation of protein kinase B (pAKT); **b**.Treatment with UO126, an inhibitor of ERK1/2 kinases, prevented FSH and FSH/IGF-I stimulation of Cyp19 expression; IGF-I stimulated AKT phosphorylation (**c**) after 1 h of incubation, but it had no effect on ERK1/2 or CREB activation. IGF-1 alone did not induce Cyp-19 gene expression (Zhou et al., 2013). A conditional knockout of IGFR1 in granulosa cells (GCs) in mice were sterile and lack of antral follicles correlated with a 90 % decrease in serum estradiol levels (Baumgarten et al., 2017); **d**. FSH down-regulates the expression of the protein tyrosine phosphatase 1B (PTP-1B) in granulosa cells, whereas okadaic acid, a phosphatase inhibitor, enhances FSH-induced Cyp19 expression and augments gonadotropin-stimulated steroidogenesis. Inhibition of a protein phosphatase, that dephosphorylates IRS-1 and which is in a complex with IGF-1R, allows it to be tyrosine phosphorylated and activate the PI3K/PKB pathway (Law et al., 2017). FSH decreases the activity of a phosphatase (MKP3(DUSP6)), which keeps ERK inactive (Donaubauer and Hunzicker-Dunn, 2016). In this setting, cAMP-dependent PKA activation **e.** plays an important role in the control of these pathways. However, data generated using the FSHR NAM, ADX68693 (which exhibits biased antagonism in permitting FSH-induced estradiol synthesis in the absence of cAMP), indicate that, as yet fully unveiled, cAMP-independent mechanisms may also be involved. Wo: Wortmannin (PI3K inhibitor).

elaborated linking the FSHR to aromatase gene expression assume activation of PKA. There is scant evidence in the literature of cAMP-independent activation of PKA. One example that has been presented is that transcriptional activity of NF-kB is regulated by the inhibitor of kB (IkkB) kinase complex primarily associated with inflammation. The IkB kinase-associated catalytic subunit of PKA (PKAc) has been reported to be activated through a cAMP–independent mechanism (Zhong et al., 1997). In this example, the catalytic subunit of PKA is maintained in an inactive state through association with IkB, IkB-a or IkB-b in an NF-kB–IkB–PKAc complex (Zhong et al., 1997). Signals that cause the degradation of IkB result in activation of PKAc in a cAMP-independent manner causing the subsequent phosphorylation of p65 (Zhong et al., 1997).

Another possibility is that downstream persistent signaling (putatively not blocked by ADX68693/ADX61623) occurs in a context where hormone-occupied receptor is highly localized to nanodomains containing PKA holoenzyme leading to activation of nuclear CYP19 expression through some as yet unexplained mechanism (Lyga et al., 2016; Lohse et al., 2023). In this regard, another possibility is that kinase anchoring proteins (AKAPs) may act as scaffolding proteins to raise local concentrations of PKA isozyme to specific sites to regulate steroidogenesis albeit in a cAMP-dependent and potentially persistent manner. For example, a mouse AKAP that interacts with the mitochondrial peripheral-type benzodiazepine receptor (PBR) and PKA regulatory subunit R1α (Prkar1α), named PBR and PKA-associated protein 7 (PAP7), was identified and shown to be involved in hormone-induced mitochondrial steroid biosynthesis (Liu et al., 2003b). PAP7 is constitutively expressed in gonads and was shown to play a role in CG-induced steroid transport and synthesis (Liu et al., 2003b; Li et al., 2001). Although PAP7 binds to the regulatory subunit of PKA, there is evidence that activation of PKA does not require dissociation of the regulatory subunits from the catalytic subunits as occurs when supraphysiological levels of cAMP rise following hormone stimulation (Smith et al., 2017). Whether PAP7 targeting of PKA plays a role in FSH action remains to be determined. However, there would have to still be an activation of PKA by cAMP and clearly progesterone synthesis is blocked by ADX68693.

AKAP13 like PAP7 also influences mitochondrial steroid synthesis (Ng et al., 2019). Recently AKAP13 has been shown to act as a scaffolding protein that plays a role in FSH-induced steroidogenesis by a physical interaction with CREB, resulting in phosphorylation of CREB by PKA (Cayton Vaught et al., 2023). Another AKAP, AKAP95 (encoded by AKAP8 gene), is known to anchor PKA in the nucleus and to phosphorylate CREB (Gu et al., 2018). Knockdown of the AKAP8 gene reduced the amount of PKA anchored in the nucleus and attenuated the phosphorylation of CREB by either FSH or direct activation of the cAMP/PKA pathway and also significantly attenuated FSH-induced CYP19A1 expression and estrogen synthesis (Gu et al., 2018). It would be interesting to determine if AKAP13 and AKAP95 play a role in aromatase induction in the face of a cAMP blockade by ADX68693, as this would suggest that very miniscule amounts of cAMP can be brought to high concentrations by a specific interaction with CREB sufficient to activate aromatase gene expression. However, what is unclear is why this does not happen with ADX68692. Whether this is due to a conformation of the receptor that permits or disallows signaling platform access is a subject for future study.

Recently it has been shown that PAP7 also functions as an acyl-CoA binding domain-containing 3 (ACBD3) Golgi scaffolding protein, which directly interacts with KDEL receptor (KDELR) and regulates its trafficking via PKA (Jia et al., 2023). The cAMP that triggers activation is related to KDELR, which does not seem to be a canonical G-protein-coupled receptor. However, the point here is that these data suggest a potential mechanism that, in the absence of cAMP (either the initial burst induced by FSH, or when blocked by FSHR-allosteric small molecule inhibitors), highly localized PKA isozyme in specific signaling platforms may potentially be activated by virtue of internalized FSHR itself or by virtue of its interaction with other proteins triggering a cAMP-like response. A couple of possibilities of such proteins includes APPL1 and 14-3-3ϖ both known interacting proteins of FSHR (discussed below) (Sposini et al., 2017, 2020; Thomas et al., 2011; Nechamen et al., 2004, 2007; Dias et al., 2010b).

Another potential mechanism of cAMP-independent signaling in granulosa cells may involve the regulatory subunit 2 (RII) of PKA, (encoded by the PRKAR2B gene) a marker of granulosa cell differentiation (Ratoosh et al., 1987). A decrease in the regulatory subunit or mutations in that gene may cause constitutive activation of PKA, which has been linked to the pathogenesis of endocrine tumors as evidenced by the classification of this, and other PKA regulatory subunit genes, as tumor suppressor genes (Sandrini et al., 2002). It has been reported that the transcription factor hypoxia inducible factor 1-alpha (HIF-1a) under hypoxic conditions can suppress expression of the PRKAR2B gene by sequestrating the repressor, Sp-1 (Lucia et al., 2020). Conversely, overexpression of PRKAR2B in human prostate cancer cell lines

increased the expression of HIF-1α (Xia et al., 2020). Incubation under hypoxic conditions or overexpression of HIF-1α significantly suppressed the expression of Prkar2b but did not significantly affect the expression of the genes encoding for the other regulatory subunits (Prkar1a, Prkar1b, and Prkar2a) and the catalytic subunit (Prkaca) (Lucia et al., 2020), whereas, HIF-1α suppression using RNA interference increased Prkar2b gene transcription (Lucia et al., 2020). Pertinently, FSH stimulation of FSHR increases HIF-1α, an effect that is primarily translational as it was not inhibited by pretreatment with the transcriptional inhibitor actinomycin (Alam et al., 2004). As FSH induces HIF-1α expression, it seems reasonable to hypothesize that would suppress the synthesis of the regulatory subunit of PKA (Lucia et al., 2020). Interestingly, mice lacking Prkar2b are fertile and long-lived (Brandon et al., 1998). Indeed, it has recently been shown that FSH regulates estradiol synthesis in hypoxic granulosa cells by activating glycolytic metabolism through the HIF-1α–AMP activated protein kinase (AMPK)-GLUT1 pathway, and that blocking HIF-1α with siRNAs blocks estradiol production despite FSH stimulation (Wu et al., 2022). Activation of this pathway may also involve β-catenin enhancement of FSH action (Parakh et al., 2006; Fan et al., 2010).

In summary, biased antagonism of steroidogenesis by small molecule antagonists of FSHR demonstrated biased signaling by FSHR and suggested that cAMP- independent or highly localized signaling platforms underpinned by anchoring proteins and/or regulatory subunit control may be at play in FSHR action.

5. Internalization and recycling of the hormone-receptor complex

In addition to illuminating the intricacies of gonadotropin-receptor-induced intracellular signaling cascades, small molecule ligands targeting the gonadotropin receptors have also been useful with regards to furthering understanding of receptor internalization and endosomal signaling. For example, using FSHR fused to enhanced-green fluorescent protein (EGFP), it was shown that both FSH as well as the Organon-manufactured small molecule FSHR allosteric agonist 214444-0 induced FSHR internalization (as is typical following agonist-induced GPCR activation). These studies illustrated that hormone binding at the ECD is not necessary for internalization to occur because allosteric activation of the TMD of FSHR by 214444-0 was necessary, and sufficient, to induce internalization. However, an open question is whether activation (ie. cAMP production) is necessary for FSHR internalization? Here, the FSHR small molecule antagonist ADX61623 again provided insight. In addition to increasing ^{125}I-hFSH binding when measured in HEK293 cells expressing FSHR, ADX61623, which blocked FSH-induced cAMP production, did not prevent internalization of the receptor-bound ^{125}I-hFSH (Dias et al., 2011).

Ample evidence has demonstrated that, after hormone-induced internalization, FSHR recycles back to the cell membrane (Kluetzman et al., 2011; Krishnamurthy et al., 2003), but the mechanisms involved were not clear. Additionally, it has been established that the FSHR is ubiquitinated (Cohen et al., 2003) so some consideration of the proteosomal pathway and the role of E3 ubiquitin ligase and how these may also be implicated in the flux of FSHR synthesis/degradation is required. The discovery that two adapter proteins, APPL1 and 14-3-3π interact with FSHR, also begged for clarification about their roles, which remained obscure (Thomas et al., 2011; Nechamen et al., 2004, 2007; Dias et al., 2010b; Cohen et al., 2004).

Some clarity has been achieved on the latter point thanks to the implementation of high-resolution imaging of gonadotropin receptors. A potential role for APPL1 in very early endosome (VEE) trafficking was discovered using this advanced imaging method, first for LHCGR (Sposini et al., 2017) and then in FSHR (Sposini et al., 2020). VEEs are smaller endosomes devoid of early endosome (EE) and intermediate EE markers such as EE antigen 1 (EEA1), Rab5, and phosphatidyl inositol-3 phosphate (PI3P) (Jean-Alphonse et al., 2014). Blocking of LHCGR internalization with Dyngo-4a, a potent dynamin inhibitor, decreased cAMP production. Furthermore, APPL1 in VEEs was found to act as a negative regulator of both LH- and FSH-induced cAMP production by LHCGR and FSHR, respectively (Sposini et al., 2017, 2020) (Fig. 4). Interestingly, although the VEE is an endosomal compartment linked to sustained ERK1/2 signaling (Jean-Alphonse et al., 2014), APPL1 did not alter LH-dependent ERK1/2 activation (Sposini et al., 2017).

The above described studies have demonstrated a critical role for receptor internalization and endosomal signaling in FSHR and LHGCR activity, and that APPL1 is essential for both rapid recycling of the receptors to the cell membrane (with LHCGR recycling driven by receptor-mediated Gαs/cAMP signaling from the VEE and PKA-dependent phosphorylation of APPL1 at serine 410) and for regulation of endosomal cAMP signaling (Sposini et al., 2017).

Given the critical nature of receptor internalization and endosomal signaling for FSHR activity, the availability of FSHR allosteric agonists suggested the potential to re-program FSHR activity via altering engagement with endosomal machinery (Sposini et al., 2020).Two chemically distinct FSHR agonists (a benzamide derivative, B3 and a thiazolidinone derivative, T1) were employed to assess whether these compounds exhibit differential abilities to alter receptor endosomal trafficking and signaling within the VEE. It was confirmed that blocking of internalization with Dyngo-4a reduced small molecule agonist-induced cAMP accumulation in cells measured with a cAMP sensor, as seen with FSH (Sposini et al., 2020). However, T1 was able to induce a greater level of cAMP than FSH and B3 suggesting that the distinct function of APPL1 to reduce endosomal cAMP generation can potentially be pharmacologically selected. Indeed, knockdown of APPL1 increased cAMP production induced by both FSH and B3, but not T1. On the other hand, APPL1 knockdown reduced recycling of FSHR induced by T1 and FSH but not by B3 (Sposini et al., 2020). It is worth noting that the chemical series from which B3 (the enantiomer of 9k) was derived, required an EC_{20} FSH concentration for stimulating primary granulosa

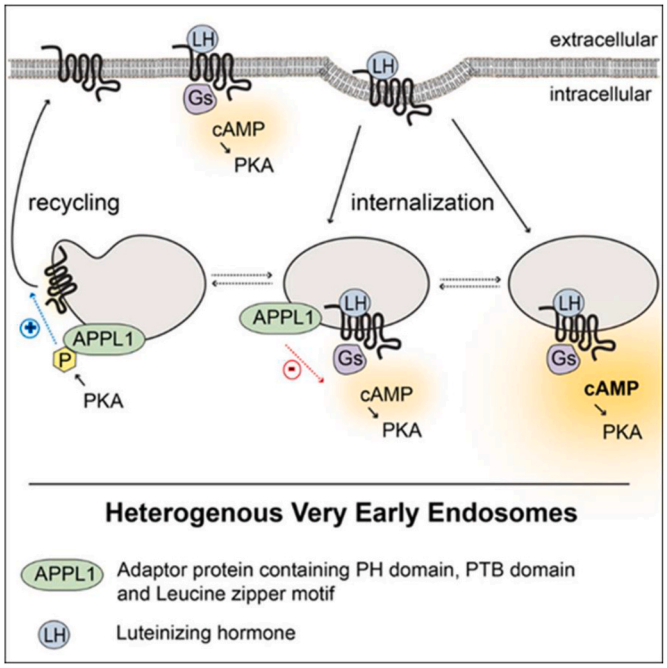


Fig. 4. APPL1 was found to play two key and distinct functions in regulating LHR activity: Required for receptor recycling and negative regulation of LH-induced endosomal cAMP (Sposini et al., 2017). Using this experimental paradigm and advanced imaging methods and FSHR small molecules, it was discovered that endosomal programming can be selectively modified by FSHR small molecule agonists (Sposini et al., 2020). Figure from (Sposini et al., 2017), reproduction licensed by Creative Commons CC-BY.

cells, thus behaving as PAM. However, B3 studied in these endosomal signaling analyses did not require any FSH to stimulate cAMP (Sposini et al., 2020).

All three small molecule agonists (214444–0, T1 and B3) induced FSHR internalization independent of FSH action. This suggested that they do so by inducing a similar conformational change in the FSHR. Furthermore, the inhibition of FSH-induced cAMP production by ADX61623 suggested that FSH-induced conformational change triggers internalization in a cAMP-independent manner suggesting that internalization is not cAMP dependent. An alternative explanation is that in the absence of G protein (in the presence of inhibitors) or following the dissociation of G protein small-molecule(agonist)-occupied FSHR will internalize independent of FSH.

In summary, implementation of gonadotropin receptor small molecule agonists and antagonists have validated critical interactions between gonadotropin receptors and previously identified adaptor proteins, and signaling and trafficking pursuant to those interactions. Potential therapies, based on modulation of interacting proteins, are currently being considered for GPCRs in general. Internalization of FSHR appears necessary for engaging Gs-protein signaling and adenylyl cyclase activation and persistent signaling involves receptor sorting and endosomal G protein signaling from the VEE. Moreover, using advanced imaging methods and FSHR small molecules, it was discovered that endosomal programming can be selectively modified by FSHR small molecules (Sposini et al., 2020). Understanding how the Addex FSHR small molecule antagonists affect these processes awaits their evaluation in sophisticated high-resolution imaging systems.

6. Hetero-oligomerization through protein-protein interactions

Recent demonstration of FSHR and LHCGR homologous and heterologous interactions studied by in situ imaging (Fanelli et al., 2020; Mazurkiewicz et al., 2015) has raised the possibilities of nuanced, fine-tuned signaling mediated through these interactions. There is also evidence that the FSHR interacts with other membrane proteins (Casarini et al., 2020; Lundin et al., 2022), in addition to canonical signaling proteins (Ulloa-Aguirre et al., 2018; Reiter et al., 2017; Kara et al., 2006) and adapter proteins (Sposini et al., 2017, 2020; Thomas et al., 2011; Dias et al., 2010b; Jonas and Hanyaloglu, 2019). These interactions may play a role in modulating FSH-induced estrogen biosynthesis and or folliculogenesis, complementing the canonical cAMP pathways to progesterone biosynthesis, providing a previously appreciated fail-safe mechanism (Pasapera et al., 2005). The availability of gonadotropin receptor small molecule agonists and antagonists enables examination of this hypothesis and whether these interactions can be differentially modulated, which, as discussed above, has been shown for APPL1/FSHR and APPL1/LHCGR interactions and endosomal signaling (Sposini et al., 2020; Casarini et al., 2020).

The observation that blocking cAMP by the FSHR NAM small molecule ADX68693 was not sufficient to block folliculogenesis, but that ADX68692, which blocks both cAMP and estradiol synthesis, was sufficient to block this process, raised three questions: First, are there alternative mechanisms for production of estradiol and, thus, folliculogenesis in the face of a block of cAMP production that should be considered? Second, might estradiol production be necessary but not sufficient for proliferation (folliculogenesis), ie., is there an, yet, unappreciated role for FSHR in activating the time-dependent induction of proliferation/folliculogenesis perhaps by interacting with and transactivating growth factor receptors such as insulin-like growth factor receptor (IGFR) or epidermal growth factor receptor (EGFR)? Thirdly, is there a difference in the gene expression profile of FSH when cAMP is blocked and estradiol is produced versus when both estradiol and cAMP production are blocked and might that inform the first question? The first question was the subject of much of the preceding discussion. Results discussed next regarding recent developments in understanding the role of growth factor receptor IGF1R as well as estrogen binding protein interactions with FSHR have provided intriguing insights into the second question.

In a recent study, FSHR-interacting proteins were identified using mass spectrometry. HEK293 cells expressing either wild-type (WT) or an inactivating variant (A189V) FSHR that results in protein misfolding and subsequent failure to traffic to the plasma membrane (Aittomaki et al., 1995), were used. Unsurprisingly, a preponderance of interacting proteins that are involved in chaperoning misfolded proteins with FSHR A189V compared to WT FSHR was discovered (Lundin et al., 2022). An unanticipated discovery also emerged showing that, upon stimulation, both the WT and variant FSHR interact with IGFR (Lundin et al., 2022). FSHR-expressing cell lines differentiated from pluripotent stem cells derived from patients with FSHR variant A189V or from an embryonic stem cell line to generate a WT FSHR expressing line were also produced as models for examination of endogenous FSHR function in vitro (Lundin et al., 2022). In this model, FSH stimulation of the WT, but not variant A189V, FSHR activated the canonical cAMP-dependent signaling pathway and downstream mediators (Lundin et al., 2022). These data potentially open new lines of inquiry on what role, if any, IGFR may play in cAMP/PKA-independent activation of aromatase induction and/or proliferation. Although laborious, this model may be used in the presence and absence of the FSHR small molecule negative allosteric modulators to determine if the interaction is differentially affected by the two Addex negative allosteric modulators. In addition, this platform may help understand the underpinning of the FSHR A189V trafficking defect and modes of action of FSHR pharmacoperones that can be used to rescue them (see below), as well as pharmacoperones targeting other receptors.

In other studies, it has also been observed that FSH induces estrogen-sensitive binding proteins in human granulosa cells and L cells (Pasapera et al., 2005), including the G protein-coupled estrogen receptor (GPER, GPR30) (Casarini et al., 2020), and the nuclear steroid hormone receptor ERβ (but not ERα), as well as the progesterone receptor (Pavlik et al., 2011). GPER is expressed in normal human ovaries (Heublein et al., 2012) and has also been implicated in cancer cell proliferation, such as in Tamoxifen resistant Her-2/neu positive ovarian epithelial cancer (Heublein et al., 2013). Indeed, GPER has been proposed as a gonadotropin receptor-dependent positive prognosticator in ovarian carcinoma patients (Heublein et al., 2013).

GPER interacts with membrane-associated guanylate kinase (MAGUK) scaffold proteins through its C-terminal PDZ motif (~SSAV), specifically with the MAGUK proteins postsynaptic density protein 95 (PSD-95) and synapse-associated protein 97 (SAP97), the latter of which anchors AKAP, AKAP5, and PKA RII (Gonzalez de Valdivia et al., 2017). Interaction of GPER with MAGUK proteins and AKAP5, constitutively inhibits cAMP in a Gαi/o-independent manner (Broselid et al., 2014). GPER also mediates survival/proliferation signals through the PI3K/Akt signaling pathway (Akt promotes cell survival by phosphorylating and inhibiting a forkhead transcription factor (Brunet et al., 1999)) and by constitutively increasing mitogenic ERK1/2 activation in a $G_{i/o}$-dependent manner (Gonzalez de Valdivia et al., 2017), as well as $G_{\beta\gamma}$-dependent mechanisms upregulating proto-oncogenes (Maggiolini et al., 2004). Given that GPER is a gonadotropin-dependent positive prognosticator in ovarian cancer, and given that GPER is also found in ovarian tissues throughout the follicular phase (Heublein et al., 2012) it seemed likely that GPER would play a role in granulosa cell proliferation.

Recently, a significant discovery characterized how FSHR and GPER form a complex that promotes follicle development/granulosa cell proliferation (Casarini et al., 2020) (Fig. 5). In that report, FSH induction of cAMP production was blocked by GPER but FSH-induced AKT activation (mediated by $G_{\beta\gamma}$-dependent Akt phosphorylation) was increased, as was cell viability (with a reduction in proapoptotic cleavage of procaspase 9). The ability of GPER to inhibit FSH-mediated cAMP signaling was confirmed by measurement of FSH-induced cAMP response element-binding protein (CREB) phosphorylation, a canonical

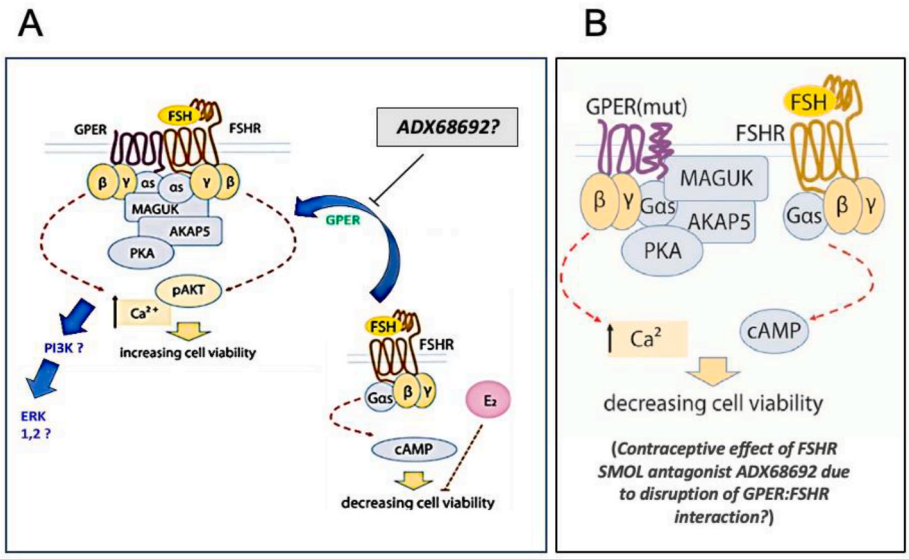


Fig. 5. ***A:*** FSHR interaction with GPER constrains FSH-induced cAMP production inhibiting differentiation and favoring proliferation. A question is whether FSHR small molecule (SMOL) antagonists ADX68692 but not ADX68693 blocks the GPER-FSHR interaction. ***B:*** Mutation of GPER prevents FSHR interaction with GPER allowing FSH-induced cAMP and decreased cell viability, suggesting that disruption of GPER-FSHR interaction by an FSHR SMOL antagonist may block proliferation of granulosa cells and hence provide contraception. Figure modified from (Casarini et al., 2020), reproduction licenced by Creative Commons CC-BY.

downstream cAMP-dependent event, that has also been reported to occur via an intracellular calcium release/ERK-dependent pathway (Zanassi et al., 2001). While it is unlikely that FSH binding to the GPER/FSHR complex can induce Cyp19a expression via the PKA pathway, its inhibition of caspase 9 cleavage reduces the influence of an apoptotic pathway and can potentially stimulate proliferation. It is tempting to speculate that the activation of Akt and ERK1/2 may also play a role in cAMP-independent estradiol production, albeit on a different timescale than the PKA-induced phosphorylation of CREB. Unfortunately, it was not determined if FSH regulated GPER-induced ERK1/2 activation or the mitogen-activated protein kinase (MAPK) signaling pathway (Casarini et al., 2020), which are both upstream of CREB phosphorylation (Bonni et al., 1999). However, the phosphorylation of Akt was inhibited by gallien, a $G_{\beta\gamma}$ inhibitor. This raises the possibility of β-arrestin-dependent FSH activation of ERK1/2 which was previously determined to be independent of PKA, and has a delayed and extended profile (Kara et al., 2006). cAMP independent β-arrestin2-dependent phosphorylation of CREB is mediated by ERK (Manson et al., 2011) and osteocalcin control of adiponectin expression is also mediated by ERK independently of PKA (Otani et al., 2015). ERK induced CREB phosphorylation may also occur through mitogen and stress activated kinases (MSK1,2) (Wiggin et al., 2002). It should be noted that mouse knockouts of MSK1,2 are fertile (Wiggin et al., 2002) and so are the GPER knockout mice (Prossnitz and Hathaway, 2015). In human granulosa cells, GPER siRNA increased FSH-mediated cAMP and progesterone biosynthesis (Casarini et al., 2020), but it remains to be determined if GPER siRNA experiments enhance FSH-induced estradiol biosynthesis. Nonetheless, the association of FSHR with GPER and the role of GPER in proliferation provided a rationale to a paradigm that might explain why ADX68693 does not block preovulatory follicle development in the face of a block of FSH-dependent cAMP, that is, via β-arrestin-mediated activation of ERK1/2, which would synergize with the FSHR/GPER activation of ERK1/2 (which would synergize with the FSHR/GPER activation of pAkt) to induce estradiol biosynthesis. One possibility underlying the inability of ADX68692 to induce such an effect is that it prevents the interaction of FSHR with the GPER complex while ADX68693 does not. However, this remains to be formally tested.

In conclusion, gonadotropin receptor small molecule modulators may be used to determine if homo- and hetero-oligomeric states of the receptor are modulated chemically. Experimental models discussed above to detect protein interactions with FSHR seem promising not only to identify interaction, but also to discover small molecules that can disrupt those interactions via induction of varied receptor conformers. Such experiments may shed light not only on biased agonism, but also on whether multimeric states and protein-protein interactions with the receptor underpin physiological manifestations of gonadotropin stimulation.

7. Biosynthesis of receptors

Following translation, nascent GPHR proteins are folded into their native conformation and undergo post-translational processing in the endoplasmic reticulum (ER) and Golgi apparatus from where they are transported to the plasma membrane to be available for interacting with their cognate ligands. Cellular quality control systems prevent upward trafficking of incorrectly folded (misfolded) receptor proteins. One of the first inactivating variants of the FSHR (A189V) was discovered in patients in Finland who presented with secondary amenorrhea before the age of 20 yr (Aittomaki et al., 1995, 1996a, 1996b; Rannikko et al., 2002). This is a rare disease manifesting as primary ovarian insufficiency, with the lack of sex steroids negatively impacting bone health (Luiro et al., 2019). Hormone replacement therapy is indicated but is not sufficient to restore fertility. However, in cases where a mutation results in misfolding that prevents posttranslational processing and maturation/trafficking to the plasma membrane [as is the case for the A198V FSHR variant (Rannikko et al., 2002) and many other GPHR variants], it was thought that rescuing the receptor using gonadotropin receptor small molecule modulators would ameliorate the insufficiency and restore natural menses. Today the use of small molecules to rescue defective trafficking of receptors in vitro is a common pre-clinical study finding, and the term pharmacoperone (from pharmacological chaperone) has been coined (Conn et al., 2014). Cells containing the FSHR A189V variant when treated with an LHCGR allosteric agonist Org 41841, were shown to increase FSH-dependent activity (Janovick et al., 2009). FSH-induced cAMP was increased about 1.4 fold and FSH binding about 3 fold. Control experiments validated that the LHCGR agonist *per se* had no direct effect on cAMP production (Janovick et al., 2009). Thus, the LHCGR small molecule could in fact cross over to FSHR and

although it could not activate it, it could still have an enhancing effect (conformer stabilization and corrected trafficking) only observed in the presence of FSH. However, other laboratory-made mutations in the FSHR ECD, which also failed to be expressed on the cell surface, were not rescued with Org 41841 (Nechamen and Dias, 2000, 2003). The FSHR A189V variant has extremely low FSH binding and undetectable cAMP production measured directly, or with the cAMP FRET vector ICUE (Indicator of cAMP Using Epac biosensor), as well as PKA activation measured with AKAR (A-Kinase Activity Reporter) FRET sensor and the PSOMLuc (CRE-dependent luciferase reporter) vector (Tranchant et al., 2011). Interestingly, cAMP-independent, β-arrestin dependent, ERK phosphorylation (resistant to H89 inhibition, a PKA inhibitor) was determined to be the predominant form of signaling induced by this variant and this phenomenon was found to be due to the low expression level of the variant and not due to the presence of the mutation itself, as WT FSHR exhibited similar preferential coupling when expressed at low plasma-membrane densities (Tranchant et al., 2011).

Another FSHR small molecule agonist, CAN1404 (Table 1), was able to rescue a host of FSHR variants that exhibited poor cell surface expression, but not all variants could be rescued (Hanyroup et al., 2021). Only successful rescue was possible with mutations that occurred in the TMD and not the ECD. That observation was consistent with the inability of Org41841 to rescue engineered mutations in the FSHR ECD that caused trafficking defects (Nechamen and Dias, 2000, 2003; Mahale et al., 2001), with the exception of the A189V mutant which was modestly rescued by this allosteric agonist (see above). Similarly, in an, as yet, unpublished study using a different panel of FSHR variants, it was found that CAN1405, another FSHR small molecule agonist, rescued some misfolded FSHR variants with TMD-located mutations, whereas those located in the ECD were virtually refractory to rescue (Ulloa-Aguirre et al., 2025b). Similar to the FSHR, rescue of cell surface expression of LHCGR has also been achieved with a thienopyrimidine, Org 42599 LHCGR small molecule (renamed to LHR-Chap) (Newton et al., 2011, 2021). Again, not all LHCGR variants could be rescued, and only TMD-located variants were responsive. Interestingly, no increase in hormone binding to WT LHCGR was observed when cells were treated with Org 42599 (Newton et al., 2011), unlike when FSHR is treated with FSHR NAMs (Dias et al., 2011, 2014) or Org 41841 and Org 214444-0 (Jiang et al., 2014b).

Recently, it has been shown that rescue of misfolded GnRH receptors (GnRHR) can be achieved with small peptidomimetic antagonist molecules that are independent of cognate GnRHR activity (Conn and Ulloa-Aguirre, 2010). High-throughput screening demonstrated the efficacy of GnRHR-specific pharmacoperones that rescued misfolded GnRHR variants from ER retention, leading to plasma membrane localization and restored signaling in vitro (Smith et al., 2020). In this remarkable finding, eight different structural classes were identified that showed rescue of the GnRHR E90K pathogenic variant, and none of the 11 lead compounds tested showed appreciable agonist or antagonist effect. Such 'neutral' pharmacoperones targeting the gonadotropin receptors have yet to be described, however this study highlights the potential for such compounds which would permit the spatial and temporal responses to endogenous hormone to be restored without impact from exogenous ligand (agonist or antagonist) activity. Furthermore, in the case of GnRHR and vasopressin V2 receptor, "rescue" of cell surface expression of variant receptors using agonist, antagonist or compounds with no functional activity, has unveiled constitutive activity of some misfolded variant receptors (Janovick et al., 2017; Janovick and Conn, 2010), suggesting another potential outcome that may still be discovered/explored for the gonadotropin receptors.

In summary, small molecule compounds can act as pharmacoperones to restore cell surface expression and function to upward trafficking-defective, misfolded gonadotropin receptors. Those variants with mutations in the TMD are more amenable to rescue by small molecules than trafficking-defective receptors with mutations in the ECD. It isn't known if mutations which specifically prevent receptor glycosylation (in the ECD) can be rescued with gonadotropin receptor small molecules, although the LHCGR allosteric agonist Org 41841, provoked a modest, but significant increase in FSHR response and binding of the A189V pathogenic variant (Janovick et al., 2009), which probably alters receptor glycosylation at Asn 191 leading to intracellular retention (Aittomaki, Herva et al., 1996). Stabilization of a receptor conformation compatible with ER export appears to underpin the rescue phenomenon (Jardon-Valadez and Ulloa-Aguirre, 2024). It isn't known if the FSHR NAMs ADX68692 or ADX68693 can also rescue gonadotropin receptors. However, their oral bioavailability and good pharmacokinetic characteristics suggest it may be worth trying. Likewise, efforts to identify 'neutral' pharmacoperones for these receptors would also be an avenue worth pursuing.

8. Post-signaling nuances of hormone/small molecule-occupied receptors

Following hormone-induced signaling, activation of gene transcription leads to the production of a variety of proteins that will participate in the selection and nurturing of preantral follicle growth and development. The canonical view is that FSH, through the FSHR-induction of cAMP synthesis, activates downstream effectors that drive preovulatory follicle growth and development of fertilization-competent oocytes (Ulloa-Aguirre et al., 2018). This view engendered selection of FSHR as a target to develop an orally active nonsteroidal drug that would block fertility in women but could also be useful to treat estrogen exacerbated endometriosis and estrogen-dependent cancer as an alternative to aromatase inhibitors. In an effort to determine proof of concept that non-steroidal FSHR small molecule antagonists are contraceptive, *in vivo* testing showed that complete block of both progesterone and estradiol production was required to effectively block folliculogenesis (Dias et al., 2010a, 2014).

The observation that ADX68692 (which blocks both estradiol and progesterone synthesis) could block formation of preovulatory follicles, but that ADX68693 (which does not block estradiol synthesis) could not, suggested that an evaluation of the landscape of rat granulosa cell transcriptome induced by FSH in the presence or absence of FSHR NAMs ADX68692 and ADX68693 might provide clues as to additional targets that might block proliferation without blocking estradiol production. Indeed, recent findings have demonstrated that induction of nuanced gene transcription is observed with glycoforms of FSH that are hypoglycosylated at the β-subunit when compared to fully glycosylated FSH (Zarinan et al., 2024). Furthermore, when fully glycosylated FSH was compared to hypoglycosylated FSH the transcriptome induced by either form of FSH differed significantly (Zarinan et al., 2024). It therefore follows that biased signaling in the presence of FSHR NAMS would likewise reveal significant differences in the FSH-induced transcriptome. Preliminary findings have provided evidence that this is so (Hernandez-Ramírez et al., 2022).

In summary, this review has highlighted how small molecules that bind to gonadotropin receptors and function as agonists or antagonists of FSH or LH action (and hence that activate or inhibit receptor function), have proven very useful in understanding the structural, functional and physiological attributes of gonadotropin receptors. Of interest, is how unexpected findings have shed light on the complexity of gonadotropin receptors and their associated signaling. This review also proposes several opportunities for future experiments that implement small molecules currently discussed. Some of these compounds are not currently available unless synthesized independently and carefully evaluated in established assays for FSH activity.

In addition, new experimental assays discussed in the review (i.e. those exploring rescue of misfolded receptors; identification of interacting proteins; modulation of known interactions such as GPER/FSHR; proliferation assays and use of high-throughput screening approaches) provide a repertoire of tools to explore the intricacies of receptor

signaling and may lead to identification of new compounds which may, or may not (as discussed in the case of the GnRHR), activate or inhibit particular functional modalities. The challenge ahead will include capturing different biased states of the receptor and oligomeric complexes of the receptor with its canonical as well as newly emerging partners. These conformational changes and large-scale molecular interactions will likely be studied by molecular dynamics simulations (Ulloa-Aguirre et al., 2025b; Jardon-Valadez and Ulloa-Aguirre, 2024) as well as determined by time-resolved cryo-EM (Feng and Frank, 2024; Seitz et al., 2022). It is clear that understanding the complexity and nuances of gonadotropin receptor signaling, and more broadly, all G protein coupled receptors, is likely to yield additional targets for therapeutic interventions (Shchepinova et al., 2020).

CRediT authorship contribution statement

James A. Dias: Writing – review & editing, Writing – original draft, Conceptualization. **Claire L. Newton:** Writing – review & editing, Writing – original draft, Conceptualization. **Alfredo Ulloa-Aguirre:** Writing – review & editing, Writing – original draft, Conceptualization.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Alfredo Ulloa-Aguirre reports financial support was provided by National Autonomous University of Mexico. Claire L. Newton reports financial support was provided by South African National Research Foundation. James A. Dias is Associated Editor for the Special Issue **"Implementation of Small Molecules to Study the Structure, Function and Cellular Physiology of GPCRs**".

Acknowledgement

The authors wish to acknowledge the support of Le Studium and the organizers of the conference: ICGR-V - 5th International Conference on Gonadotropins and Receptors, March 12, - March 15, 2024, Tours France, and for the encouragement to prepare this review. Studies in the laboratory of A.U.-A. have been supported by funding from the Coordinación de la Investigación Científica and the Programa de Apoyo a Proyectos de Investigación e Innovación Tecnológica (PAPIIT grant IN208323), UNAM, Mexico. Studies in the laboratory of C.L.N. have been supported by funding from the South African National Research Foundation (CSUR grant 94008).

References

Agrawal, G., Dighe, R.R., 2009. Critical involvement of the Hinge region of the follicle-stimulating hormone receptor in the activation of the receptor. J. Biol. Chem. 284, 2636–2647 doi: M808199200 [pii] 10.1074/jbc.M808199200.

Aittomaki, K., Lucena, J.L., Pakarinen, P., et al., 1995. Mutation in the follicle-stimulating hormone receptor gene causes hereditary hypergonadotropic ovarian failure. Cell 82, 959–968. https://doi.org/10.1016/0092-8674(95)90275-9.

Aittomaki, K., Herva, R., Stenman, U.H., et al., 1996a. Clinical features of primary ovarian failure caused by a point mutation in the follicle-stimulating hormone receptor gene. J. Clin. Endocrinol. Metab. 81, 3722–3726. https://doi.org/10.1210/jcem.81.10.8855829.

Aittomaki, K., Tapanainen, J., Huhtaniemi, I., de la Chapelle, A., 1996b. [Inherited primary amenorrhea. The first gynecological disease of Finnish heritage]. Duodecim 112, 9–11. https://www.ncbi.nlm.nih.gov/pubmed/10590594.

Alam, H., Maizels, E.T., Park, Y., et al., 2004. Follicle-stimulating hormone activation of hypoxia-inducible factor-1 by the phosphatidylinositol 3-kinase/AKT/Ras homolog enriched in brain (Rheb)/mammalian target of rapamycin (mTOR) pathway is necessary for induction of select protein markers of follicular differentiation. J. Biol. Chem. 279, 19431–19440. https://doi.org/10.1074/jbc.M401235200.

Anderson, R.C., Newton, C.L., Millar, R.P., 2018. Small molecule follicle-stimulating hormone receptor agonists and antagonists. Front. Endocrinol. 9, 757. https://doi.org/10.3389/fendo.2018.00757.

Arey, B.J., Deecher, D.C., Shen, E.S., et al., 2002. Identification and characterization of a selective, nonpeptide follicle-stimulating hormone receptor antagonist. Endocrinology 143, 3822–3829. http://www.ncbi.nlm.nih.gov/pubmed/12239093.

Arey, B.J., Yanofsky, S.D., Claudia Perez, M., et al., 2008. Differing pharmacological activities of thiazolidinone analogs at the FSH receptor. Biochem. Biophys. Res. Commun. 368, 723–728. https://doi.org/10.1016/j.bbrc.2008.01.119.

Ayoub, M.A., Landomiel, F., Gallay, N., et al., 2015. Assessing gonadotropin receptor function by resonance energy transfer-based assays. Front. Endocrinol. 6, 130. https://doi.org/10.3389/fendo.2015.00130.

Ayoub, M.A., Yvinec, R., Jegot, G., et al., 2016. Profiling of FSHR negative allosteric modulators on LH/CGR reveals biased antagonism with implications in steroidogenesis. Mol. Cell. Endocrinol. 436, 10–22. https://doi.org/10.1016/j.mce.2016.07.013.

Ballesteros, J.A., Weinstein, H., 1995. Integrated methods for the construction of three-dimensional models and computational probing of structure-function relations in G protein-coupled receptors. Methods Neurosci. 25, 366–428.

Banerjee, A.A., Mahale, S.D., 2015. Role of the extracellular and intracellular loops of follicle-stimulating hormone receptor in its function. Front. Endocrinol. 6, 110. https://doi.org/10.3389/fendo.2015.00110.

Baumgarten, S.C., Armouti, M., Ko, C., Stocco, C., 2017. IGF1R expression in ovarian granulosa cells is essential for steroidogenesis, follicle survival, and fertility in female mice. Endocrinology 158, 2309–2318. https://doi.org/10.1210/en.2017-00146.

Bonger, K.M., van den Berg, R.J., Knijnenburg, A.D., et al., 2009. Discovery of selective luteinizing hormone receptor agonists using the bivalent ligand method. ChemMedChem 4, 1189–1195. https://doi.org/10.1002/cmdc.200900058.

Bonni, A., Brunet, A., West, A.E., Datta, S.R., Takasu, M.A., Greenberg, M.E., 1999. Cell survival promoted by the Ras-MAPK signaling pathway by transcription-dependent and -independent mechanisms. Science 286, 1358–1362. https://doi.org/10.1126/science.286.5443.1358.

Brandon, E.P., Logue, S.F., Adams, M.R., et al., 1998. Defective motor behavior and neural gene expression in RIIbeta-protein kinase A mutant mice. J. Neurosci. : Off. J. Soc. Neurosci. 18, 3639–3649. https://doi.org/10.1523/JNEUROSCI.18-10-03639.1998.

Broselid, S., Berg, K.A., Chavera, T.A., et al., 2014. G protein-coupled receptor 30 (GPR30) forms a plasma membrane complex with membrane-associated guanylate kinases (MAGUKs) and protein kinase A-anchoring protein 5 (AKAP5) that constitutively inhibits cAMP production. J. Biol. Chem. 289, 22117–22127. https://doi.org/10.1074/jbc.M114.566893.

Brunet, A., Bonni, A., Zigmond, M.J., et al., 1999. Akt promotes cell survival by phosphorylating and inhibiting a Forkhead transcription factor. Cell 96, 857–868. https://doi.org/10.1016/s0092-8674(00)80595-4.

Bruser, A., Schulz, A., Rothemund, S., et al., 2016. The activation mechanism of glycoprotein hormone receptors with implications in the cause and therapy of endocrine diseases. J. Biol. Chem. 291, 508–520. https://doi.org/10.1074/jbc.M115.701102.

Casarini, L., Lazzaretti, C., Paradiso, E., et al., 2020. Membrane estrogen receptor (GPER) and follicle-stimulating hormone receptor (FSHR) heteromeric complexes promote human ovarian follicle survival. iScience 23, 101812. https://doi.org/10.1016/j.isci.2020.101812.

Cassier, E., Gallay, N., Bourquard, T., et al., 2017. Phosphorylation of beta-arrestin2 at Thr(383) by MEK underlies beta-arrestin-dependent activation of Erk1/2 by GPCRs. eLife 6. https://doi.org/10.7554/eLife.23777.

Cayton Vaught, K.C., Hazimeh, D., Carter, A.S., et al., 2023. AKAP13 enhances CREB1 activation by FSH in granulosa cells. Reprod. Sci. 30, 1528–1539. https://doi.org/10.1007/s43032-022-01097-5.

Cohen, B.D., Bariteau, J.T., Magenis, L.M., Dias, J.A., 2003. Regulation of follitropin receptor cell surface residency by the ubiquitin-proteasome pathway. Endocrinology 144, 4393–4402. http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=12960054.

Cohen, B.D., Nechamen, C.A., Dias, J.A., 2004. Human follitropin receptor (FSHR) interacts with the adapter protein 14-3-3tau. Mol. Cell. Endocrinol. 220, 1–7. http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=15196694.

Conn, P.M., Ulloa-Aguirre, A., 2010. Trafficking of G-protein-coupled receptors to the plasma membrane: insights for pharmacoperone drugs. Trends Endocrinol. Metabol.: TEM 21, 190–197. https://doi.org/10.1016/j.tem.2009.11.003.

Conn, P.M., Ulloa-Aguirre, A., Janovick, J.A., 2014. "Pharmacoperone": what's in a word? Pharmacol. Res. 83, 1–2. https://doi.org/10.1016/j.phrs.2013.11.005.

Dias, J.A., Reichert Jr., L.E., 1982. Characterization of a follitropin-binding component prepared from immature bovine testes in the absence of detergent. J. Biol. Chem. 257, 613–620.

Dias, J.A., Huston, J.S., Reichert Jr., L.E., 1981. Effect of the structure-stabilizing agent glycerol on detergent-solubilized follicle-stimulating hormone receptors from calf testis. Endocrinology 109, 736–742. https://doi.org/10.1210/endo-109-3-736.

Dias, J.A., Bonnet, B., Weaver, B.A., et al., 2010a. A negative allosteric modulator demonstrates biased antagonism of the follicle stimulating hormone receptor. Mol. Cell. Endocrinol. 333, 143–150. https://doi.org/10.1016/j.mce.2010.12.023.

Dias, J.A., Mahale, S.D., Nechamen, C.A., Davydenko, O., Thomas, R.M., Ulloa-Aguirre, A., 2010b. Emerging roles for the FSH receptor adapter protein APPL1 and overlap of a putative 14-3-3tau interaction domain with a canonical G-protein interaction site. Mol. Cell. Endocrinol. 329, 17–25. http://www.ncbi.nlm.nih.gov/pubmed/20600589.

Dias, J.A., Bonnet, B., Weaver, B.A., et al., 2011. A negative allosteric modulator demonstrates biased antagonism of the follicle stimulating hormone receptor. Mol. Cell. Endocrinol. 333, 143–150. https://doi.org/10.1016/j.mce.2010.12.023.

Dias, J.A., Campo, B., Weaver, B.A., et al., 2014. Inhibition of follicle-stimulating hormone induced preovulatory follicles in rats treated with a nonsteroidal negative

allosteric modulator of follicle-stimulating hormone receptor. Biol. Reprod. 9019, 1–11. https://doi.org/10.1095/biolreprod.113.109397.
Donaubauer, E.M., Hunzicker-Dunn, M.E., 2016. Extracellular signal-regulated kinase (ERK)-dependent phosphorylation of Y-Box-binding protein 1 (YB-1) enhances gene expression in granulosa cells in response to follicle-stimulating hormone (FSH). J. Biol. Chem. 291, 12145–12160. https://doi.org/10.1074/jbc.M115.705368.
Donaubauer, E.M., Law, N.C., Hunzicker-Dunn, M.E., 2016a. Follicle-Stimulating Hormone (FSH) dependent regulation of Extracellular Regulated Kinase (ERK) phosphorylation by MAP kinase phosphatase MKP3. J. Biol. Chem. https://doi.org/10.1074/jbc.M116.733972.
Donaubauer, E.M., Law, N.C., Hunzicker-Dunn, M.E., 2016b. Follicle-Stimulating hormone (FSH)-dependent regulation of Extracellular Regulated Kinase (ERK) phosphorylation by the mitogen-activated protein (MAP) kinase phosphatase MKP3. J. Biol. Chem. 291, 19701–19712 doi: M116.733972 [pii];10.1074/jbc.M116.733972 [doi].
Duan, J., Xu, P., Cheng, X., et al., 2021. Structures of full-length glycoprotein hormone receptor signalling complexes. Nature 598, 688–692. https://doi.org/10.1038/s41586-021-03924-2.
Duan, J., Liu, H., Zhao, F., et al., 2023a. GPCR activation and GRK2 assembly by a biased intracellular agonist. Nature 620, 676–681. https://doi.org/10.1038/s41586-023-06395-9.
Duan, J., Xu, P., Zhang, H., et al., 2023b. Mechanism of hormone and allosteric agonist mediated activation of follicle stimulating hormone receptor. Nat. Commun. 14, 519. https://doi.org/10.1038/s41467-023-36170-3.
Escamilla-Hernandez, R., Little-Ihrig, L., Orwig, K.E., Yue, J., Chandran, U., Zeleznik, A. J., 2008. Constitutively active protein kinase A qualitatively mimics the effects of follicle-stimulating hormone on granulosa cell differentiation. Mol. Endocrinol. 22, 1842–1852. https://doi.org/10.1210/me.2008-0103.
Fan, Q.R., Hendrickson, W.A., 2005. Structure of human follicle-stimulating hormone in complex with its receptor. Nature 433, 269–277. http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=15662415.
Fan, H.Y., O'Connor, A., Shitanaka, M., Shimada, M., Liu, Z., Richards, J.S., 2010. Beta-catenin (CTNNB1) promotes preovulatory follicular development but represses LH-mediated ovulation and luteinization. Mol. Endocrinol. 24, 1529–1542. https://doi.org/10.1210/me.2010-0141.
Fanelli, F., Hanyaloglu, A.C., Jonas, K., 2020. Integrated structural modeling and super-resolution imaging resolve GPCR oligomers. Prog. Mol. Biol. Transl. Sci. 169, 151–179. https://doi.org/10.1016/bs.pmbts.2019.11.005.
Feng, X., Frank, J., 2024. Time-resolved cryo-EM (TRCEM) sample preparation using a PDMS-based microfluidic chip assembly. bioRxiv. https://doi.org/10.1101/2024.12.08.627437.
Fredriksson, R., Lagerstrom, M.C., Lundin, L.G., Schioth, H.B., 2003. The G-protein-coupled receptors in the human genome form five main families. Phylogenetic analysis, paralogon groups, and fingerprints. Mol. Pharmacol. 63, 1256–1272. http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=12761335.
Gonzalez de Valdivia, E., Broselid, S., Kahn, R., Olde, B., Leeb-Lundberg, L.M.F., 2017. G protein-coupled estrogen receptor 1 (GPER1)/GPR30 increases ERK1/2 activity through PDZ motif-dependent and -independent mechanisms. J. Biol. Chem. 292, 9932–9943. https://doi.org/10.1074/jbc.M116.765875.
Goodman Jr., O.B., Krupnick, J.G., Gurevich, V.V., Benovic, J.L., Keen, J.H., 1997. Arrestin/clathrin interaction. Localization of the arrestin binding locus to the clathrin terminal domain. J. Biol. Chem. 272, 15017–15022. http://www.ncbi.nlm.nih.gov/pubmed/9169477.
Gu, Y., Xu, W., Zhuang, B., Fu, W., 2018. Role of A-kinase anchoring protein 95 in the regulation of cytochrome P450 family 19 subfamily A member 1 (CYP19A1) in human ovarian granulosa cells. Reprod. Fertil. Dev. 30, 1128–1136. https://doi.org/10.1071/RD17313.
Guner, J.Z., Monsivais, D., Yu, H., et al., 2023. Oral follicle-stimulating hormone receptor agonist affects granulosa cells differently than recombinant human FSH. Fertil. Steril. 120, 1061–1070. https://doi.org/10.1016/j.fertnstert.2023.07.024.
Hanyroup, S., Anderson, R.C., Nataraja, S., Yu, H.N., Millar, R.P., Newton, C.L., 2021. Rescue of cell surface expression and signaling of mutant follicle-stimulating hormone receptors. Endocrinology 162. https://doi.org/10.1210/endocr/bqab134.
Hayes, E., Winston, N., Stocco, C., 2024. Molecular crosstalk between insulin-like growth factors and follicle-stimulating hormone in the regulation of granulosa cell function. Reprod. Med. Biol. 23, e12575. https://doi.org/10.1002/rmb2.12575.
Hernandez-Ramírez, L., Espinal-Enriquez, J., De Anda-Jáuregui, G., et al., 2022. Impact of small negative allosteric modulators (NAM) of the follicle-stimulating hormone (FSH) receptor on the dynamics of the transcriptome in rat granulosa cells exposed to different FSH glycosylation variants. J. Endocr. Soc. A589–A590.
Heublein, S., Lenhard, M., Vrekoussis, T., et al., 2012. The G-protein-coupled estrogen receptor (GPER) is expressed in normal human ovaries and is upregulated in ovarian endometriosis and pelvic inflammatory disease involving the ovary. Reprod. Sci. 19, 1197–1204. https://doi.org/10.1177/1933719112446085.
Heublein, S., Mayr, D., Vrekoussis, T., et al., 2013. The G-protein coupled estrogen receptor (GPER/GPR30) is a gonadotropin receptor dependent positive prognosticator in ovarian carcinoma patients. PLoS One 8, e71791. https://doi.org/10.1371/journal.pone.0071791.
Huhtaniemi, I., 2015. A short evolutionary history of FSH-stimulated spermatogenesis. Hormones (Athens) 14, 468–478. https://doi.org/10.14310/horm.2002.1632.
Janovick, J.A., Conn, P.M., 2010. Salt bridge integrates GPCR activation with protein trafficking. Proc. Natl. Acad. Sci. USA 107, 4454–4458. https://doi.org/10.1073/pnas.0914261107.
Janovick, J.A., Maya-Nunez, G., Ulloa-Aguirre, A., et al., 2009. Increased plasma membrane expression of human follicle-stimulating hormone receptor by a small molecule thienopyr(im)idine. Mol. Cell. Endocrinol. 298, 84–88. https://doi.org/10.1016/j.mce.2008.09.015.
Janovick, J.A., Spicer, T.P., Bannister, T.D., Scampavia, L., Conn, P.M., 2017. Pharmacoperone rescue of vasopressin 2 receptor mutants reveals unexpected constitutive activity and coupling bias. PLoS One 12, e0181830. https://doi.org/10.1371/journal.pone.0181830.
Jardon-Valadez, E., Ulloa-Aguirre, A., 2024. Tracking conformational transitions of the gonadotropin hormone receptors in a bilayer of (SDPC) poly-unsaturated lipids from all-atom molecular dynamics simulations. PLoS Comput. Biol. 20, e1011415. https://doi.org/10.1371/journal.pcbi.1011415.
Jean-Alphonse, F., Bowersox, S., Chen, S., Beard, G., Puthenveedu, M.A., Hanyaloglu, A. C., 2014. Spatially restricted G protein-coupled receptor activity via divergent endocytic compartments. J. Biol. Chem. 289, 3960–3977. https://doi.org/10.1074/jbc.M113.526350.
Jia, J., Tang, S., Yue, X., et al., 2023. An A-kinase anchoring protein (ACBD3) coordinates traffic-induced PKA activation at the Golgi. J. Biol. Chem. 299, 104696. https://doi.org/10.1016/j.jbc.2023.104696.
Jiang, X., Dreano, M., Buckler, D.R., et al., 1995. Structural predictions for the ligand-binding region of glycoprotein hormone receptors and the nature of hormone-receptor interactions. Structure 3, 1341–1353. https://doi.org/10.1016/s0969-2126(01)00272-6.
Jiang, X., Liu, H., Chen, X., et al., 2012. Structure of follicle-stimulating hormone in complex with the entire ectodomain of its receptor. Proc. Natl. Acad. Sci. USA 109, 12491–12496. https://doi.org/10.1073/pnas.1206643109.
Jiang, X., Fischer, D., Chen, X., et al., 2014a. Evidence for follicle-stimulating hormone receptor as a functional trimer. J. Biol. Chem. 289, 14273–14282. https://doi.org/10.1074/jbc.M114.549592.
Jiang, X., Dias, J.A., He, X., 2014b. Structural biology of glycoprotein hormones and their receptors: insights to signaling. Mol. Cell. Endocrinol. 382, 424–451. https://doi.org/10.1016/j.mce.2013.08.021.
Jonas, K.C., Hanyaloglu, A.C., 2019. Analysis of spatial assembly of GPCRs using photoactivatable dyes and localization microscopy. Methods Mol. Biol. 1947, 337–348. https://doi.org/10.1007/978-1-4939-9121-1_19.
Kara, E., Crepieux, P., Gauthier, C., et al., 2006. A phosphorylation cluster of five serine and threonine residues in the C-terminus of the follicle-stimulating hormone receptor is important for desensitization but not for beta-arrestin-mediated ERK activation. Mol. Endocrinol. 20, 3014–3026. http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=16887887.
Kene, P.S., Dighe, R.R., Mahale, S.D., 2005. Delineation of regions in the extracellular domain of follicle-stimulating hormone receptor involved in hormone binding and signal transduction. Am. J. Reprod. Immunol. 54, 38–48. https://doi.org/10.1111/j.1600-0897.2005.00285.x.
Kluetzman, K.S., Thomas, R.M., Nechamen, C.A., Dias, J.A., 2011. Decreased degradation of internalized follicle-stimulating hormone caused by mutation of aspartic acid 6.30 (550) in a protein kinase-CK2 consensus sequence in the third intracellular loop of human follicle-stimulating hormone receptor. Biol. Reprod. 84, 1154–1163. https://doi.org/10.1095/biolreprod.110.087965.
Krishnamurthy, H., Kishi, H., Shi, M., et al., 2003. Postendocytotic trafficking of the follicle-stimulating hormone (FSH)-FSH receptor complex. Mol. Endocrinol. 17, 2162–2176. http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=12907758.
Law, N.C., Donaubauer, E.M., Zeleznik, A.J., Hunzicker-Dunn, M., 2017. How protein kinase A activates canonical tyrosine kinase signaling pathways to promote granulosa cell differentiation. Endocrinology 158, 2043–2051. https://doi.org/10.1210/en.2017-00163.
Lazzaretti, C., Simoni, M., Casarini, L., Paradiso, E., 2023. Allosteric modulation of gonadotropin receptors. Front. Endocrinol. 14, 1179079. https://doi.org/10.3389/fendo.2023.1179079.
Li, H., Degenhardt, B., Tobin, D., Yao, Z.X., Tasken, K., Papadopoulos, V., 2001. Identification, localization, and function in steroidogenesis of PAP7: a peripheral-type benzodiazepine receptor- and PKA (RIalpha)-associated protein. Mol. Endocrinol. 15, 2211–2228. https://doi.org/10.1210/mend.15.12.0736.
Liu, A.M., Ho, M.K., Wong, C.S., Chan, J.H., Pau, A.H., Wong, Y.H., 2003a. Galpha(16/z) chimeras efficiently link a wide range of G protein-coupled receptors to calcium mobilization. J. Biomol. Screen 8, 39–49. https://doi.org/10.1177/1087057102239665.
Liu, J., Li, H., Papadopoulos, V., 2003b. PAP7, a PBR/PKA-RIalpha-associated protein: a new element in the relay of the hormonal induction of steroidogenesis. J. Steroid Biochem. Mol. Biol. 85, 275–283. https://doi.org/10.1016/s0960-0760(03)00213-9.
Lohse, M.J., Bock, A., Zaccolo, M., 2023. G protein-coupled receptor signaling: new insights define cellular nanodomains. Annu. Rev. Pharmacol. Toxicol. https://doi.org/10.1146/annurev-pharmtox-040623-115054.
Loozen, H.J., Timmers, C.M., 2010. (DIHYDRO) IMIDAZOISO (5, I-A) QUINOLINES AS FSH RECEPTOR AGONISTS FOR THE TREATMENT OF FERTILITY DISORDERSWO 2010/136438 Al. inventors.
Lucia, K., Wu, Y., Garcia, J.M., et al., 2020. Hypoxia and the hypoxia inducible factor 1alpha activate protein kinase A by repressing RII beta subunit transcription. Oncogene 39, 3367–3380. https://doi.org/10.1038/s41388-020-1223-6.
Luiro, K., Aittomaki, K., Jousilahti, P., Tapanainen, J.S., 2019. Long-term health of women with genetic POI due to FSH-resistant ovaries. Endocr Connect 8, 1354–1362. https://doi.org/10.1530/EC-19-0244.
Lundin, K., Sepponen, K., Vayrynen, P., et al., 2022. Human pluripotent stem cell-derived cells endogenously expressing follicle-stimulating hormone receptors: modeling the function of an inactivating receptor mutation. Mol. Hum. Reprod. 28. https://doi.org/10.1093/molehr/gaac012.

Lyga, S., Volpe, S., Werthmann, R.C., et al., 2016. Persistent cAMP signaling by internalized LH receptors in ovarian follicles. Endocrinology 157, 1613–1621. https://doi.org/10.1210/en.2015-1945.

Maclean, D., Holden, F., Davis, A.M., et al., 2004. Agonists of the follicle stimulating hormone receptor from an encoded thiazolidinone library. J. Comb. Chem. 6, 196–206. https://doi.org/10.1021/cc0300154.

Maggiolini, M., Vivacqua, A., Fasanella, G., Recchia, A.G., Sisci, D., Pezzi, V., et al., 2004. The G protein-coupled receptor GPR30 mediates c-fos up-regulation by 17beta-estradiol and phytoestrogens in breast cancer cells. J. Biol. Chem. 279, 27008–27016. https://doi.org/10.1074/jbc.M403588200.

Mahale, S.D., Cavanagh, J., Schmidt, A., MacColl, R., Dias, J.A., 2001. Autologous biological response modification of the gonadotropin receptor. J. Biol. Chem. 276, 12410–12419.

Mannes, M., Martin, C., Menet, C., Ballet, S., 2022. Wandering beyond small molecules: peptides as allosteric protein modulators. Trends Pharmacol. Sci. 43, 406–423. https://doi.org/10.1016/j.tips.2021.10.011.

Manson, M.E., Corey, D.A., Rymut, S.M., Kelley, T.J., 2011. beta-arrestin-2 regulation of the cAMP response element binding protein. Biochemistry 50, 6022–6029. https://doi.org/10.1021/bi200015h.

Mazurkiewicz, J.E., Herrick-Davis, K., Barroso, M., et al., 2015. Single-molecule analyses of fully functional fluorescent protein-tagged follitropin receptor reveal homodimerization and specific heterodimerization with lutropin receptor. Biol. Reprod. 92, 100 doi: biolreprod.114.125781 [pii];10.1095/biolreprod.114.125781 [doi].

Mueller, S., Jaeschke, H., Gunther, R., Paschke, R., 2009. The Hinge region: an important receptor component for GPHR function. Trends Endocrinol. Metabol.: TEM 21, 111–122 doi: S1043-2760(09)00150-7 [pii]10.1016/j.tem.2009.09.001.

Nataraja, S., Sriraman, V., Palmer, S., 2018. Allosteric regulation of the follicle-stimulating hormone receptor. Endocrinology 159, 2704–2716 doi: 5001727 [pii]; 10.1210/en.2018-00317 [doi].

Nataraja, S., Yu, H., Guner, J., Palmer, S., 2020. Discovery and preclinical development of orally active small molecules that exhibit highly selective follicle stimulating hormone receptor agonism. Front. Pharmacol. 11, 602593. https://doi.org/10.3389/fphar.2020.602593.

Nechamen, C.A., Dias, J.A., 2000. Human follicle stimulating hormone receptor trafficking and hormone binding sites in the amino terminus. Mol. Cell. Endocrinol. 166, 101–110 doi: S0303-7207(00)00281-1 [pii].

Nechamen, C.A., Dias, J.A., 2003. Point mutations in follitropin receptor result in ER retention. Mol. Cell. Endocrinol. 201, 123–131. http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=12706300.

Nechamen, C.A., Thomas, R.M., Cohen, B.D., et al., 2004. Human follicle-stimulating hormone (FSH) receptor interacts with the adaptor protein APPL1 in HEK 293 cells: potential involvement of the PI3K pathway in FSH signaling. Biol. Reprod. 71, 629–636. http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=15070827.

Nechamen, C.A., Thomas, R.M., Dias, J.A., 2007. APPL1, APPL2, Akt2 and FOXO1a interact with FSHR in a potential signaling complex. Mol. Cell. Endocrinol. 260–262, 93–99. http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=17030088.

Newton, C.L., Whay, A.M., McArdle, C.A., et al., 2011. Rescue of expression and signaling of human luteinizing hormone G protein-coupled receptor mutants with an allosterically binding small-molecule agonist. Proc. Natl. Acad. Sci. USA 108, 7172–7176 doi: 1015723108 [pii]10.1073/pnas.1015723108.

Newton, C.L., Anderson, R.C., Kreuchwig, A., Krause, G., Katz, A.A., Millar, R.P., 2021. Rescue of function of mutant luteinising hormone receptors with deficiencies in cell surface expression, hormone binding and hormone signalling. Neuroendocrinology. https://doi.org/10.1159/000508000.

Ng, S.S.M., Jorge, S., Malik, M., et al., 2019. A-Kinase anchoring protein 13 (AKAP13) augments progesterone signaling in uterine fibroid cells. J. Clin. Endocrinol. Metab. 104, 970–980. https://doi.org/10.1210/jc.2018-01216.

Oduwole, O.O., Huhtaniemi, I.T., Misrahi, M., 2021. The roles of luteinizing hormone, follicle-stimulating hormone and testosterone in spermatogenesis and folliculogenesis revisited. Int. J. Mol. Sci. 22. https://doi.org/10.3390/ijms222312735.

Otani, T., Mizokami, A., Hayashi, Y., et al., 2015. Signaling pathway for adiponectin expression in adipocytes by osteocalcin. Cell. Signal. 27, 532–544. https://doi.org/10.1016/j.cellsig.2014.12.018.

Parakh, T.N., Hernandez, J.A., Grammer, J.C., et al., 2006. Follicle-stimulating hormone/cAMP regulation of aromatase gene expression requires beta-catenin. Proc. Natl. Acad. Sci. USA 103, 12435–12440. https://doi.org/10.1073/pnas.0603006103.

Pasapera, A.M., Jimenez-Aguilera Mdel, P., Chauchereau, A., et al., 2005. Effects of FSH and 17beta-estradiol on the transactivation of estrogen-regulated promoters and cell proliferation in L cells. J. Steroid Biochem. Mol. Biol. 94, 289–302. http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=15857748.

Pavlik, R., Wypior, G., Hecht, S., et al., 2011. Induction of G protein-coupled estrogen receptor (GPER) and nuclear steroid hormone receptors by gonadotropins in human granulosa cells. Histochem. Cell Biol. 136, 289–299. https://doi.org/10.1007/s00418-011-0846-7.

Prabhudesai, K.S., Aathi, M.S., Dighe, V., Idicula-Thomas, S., 2021. A 5-mer peptide derived from Hinge region of hFSHR can function as positive allosteric modulator in vivo. Biochim. Biophys. Acta Biomembr. 1863, 183492. https://doi.org/10.1016/j.bbamem.2020.183492.

Prossnitz, E.R., Hathaway, H.J., 2015. What have we learned about GPER function in physiology and disease from knockout mice? J. Steroid Biochem. Mol. Biol. 153, 114–126. https://doi.org/10.1016/j.jsbmb.2015.06.014.

Rannikko, A., Pakarinen, P., Manna, P.R., et al., 2002. Functional characterization of the human FSH receptor with an inactivating Ala189Val mutation. Mol. Hum. Reprod. 8, 311–317. http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=11912278.

Ratoosh, S.L., Lifka, J., Hedin, L., Jahsen, T., Richards, J.S., 1987. Hormonal regulation of the synthesis and mRNA content of the regulatory subunit of cyclic AMP-dependent protein kinase type II in cultured rat ovarian granulosa cells. J. Biol. Chem. 262, 7306–7313. https://www.ncbi.nlm.nih.gov/pubmed/3034888.

Reiter, E., Ayoub, M.A., Pellissier, L.P., et al., 2017. beta-arrestin signalling and bias in hormone-responsive GPCRs. Mol. Cell. Endocrinol. 449, 28–41 doi: S0303-7207(17)30074-6 [pii];10.1016/j.mce.2017.01.052 [doi].

Richards, J.S., Pangas, S.A., 2010. The ovary: basic biology and clinical implications. J. Clin. Investig. 120, 963–972. https://doi.org/10.1172/JCI41350.

Ryu, K., Gilchrist, R.L., Tung, C.S., Ji, I., Ji, T.H., 1998. High affinity hormone binding to the extracellular N-terminal exodomain of the follicle-stimulating hormone receptor is critically modulated by exoloop 3. J. Biol. Chem. 273, 28953–28958. http://www.ncbi.nlm.nih.gov/pubmed/9786899.

Sandrini, F., Matyakhina, L., Sarlis, N.J., et al., 2002. Regulatory subunit type I-alpha of protein kinase A (PRKAR1A): a tumor-suppressor gene for sporadic thyroid cancer. Genes Chromosomes Cancer 35, 182–192. https://doi.org/10.1002/gcc.10112.

Schmidt, A., MacColl, R., Lindau-Shepard, B., Buckler, D.R., Dias, J.A., 2001. Hormone-induced conformational change of the purified soluble hormone binding domain of follitropin receptor complexed with single chain follitropin. J. Biol. Chem. 276, 23373–23381. http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=11313343.

Seitz, E., Acosta-Reyes, F., Maji, S., Schwander, P., Frank, J., 2022. Recovery of Conformational Continuum from single-particle Cryo-EM images: optimization of ManifoldEM informed by ground truth. IEEE Trans Comput Imag. 8, 462–478. https://doi.org/10.1109/tci.2022.3174801.

Shchepinova, M.M., Hanyaloglu, A.C., Frost, G.S., Tate, E.W., 2020. Chemical biology of noncanonical G protein-coupled receptor signaling: toward advanced therapeutics. Curr. Opin. Chem. Biol. 56, 98–110. https://doi.org/10.1016/j.cbpa.2020.04.012.

Smith, F.D., Esseltine, J.L., Nygren, P.J., et al., 2017. Local protein kinase A action proceeds through intact holoenzymes. Science 356, 1288–1293. https://doi.org/10.1126/science.aaj1669.

Smith, E., Janovick, J.A., Bannister, T.D., et al., 2020. Rescue of mutant gonadotropin-releasing hormone receptor function independent of cognate receptor activity. Sci. Rep. 10, 10579. https://doi.org/10.1038/s41598-020-67473-w.

Smits, G., Campillo, M., Govaerts, C., et al., 2003. Glycoprotein hormone receptors: determinants in leucine-rich repeats responsible for ligand specificity. EMBO J. 22, 2692–2703. https://doi.org/10.1093/emboj/cdg260.

Sposini, S., Hanyaloglu, A.C., 2017. Spatial encryption of G protein-coupled receptor signaling in endosomes; Mechanisms and applications. Biochem. Pharmacol. 143, 1–9. https://doi.org/10.1016/j.bcp.2017.04.028.

Sposini, S., Jean-Alphonse, F.G., Ayoub, M.A., et al., 2017. Integration of GPCR signaling and sorting from very early endosomes via opposing APPL1 mechanisms. Cell Rep. 21, 2855–2867. https://doi.org/10.1016/j.celrep.2017.11.023.

Sposini, S., De Pascali, F., Richardson, R., et al., 2020. Pharmacological programming of endosomal signaling activated by small molecule ligands of the follicle stimulating hormone receptor. Front. Pharmacol. 11, 593492. https://doi.org/10.3389/fphar.2020.593492.

Sriraman, V., Denis, D., de Matos, D., Yu, H., Palmer, S., Nataraja, S., 2014. Investigation of a thiazolidinone derivative as an allosteric modulator of follicle stimulating hormone receptor: evidence for its ability to support follicular development and ovulation. Biochem. Pharmacol. 89, 266–275. https://doi.org/10.1016/j.bcp.2014.02.023.

Thomas, R.M., Nechamen, C.A., Mazurkiewicz, J.E., Ulloa-Aguirre, A., Dias, J.A., 2011. The adapter protein APPL1 links FSH receptor to inositol 1,4,5-trisphosphate production and is implicated in intracellular Ca(2+) mobilization. Endocrinology 152, 1691–1701 doi: en.2010-1353 [pii];10.1210/en.2010-1353 [doi].

Timmers, C.M., Loozen, H.J.J., 2011. Dihydrobenzoindazoles. inventors.

Timossi, C.M., Barrios-de-Tomasi, J., Gonzalez-Suarez, R., et al., 2000. Differential effects of the charge variants of human follicle-stimulating hormone. J. Endocrinol. 165, 193–205. http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=10810283.

Tranchant, T., Durand, G., Gauthier, C., et al., 2011. Preferential beta-arrestin signalling at low receptor density revealed by functional characterization of the human FSH receptor A189 V mutation. Mol. Cell. Endocrinol. 331, 109–118. https://doi.org/10.1016/j.mce.2010.08.016.

Ulloa-Aguirre, A., Conn, P.M., 2016. Pharmacoperones as a new therapeutic approach: in vitro identification and in vivo validation of bioactive molecules. Curr. Drug Targets 17, 1471–1481. https://doi.org/10.2174/1389450117666160307143345.

Ulloa-Aguirre, A., Zarinan, T., 2016. The follitropin receptor: matching structure and function. Mol. Pharmacol. 90, 596–608. https://doi.org/10.1124/mol.116.104398.

Ulloa-Aguirre, A., Crepieux, P., Poupon, A., Maurel, M.C., Reiter, E., 2011. Novel pathways in gonadotropin receptor signaling and biased agonism. Rev. Endocr. Metab. Disord. 12, 259–274. https://doi.org/10.1007/s11154-011-9176-2.

Ulloa-Aguirre, A., Dias, J.A., Bousfield, G.R., 2017. Gonadotropins. Endocrinology of the Testis and Male Reproduction. Springer Int. Publishing, pp. 71–122.

Ulloa-Aguirre, A., Reiter, E., Crepieux, P., 2018. FSH receptor signaling: complexity of interactions and signal diversity. Endocrinology 159, 3020–3035. https://doi.org/10.1210/en.2018-00452.

Ulloa-Aguirre, A., Zarinan, T., Dias, J.A., Kumar, T.R., Bousfield, G.R., 2025a. Biased signaling by human follicle-stimulating hormone variants. Pharmacol. Therapeut. 268, 108821. https://doi.org/10.1016/j.pharmthera.2025.108821.

Ulloa-Aguirre, A., Jardón-Valadez, E., Zarinan, T., Gutiérrez-Sagal, R., Nataraja, S., Yu, H., 2025b. Structural features and functional effects of the follicle-stimulating hormone receptor (FSHR) allosteric agonist CAN1405 as disclosed by in silico and in vitro studies. J. Endocr. Soc. 9 (Suppl. 1), A1030–A1031. https://doi.org/10.1210/jendso/bvaf149.

Ulloa-Aguirre, A., Anderson, R.C., Zarinan, T., Gutierrez-Sagal, R., Jardon-Valadez, E., Newton, C.L., 2025c. Folding, misfolding, and regulation of intracellular traffic of G protein-coupled receptors involved in the hypothalamic-pituitary-gonadal axis. Andrology. https://doi.org/10.1111/andr.70018.

van Koppen, C.J., Verbost, P.M., van de Lagemaat, R., et al., 2013. Signaling of an allosteric, nanomolar potent, low molecular weight agonist for the follicle-stimulating hormone receptor. Biochem. Pharmacol. 85, 1162–1170. https://doi.org/10.1016/j.bcp.2013.02.001.

van Straten, N.C., van Berkel, T.H., Demont, D.R., et al., 2005. Identification of substituted 6-amino-4-phenyltetrahydroquinoline derivatives: potent antagonists for the follicle-stimulating hormone receptor. J. Med. Chem. 48, 1697–1700. https://doi.org/10.1021/jm049676l.

Wess, J., Oteng, A.B., Rivera-Gonzalez, O., Gurevich, E.V., Gurevich, V.V., 2023. beta-Arrestins: structure, function, physiology, and pharmacological perspectives. Pharmacol. Rev. 75, 854–884. https://doi.org/10.1124/pharmrev.121.000302.

Wiggin, G.R., Soloaga, A., Foster, J.M., Murray-Tait, V., Cohen, P., Arthur, J.S., 2002. MSK1 and MSK2 are required for the mitogen- and stress-induced phosphorylation of CREB and ATF1 in fibroblasts. Mol. Cell Biol. 22, 2871–2881. https://doi.org/10.1128/MCB.22.8.2871-2881.2002.

Wortmann, L., Muhn, H.-P., Menzenbach, B., et al., 2008. 1,2-Diarylacetylene Derivatives of Acyltryptophanols. inventors.

Wortmann, L., Lindenthal, B., Muhn, P., et al., 2019. Discovery of BAY-298 and BAY-899: Tetrahydro-1,6-naphthyridine-based, potent, and selective antagonists of the luteinizing hormone receptor which reduce sex hormone levels in vivo. J. Med. Chem. 62, 10321–10341. https://doi.org/10.1021/acs.jmedchem.9b01382.

Wrobel, J., Green, D., Jetter, J., et al., 2002. Synthesis of (bis)sulfonic acid, (bis) benzamides as follicle-stimulating hormone (FSH) antagonists. Bioorg. Med. Chem. 10, 639–656. http://www.ncbi.nlm.nih.gov/pubmed/11814852.

Wu, G., Li, C., Tao, J., et al., 2022. FSH mediates estradiol synthesis in hypoxic granulosa cells by activating glycolytic metabolism through the HIF-1alpha-AMPK-GLUT1 signaling pathway. J. Biol. Chem. 298, 101830. https://doi.org/10.1016/j.jbc.2022.101830.

Xia, L., Sun, J., Xie, S., et al., 2020. PRKAR2B-HIF-1alpha loop promotes aerobic glycolysis and tumour growth in prostate cancer. Cell Prolif. 53, e12918. https://doi.org/10.1111/cpr.12918.

Yanofsky, S.D., Shen, E.S., Holden, F., et al., 2006. Allosteric activation of the follicle-stimulating hormone (FSH) receptor by selective, nonpeptide agonists. J. Biol. Chem. 281, 13226–13233. https://doi.org/10.1074/jbc.M600601200.

Yu, H.N., Richardson, T.E., Nataraja, S., et al., 2014. Discovery of substituted benzamides as follicle stimulating hormone receptor allosteric modulators. Bioorg. Med. Chem. Lett 24, 2168–2172. https://doi.org/10.1016/j.bmcl.2014.03.018.

Zanassi, P., Paolillo, M., Feliciello, A., Avvedimento, E.V., Gallo, V., Schinelli, S., 2001. cAMP-dependent protein kinase induces cAMP-response element-binding protein phosphorylation via an intracellular calcium release/ERK-dependent pathway in striatal neurons. J. Biol. Chem. 276, 11487–11495. https://doi.org/10.1074/jbc.M007631200.

Zarinan, T., Butnev, V.Y., Gutierrez-Sagal, R., et al., 2020. In vitro impact of FSH glycosylation variants on FSH receptor-stimulated signal transduction and functional selectivity. J. Endocr. Soc. 4. https://doi.org/10.1210/jendso/bvaa019 bvaa019.

Zarinan, T., Mayorga, J., Jardon-Valadez, E., et al., 2021. A novel mutation in the FSH receptor (I423T) affecting receptor activation and leading to primary ovarian failure. J. Clin. Endocrinol. Metab. 106, e534–e550. https://doi.org/10.1210/clinem/dgaa782.

Zarinan, T., Espinal-Enriquez, J., De Anda-Jauregui, G., et al., 2024. Differential effects of follicle-stimulating hormone glycoforms on the transcriptome profile of cultured rat granulosa cells as disclosed by RNA-seq. PLoS One 19, e0293688. https://doi.org/10.1371/journal.pone.0293688.

Zeleznik, A.J., Saxena, D., Little-Ihrig, L., 2003. Protein kinase B is obligatory for follicle-stimulating hormone-induced granulosa cell differentiation. Endocrinology 144, 3985–3994. http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=12933673.

Zhan, T., Zhang, J., Zhang, Y., et al., 2024. A dose-response study on functional and transcriptomic effects of FSH on Ex vivo mouse folliculogenesis. Endocrinology 165. https://doi.org/10.1210/endocr/bqae054.

Zhong, H., SuYang, H., Erdjument-Bromage, H., Tempst, P., Ghosh, S., 1997. The transcriptional activity of NF-kappaB is regulated by the IkappaB-associated PKAc subunit through a cyclic AMP-independent mechanism. Cell 89, 413–424. https://doi.org/10.1016/s0092-8674(00)80222-6.

Zhou, P., Baumgarten, S.C., Wu, Y., et al., 2013. IGF-I signaling is essential for FSH stimulation of AKT and steroidogenic genes in granulosa cells. Mol. Endocrinol. 27, 511–523. https://doi.org/10.1210/me.2012-1307.