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Induction of sperm hypermotility through membrane progestin receptor alpha (mPRα): A teleost model of rapid, multifaceted, nongenomic progestin signaling

Wenxian Tan[a,b], Yefei Pang[a], Christopher Tubbs[a,c], Peter Thomas[a,*]

[a] The University of Texas at Austin Marine Science Institute, 750 Channel View Drive, Port Aransas, TX 78373, United States
[b] Huston-Tillotson University, 900 Chicon Street, Austin, TX 78702, United States
[c] San Diego Zoo Global, Institute for Conservation Research, 15600 San Pasqual Valley Road, Escondido, CA 92027, United States

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ABSTRACT

Rapid progestin effects on sperm physiology have been described in a variety of vertebrate species. Here, we briefly review the signaling pathways mediating rapid progestin induction of sperm hypermotility and increased fertility in two teleost species, Atlantic croaker and southern flounder. Acute *in vitro* treatment of teleost sperm with the progestin hormone, 20β-S, causes activation of progestin membrane receptor alpha (mPRα, or Paqr7) coupled to a stimulatory olfactory G protein (G_{olf}), resulting in increased cAMP and calcium concentrations and hypermotility upon activation in a hyperosmotic medium. Pharmacological tools were used to investigate the involvement of mPRα and several intracellular signaling pathways in the hypermotility response. Evidence was obtained using the specific mPRα agonist, Org OD 02-0, that this progestin action is mediated through mPRα and not through the nuclear PR. The results indicate that progestins induce hypermotility through activation of a membrane adenylyl cyclase (Acy)/cAMP pathway, an epidermal growth factor receptor (Egfr)/Mapkinase pathway, and a Pi3kinase/Akt/phosphodiesterase (Pde) pathway which result in increased sperm calcium concentrations within 10 s. The finding that inhibition of any one of these pathways is sufficient to prevent hypermotility along with the calcium increase suggests that activation of all of them and the associated calcium increase are required for the progestin hypermotility response. On the basis of these findings a model of progestin induction of sperm hypermotility in teleosts is proposed. As teleosts lack CatSper, the model described here is a non-CatSper mediated one and may therefore be applicable to a wide variety of nonmammalian vertebrates.

1. Introduction

Rapid, nongenomic progestin actions at physiological nanomolar concentrations on sperm physiology have been described in a variety of vertebrate species including the induction of hypermotility in teleost fish (Harper et al., 2004; Lishko et al., 2011; Sagare-Patil et al., 2012; Thomas, 2003; 2012). However, the mechanisms governing these effects are still not clearly elucidated, especially in teleosts, amphibians, and birds, which do not express CatSper, the calcium channel mediating progesterone stimulation of sperm motility in mammals (Cai and Clapham, 2008). In contrast to many internally fertilizing species, whose sperm acquire motility during passage through the epididymis (Cobellis et al., 2010), sperm of most teleost species with external fertilization are immotile in the seminal fluid and motility is triggered by the sudden change in external osmolality upon release of sperm into the aquatic environment (Cosson et al., 2008). Pre-treatment of sperm from teleosts belonging to two divergent families; Sciaenidae – Atlantic croaker (*Micropogonias undulatus*), spotted seatrout (*Cynoscion nebulosus*) and red drum (*Sciaenops ocellatus*), and Achiropsettidae – southern flounder (*Paralichthys lethostigma*) with the endogenous teleost progestin hormone, 17, 20β, 21-trihydroxy-4-pregnen-3-one (20β-S), increases sperm hypermotility, which is characterized by higher sperm swimming velocity upon activation with a high osmolality activation buffer (Thomas et al., 2004; Tubbs and Thomas, 2008, 2009; Tubbs et al., 2011). Moreover, the induction of hypermotility in both Atlantic croaker and southern flounder sperm is associated with a dramatic increase in fertilization success (Tubbs and Thomas, 2009; Tan et al., 2014). The essential role of membrane progestin receptor-alpha (mPRα, also known as Paqr7) in mediating sperm hypermotility has been investigated extensively in Atlantic croaker and southern flounder (Tubbs

* Corresponding author.
E-mail address: peter.thomas@utexas.edu (P. Thomas).

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and Thomas, 2009; Tan and Thomas, 2014; Tan et al., 2014). The signaling pathways mediating progestin induction of sperm motility can be studied in isolation in teleost sperm without concern for those mediating progestin induction of the acrosome reaction, because the acrosome is absent in teleost sperm. Here, we discuss the role of mPRα in mediating progestin stimulated sperm hypermotility in both teleost species, as well as the intracellular mechanisms that regulate this event.

Vertebrate sperm are good models for examining nongenomic steroid actions in isolation because sperm are transcriptionally inactive due to chromatin condensation. However, this characteristic of sperm precludes the use of gene silencing procedures to knockdown expression of mPRα and other receptors for loss-of-function experiments in order to confirm their physiological roles. The recent discovery of two synthetic progestins displaying potent mPRα agonist activities in G protein and mitogen-activated protein kinase (MAPK) activation assays in mPRα-transfected cells, but showing no activity in a nuclear progesterone receptor transactivation assay, provides powerful tools for identifying progestin functions mediated by mPRα in sperm (Kelder et al., 2010). Thus, one of these compounds, 10-ethenyl-19-norprogesterone (Org OD 02-0), was used to confirm that progestin activation of teleost sperm hypermotility is mediated through mPRα.

2. Membrane progestin receptor (mPRα) as mediator of sperm hypermotility in teleosts

Membrane progestin receptors, hypothesized to be the progestin-binding entities that mediate sperm hypermotility, have been biochemically characterized on sperm plasma membranes of spotted seatrout (Thomas et al., 1997), Atlantic croaker (Thomas et al., 2005) and southern flounder (Tubbs et al., 2011). The novel membrane receptor membrane progestin receptor alpha (mPRα, also known as Paqr7), originally identified on spotted seatrout ovaries and sperm (Zhu et al., 2003), has characteristics of a progestin and adipoQ receptor (PAQR) (Thomas et al., 2007), and has been proposed as a candidate for the receptor mediating progestin induction of sperm hypermotility. The mPRα protein is highly expressed on the plasma membrane and localized to the sperm midpiece and flagella in these three teleost species (Zhu et al., 2003; Tubbs and Thomas, 2008, 2009; Tubbs et al., 2011; Fig. 1A) and humans (Thomas et al., 2009). Both sperm mPRα expression and sperm motility are upregulated in croaker and flounder by *in vivo* treatments with LHRHa (Tubbs and Thomas, 2009; Tan et al., 2014). Furthermore, there is a positive correlation between the abundance of mPRα on the plasma membranes of croaker and flounder sperm and increases in sperm swimming velocity in response *in vitro* treatment with a physiological concentration of 20β-S (Tubbs and Thomas, 2009; Tubbs et al., 2011; Fig. 1C). These findings were replicated in both of these species using Org OD-02-0 (Tan and Thomas, 2014, 2015; Tan et al., 2014), confirming that the progestin stimulation of sperm motility in both croaker and flounder species is mediated by mPRα. Interestingly, hypermotility is also positively correlated with high mPRα abundance sperm of another flatfish species, turbot (Feng et al., 2018). Although there was considerable debate initially over the role of mPRs in progestin signaling in vertebrate cells, it is now widely accepted that mPRs are high affinity, specific progestin receptors (Smith et al., 2008; Gellersen et al., 2009; Thomas, 2012),

3. Teleost sperm expresses the stimulatory olfactory G protein (G_{olf})

G protein activation has been demonstrated through all five mPR subtypes in a variety of vertebrate cells (Thomas et al., 2007; Pang and Thomas, 2011; Pang et al., 2013). However, the finding that progestin signaling through recombinant mPRs has also been observed in a heterologous yeast expression system that does not naturally express G proteins suggests that mPRs potentially could also signal through G protein-independent mechanisms (Smith et al., 2008). Membrane

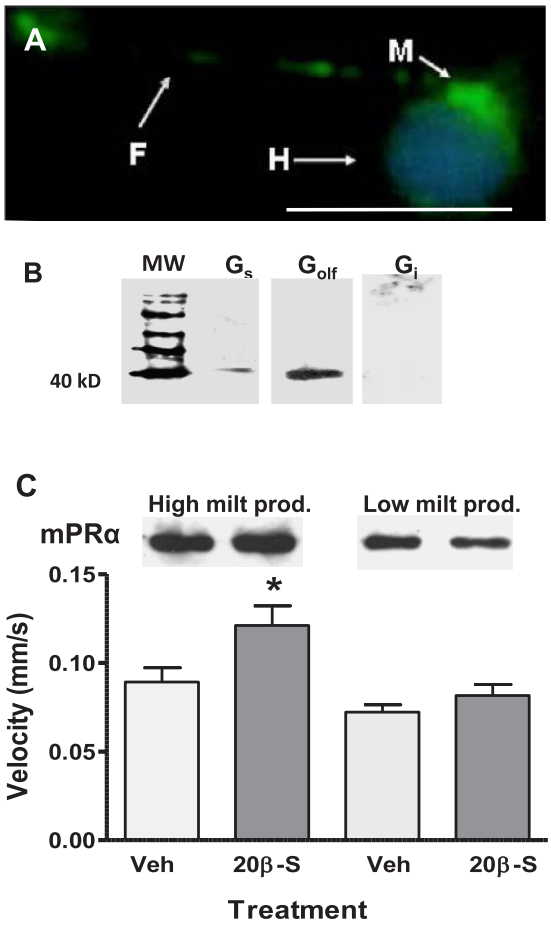

Fig. 1. Detection of membrane progestin receptor alpha (mPRα) (A) and olfactory G-protein, G_{olf} (B) on flounder sperm by immunocytochemistry and Western blot analysis, respectively. A. M, midpiece; F, flagella; H, head (stained blue with DAPI); bar-5 μm. B. MW, molecular weight marker, G_s, stimulatory G protein, G_i, inhibitory protein. Image A from Tubbs et al. (2011), General and Comparative Endocrinology, with permission. Image B from Tan et al. (2014), General and Comparative Endocrinology, with permission. C. Effects of mPRα expression on flounder sperm on 20β-S-induction of hypermotility. Top, representative Western blots of mPRα expression in sperm from males with high and low milt production; Bottom, Effects of mPRα expression on flounder sperm from high and low milt donors on hypermotility response to 20β-S ($^*P < 0.05$ compared to vehicle control, Student's *t* test, N = 6). Image C reproduced from Tubbs et al. (2011), General and Comparative Endocrinology, with permission.

progesterone alpha is coupled to and activates a pertussis toxin-sensitive inhibitory G protein (G_i) in the majority of the tissues examined, including teleost ovaries (Thomas, 2012). However, both croaker and flounder sperm membranes display high expression of the stimulatory olfactory G protein (G_{olf}) which is co-localized with mPRα on the midpiece and flagella in croaker (Tubbs and Thomas, 2009; Tan et al., 2014; Fig. 1A, B). Moreover, G_{olf} co-immunoprecipitates with mPRα from croaker sperm membrane fractions and is activated in response to 20β-S treatment (Tubbs and Thomas, 2009), suggesting this stimulatory G protein is coupled to mPRα and plays a role in progestin stimulation of teleost sperm hypermotility.

4. Membrane-bound adenylyl cyclase and cAMP levels are involved in mediating teleost sperm hypermotility

Cyclic adenosine monophosphate (cAMP) has been known since early studies in the 1970s and 80s to have an essential role in initiation of vertebrate sperm motility (Garbers et al., 1971; Morisawa, 1985).

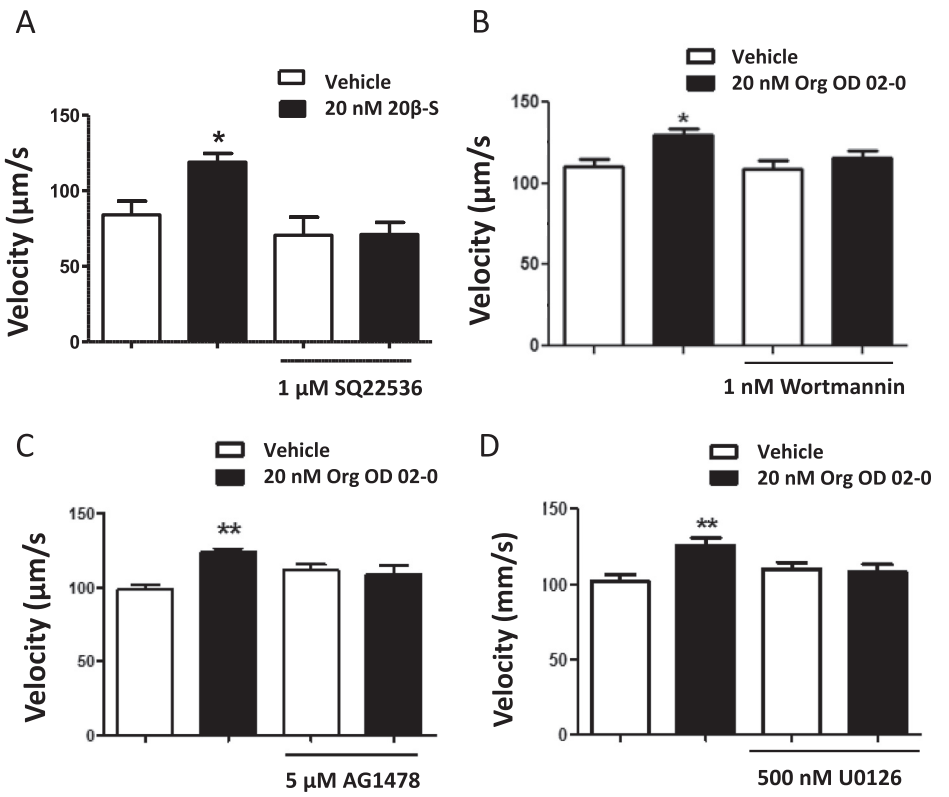

Fig. 2. Effects of 30 min preincubation with inhibitors of various signaling pathways on progestin-induced hypermotility of Atlantic croaker sperm. A. Effects of pretreatment with Acy inhibitor, SQ22536 (1 μM) on 20β-S-induced (20 nM) sperm hypermotility. Figure A repeat of experiment in Tubbs and Thomas (2009), Endocrinology. B. Effects of pretreatment with PI3K inhibitor, Wortmannin (1 nM) on the sperm motility response to 20 nM Org OD 02-0. Figure B reproduced from Tan and Thomas (2014), Biology of Reproduction, with permission. C. Effects of pretreatment with the EGFR inhibitor, AG1478 (5 μM) on the motility response. D. Effects of pretreatment with MEK1/2 inhibitor, U0126 (500 nM) on the motility response. Asterisks denote progestin-induced values significantly different from respective controls ($^{*}P < 0.05$, $^{**}P < 0.01$, Students t test, N = 8–15). Figures C and D reproduced from Tan and Thomas (2015), Molecular and Cellular Endocrinology, with permission.

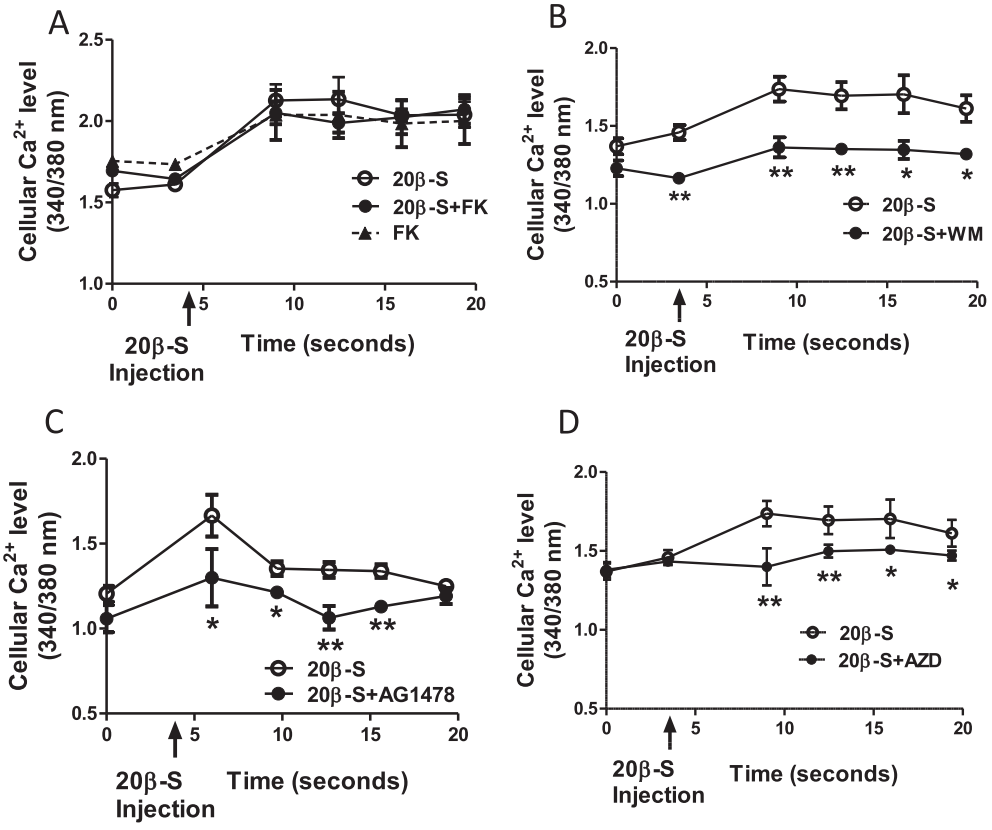

Fig. 3. Effects of 30-minute preincubations with inhibitors and pretreatment with an activator of various signaling pathways on 20 nM (C) and −100 nM (A, B, D) 20β-S-induced free Ca^{2+} increase in Atlantic croaker sperm. A. Effects of pretreatment with Acy stimulator, forskolin (FK, 10 μM) on Ca^{2+} levels. B. Effects of pretreatment with PI3K inhibitor, Wortmannin (WM, 10 nM) on Ca^{2+} levels. C. Effects of treatment with an EGFR inhibitor AG1478 (10 mM) on Ca^{2+} levels. D. Effects of pretreatment with MEK1/2 inhibitor, AZD6244 (AZD, 1 μM) on Ca^{2+} levels. 20β-S injection, indicates time when 20β-S is injected into the treatment well. Intracellular Ca^{2+} concentrations were measured using a Ca^{2+}-fluorescent dye, Fura − 2/AM, following the procedures described previously by Alasmari et al., 2013 with few modifications. Fura-2/AM (2 μM) was loaded into croaker sperm (5×10^7 cells/ml) by incubation in predilutant for 1 h. Aliquots of Fura2/AM loaded sperm was transferred to a 96-well plate, and the increase in Ca^{2+} concentrations in response to various treatments compared to vehicle controls was measured over 20 sec using a fluorescence plate reader equipped with dual injectors to add the compounds. The results were analyzed with data analysis software (MARS, CLARIOstar). Ca^{2+} concentrations were calculated from the ratios of the fluorescence emission readings at 340 and 380 nm and recorded at 510 nm. Results represent means ± SEM of 6 measurements. Asterisks denote means significantly different from the respective 20β-S values at each time point analyzed by Student's t test. $^{*}P < 0.05$, $^{**}P < 0.01$. All the experiments were repeated 3 or more times and similar results were obtained on each occasion.

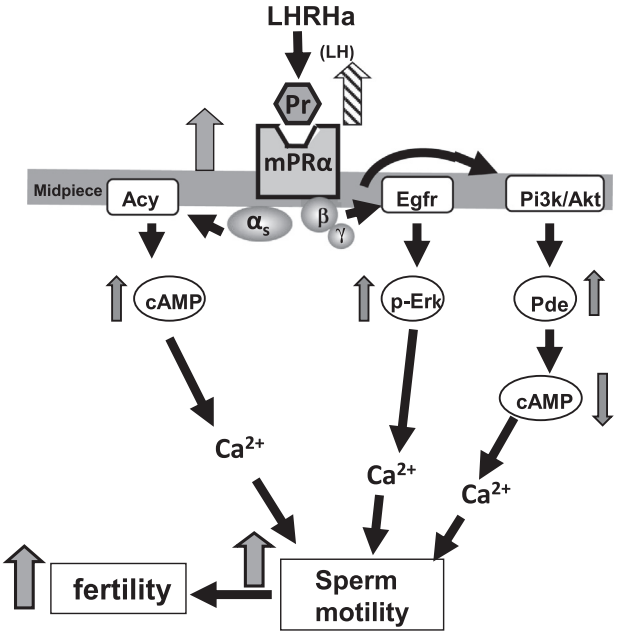

Fig. 4. Proposed model of mPRα-dependent signaling pathways mediating stimulation of sperm motility in Atlantic croaker and southern flounder. Expression of mPRα on the sperm plasma membrane and sperm motility are upregulated by *in vivo* treatments with LHRHa, presumably through upregulation of LH secretion and 20β-S production. Progestin binding to mPRα causes activation of a stimulatory G protein, G_{olf}. The α subunit of G_{olf} activates the Acy/cAMP pathway resulting in increases in cAMP and Ca^{2+} concentrations, and sperm motility. Signaling through the Egfr/p-Erk and Pi3k/Akt/Pde pathways are also required for progestin-induced increases in sperm Ca^{2+} concentrations and sperm velocity, presumably mediated through the G_{olf} βγ subunit. Notably, activation of all three signaling pathways is required for the sperm Ca^{2+} and hypermotility responses to progestins since inhibitors of any of these pathways will abolish both of them. The progestin-induced hypermotility is associated with increases in sperm fertilization success.

The finding that mPRα is coupled to G_{olf} in Atlantic croaker sperm is consistent with results showing that acute progestin treatment of croaker sperm causes a rapid increase in cAMP production by sperm membranes. Moreover, this increase in cAMP was blocked by pretreatment of croaker sperm with the membrane-bound adenylyl cyclase (Acy) inhibitor, dd-Ado, which also blocked progestin-induced hypermotility (Tubbs and Thomas, 2009). Similarly, there is high expression of G_{olf} on flounder sperm plasma membrane fractions (Fig. 1B) and pretreatment with dd-Ado prevented flounder sperm hypermotility (Tubbs et al., 2011) and cAMP responses to progestin treatments (Tan et al., 2014). Treatment of sperm with forskolin, a stimulator of Acy activity, results in increased sperm motility in croaker (Tubbs and Thomas, 2009), whereas treatment with the Acy inhibitor, SQ22536 (1 μM), blocked 20β-S stimulation of croaker sperm motility (Tubbs and Thomas, 2009; Fig. 2A). These findings suggest that progestins induce hypermotility in both croaker and flounder sperm through activation of the G_{olf} α-subunit resulting in increases in the activity of Acy and elevated cAMP levels.

5. Activation of the Pi3k/Akt pathway by progestins results in elevated phosphorylation of Akt and increased teleost sperm hypermotility

Initial studies of signaling cascades initiated by progestin activation in croaker sperm focused on the pathways mediated by the G_{olf} α-subunit (Tubbs and Thomas, 2009). More recently, the role of the G_{olf} βγ-subunit was examined in croaker sperm (Tan and Thomas, 2014, 2015). The phosphatidylinositol 3-kinase (PI3K)/AKT pathway is one signaling cascade commonly associated with βγ-subunit signaling (Stephens et al., 1997; Djordjevic and Driscoll, 2002. The PI3K/AKT pathway has been implicated in mediating important proxies of male fertility in various tetrapods including the regulation of boar sperm viability (Aparicio et al., 2007) and regulation of sperm motility in hamsters (Nagdas et al., 2002) and chickens (Ashizawa et al., 2008). Evidence exists in croaker sperm (Tan and Thomas, 2014) and in human sperm (Sagare-Patil et al., 2012) for activation of Akt by progestins at both low (100 nM) and high (5–10 μM) concentrations, respectively. More recently, the Pi3k/Akt pathway and the resulting phosphorylation of Akt has also been shown to be involved in leucine upregulation of zebrafish (*Danio rerio*) sperm motility (Zhang et al., 2017).

Selective inhibition of croaker sperm Pi3k (Wortmannin, 1 nM and LY294002, 25 μM) and Akt (ML-9, 25 μM) effectively abolished progestin-initiated sperm hypermotility (Tan and Thomas, 2014; Fig. 2B), suggesting that both Pi3k and Akt are necessary components mediating sperm hypermotility in this species. Similar results were observed with leucine-induced hypermotility in zebrafish sperm pretreated with LY294002 (Zhang et al., 2017), which suggests this signaling cascade may be highly conserved in different fish species.

Western blotting of croaker sperm membrane fractions using antibodies against Akt and its activated phosphorylated form (P-Akt) indicated that both forms were present on the plasma membrane. Furthermore, activation of croaker sperm with 20β-S resulted in increased amounts of P-Akt, while preincubation of sperm with Wortmannin (10 nM) eliminated that response, indicating that Akt is downstream of Pi3k signaling (Tan and Thomas, 2014). Similarly, progestins have also been shown to increase sperm P-AKT levels in humans (Sagare-Patil et al., 2013). Taken together, these findings indicate that sperm hypermotility in croaker, and likely in other teleosts as well, is at least partially under the control of the Pi3k/Akt pathway, with progestin stimulation of mPRα initiating the increase of intracellular P-Akt resulting in increased sperm motility.

6. Progestin stimulation of croaker sperm hypermotility is mediated through activation of phosphodiesterases (Pdes)

As previously stated, earlier research on G_{olf} α-subunit activation through mPRα indicated that its stimulation of hypermotility is at least partly mediated by the increased production of cAMP by Acy. Intriguingly, progestin treatment also increases phosphodiesterase (Pde) activity and pretreatment with inhibitors of PDE3, Cilostamide (100 nM), and PDE4, Rolipram (1 μM), increase cAMP levels and block progestin stimulation of croaker sperm motility, indicating that increased Pde activity and elevated breakdown of cAMP is also required for the hypermotility response (Tan and Thomas, 2014). In addition, it was demonstrated in the same study that pretreatment with the PI3K inhibitor, Wortmannin, abrogated the progestin-induced increase in Pde activity, suggesting that Pdes are at least partially under Pi3k/Akt control. The results suggest that mPRα-mediated progestin stimulation of sperm motility in croaker through Pi3k/Akt is dependent upon maintenance of Pde activity and a reduction in internal cAMP concentrations. Therefore, progestin-induced sperm hypermotility in croaker may be under the control of both the production of cAMP by Acy, as well as the breakdown of cAMP by Pdes.

7. Sperm hypermotility in Atlantic croaker involves the activation Egfr, ErbB2, and Mapk pathways

Activation of epidermal growth factor receptor (EGFR), ErbB2, another member of the EGFR family, and mitogen-activated protein kinase (MAPK, extracellular-related kinase 1 and 2, Erk1/2, also known as Mapk1/3) pathways through mPRα have been identified in various vertebrate cells, including croaker ovarian follicle cells and oocytes (Zuo et al., 2010; Dressing et al., 2010, 2012; Peyton and Thomas,

2011; Aizen et al., 2018), suggesting that these signaling pathways may also mediate sperm hypermotility in teleosts. Transactivation of EGFR through activation of stimulatory G protein βγ-subunits has been demonstrated with G protein-coupled estrogen receptor-1, (GPER-1) (Filardo et al., 2002). Src-related tyrosine kinases (Src) are activated, which in turn signal matrix metalloproteinases (MMP) to split pro-heparin-binding epidermal growth factor (pro-HB-EGF). The cleaved pro-HB-EGF then binds to EGFR, thus activating the receptor and increasing the activities of downstream MAPKs (Filardo and Thomas, 2005). Src has been localized to the head and flagellum of human sperm (Lawson et al., 2008). Moreover, EGFR has been shown to be involved in boar sperm motility (Oliva-Hernandez and Perez-Gutierrez, 2008) and a comprehensive proteomic analysis demonstrated that EGF signaling is important in high fertility bovine sperm (Peddinti et al., 2008). Furthermore, the mPRα protein has a similar location as EGFR and Src on vertebrate sperm, with high concentrations on the midpiece and some expression on the flagellum and head of human (Thomas et al., 2009), croaker (Fig. 1A), and southern flounder (Tubbs et al., 2011) sperm.

The finding that acute treatment of croaker sperm with human recombinant epidermal growth factor (EGF; 100 nM) mimicked the stimulatory actions of the endogenous croaker progestin, 20β-S on croaker sperm, significantly increasing sperm swimming speed compared to vehicle-treated controls, suggests that this stimulatory effect involves the release of intracellular epidermal growth factors and activation of Egfr (Tan and Thomas, 2015). This role of Egfr was confirmed using both inhibitors of upstream regulators of EGFR, Src with PP2 (10 μM) and MMP with Ilomastat (10 μM) and inhibitors of EGFR tyrosine kinase, AG1478 (5 μM) and RG13022 (50 μM), which significantly abrogated stimulation of sperm motility by 20β-S and Org OD 02-0 (Tan and Thomas, 2015; Fig. 2C). Interestingly, pretreatment with 5 nM AG879, a human epidermal growth factor receptor 2 (ERBB2) tyrosine kinase inhibitor, also significantly reduced progestin stimulation of sperm hypermotility in the same study. Furthermore, progestin stimulation of croaker sperm hypermotility was also abolished by preincubation with the human MAPK (MEK1/2) inhibitor U0126 (500 nM), Fig. 2D). Western blot analysis of croaker sperm plasma membrane fractions showed that both extracellular-related kinase 1 and 2 (Erk1/2) and its activated phosphorylated form, P-Erk1/2 were present and that P-Erk1/2 expression was increased after 20β-S treatment (Tan and Thomas, 2015). Taken together, these results suggest that progestin-mediated hypermotility via mPRα in Atlantic croaker sperm also involves activation of the Egfr, ErbB2 and Mapk pathways.

8. Role of intracellular calcium (Ca^{2+}) in mediating progestin induction of teleost sperm motility

Calcium (Ca^{2+}) has a fundamental regulatory role in the activation of vertebrate sperm motility and is mediated through activation of Ca^{2+} channels (Cosson et al., 1989; Suarez, 2008; Darszon et al., 2011; Lesich et al., 2012). Activation of Ca^{2+} channels is also required for induction of croaker sperm motility by exposure to hyperosmotic media, since it is inhibited by pretreatment with blockers of L-type Ca^{2+} channels, verapamil and nifedipine (Detweiler and Thomas, 1998). Treatment with 20β-S causes a rapid increase in sperm Ca^{2+} levels (Thomas, 2003), which is diminished by pretreatment with these Ca^{2+} channel blockers as well as the sperm hypermotility response (Supplementary Fig. 1A, B). These results indicate that an increase in Ca^{2+} channel activity resulting in elevated intracellular Ca^{2+} concentrations is an important component of progestin-stimulation of sperm hypermotility.

Recent extensive unpublished studies have investigated the potential roles of the Acy/cAMP, Pi3k/Akt, and EGF/Mapk pathways regulating sperm hypermotility in mediating the progestin-induced increase in Ca^{2+} concentrations in croaker sperm. Treatment of croaker sperm with the Acy activator, forskolin, resulted in a rapid increase in intracellular Ca^{2+} similar to that induced by 20β-S (Fig. 3A), which suggests that progestin stimulation of both intracellular Ca^{2+} influx and hypermotility involves activation of the Acy/cAMP pathway. However, as discussed before, progestin-stimulated sperm hypermotility in croaker also appears be under the control of Pdes, which in turn are at least partially under Pi3k control. Pretreatment with Wortmannin, a PI3K inhibitor, significantly reduced the increase in the intracellular Ca^{2+} response to 20β-S treatment (Fig. 3B), indicating that progestin induction of sperm hypermotility and Ca^{2+} influx also involves the activation of the Pi3k/Akt pathway and Pdes. Rapid fluctuations of Ca^{2+} concentrations have been reported in hyperactivated mammalian sperm that correlate with tail beat frequency (Suarez et al., 1993; Harper et al., 2004). Although a potential role for cAMP fluctuations in mediating these rapid Ca^{2+} fluctuations has not been demonstrated in sperm, Ca^{2+} oscillations paired with transient changes in cAMP concentrations have been reported in other cell types (Gorbunova and Spitzer, 2002; Dyachok et al., 2006), suggesting interdependency between oscillation patterns of Ca^{2+} and cAMP (Gorbunova and Spitzer, 2002; Zaccolo and Pozzan, 2003; Borodinsky and Spitzer, 2006). The interesting possibility that activation of both Acy and Pdes in croaker sperm induces cAMP fluctuations and that these in turn are associated with oscillations in Ca^{2+} levels warrants investigation.

Pretreatment of croaker sperm with AG1478, the EGFR tyrosine kinase inhibitor, blocked the progestin-induced sperm increase in Ca^{2+} increase (Fig. 3C), indicating that the progestin-stimulated teleost sperm hypermotility and Ca^{2+} increase are also dependent on the Egfr pathway (Tan and Thomas, 2015). Finally, pretreatment with the MEK1/2 inhibitor, AZD6244, also blocks the increase in Ca^{2+} in croaker sperm in response to Org OD 02-0 (Fig. 3D). Taken together, these results indicate that progestin-stimulation of sperm motility through Egfr and Mapk also involves a rapid increase in sperm Ca^{2+} concentrations.

9. Summary and future directions

Here we present an extended examination of the role of mPRα, spanning over two decades research, in mediating hypermotility in teleost sperm. While teleosts are an immensely diverse vertebrate group, the similar and consistent findings in both Atlantic croaker and southern flounder suggests the involvement of a highly conserved signaling pathway in their sperm physiology. On the basis of these findings a general model of progestin upregulation of sperm motility in teleosts through activation of mPRα and multiple signaling pathways is proposed (Fig. 4). However, the interactions between these different signaling pathways as well as the identities of the Ca^{2+} channels involved remain unclear and require additional study. Interestingly, CatSper, the calcium channel mediating progesterone stimulation of sperm motility in mammals (Lishko et al., 2011; Strunker et al., 2011), is not present on the sperm of teleosts, jawless fishes (agnathans), birds and amphibians (Cai and Clapham, 2008). Thus, the CatSper-independent teleost sperm model described here represents an alternative mechanism of progestin stimulation of sperm motility that may be applicable to a wide variety of nonmammalian vertebrates.

Another mechanism of progestin induction of teleost sperm hypermotility was proposed in the early 1990s based on initial evidence obtained in Japanese eel and masu salmon (Miura et al., 1991, 1992). The authors reported that two daily injections of the teleost progestin hormone, 17, 20β-dihydroxy-4-pregnen-3-one (DHP), caused a marked induction of sperm motility in masu salmon, which was mediated by a DHP-induced increase in sperm duct pH. Subsequent *in vitro* experiments demonstrating that sperm cAMP levels were rapidly increased in response to an increase in pH provide a plausible explanation of how the sperm become motile (Miura et al., 1992). Unfortunately, there have been no further reports of studies on this proposed mechanism of progestin stimulation of sperm hypermotility, so its importance remains unclear. The possibility that progestin induction of sperm motility in Atlantic croaker, southern flounder and other teleost species is under dual control through both mechanisms needs to be explored.

Finally, development of commercial aquaculture for many marine flatfish species such as southern flounder is severely limited by poor male reproductive performance, especially reductions in sperm motility and fertility, but the underlying causes are largely unknown. A clearer understanding of the mechanisms regulating sperm motility would enable the development of more reliable and efficient methods to improve sperm quality and spawning of southern flounder and other teleost species. Progestins activate multiple signaling pathways through mPRα in southern flounder sperm to induce calcium influx and hypermotility and enhance fertility. The potential for developing practical methods to increase the reproductive performance of male flounder broodstock through pharmacological stimulation of these pathways is currently being investigated.

Disclosure statement

The authors have nothing to disclose.

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