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Involvement of epidermal growth factor receptors and mitogen-activated protein kinase in progestin-induction of sperm hypermotility in Atlantic croaker through membrane progestin receptor-alpha ☆

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ABSTRACT

The intracellular pathways mediating rapid, nongenomic progestin stimulation of sperm motility remain unclear. The role of epidermal growth factor receptors (Egfr and ErbB2) and mitogen-activated protein kinase (Mapk) in membrane progestin receptor-alpha (mPRα)-mediated progestin stimulation of sperm hypermotility was examined in a teleost, Atlantic croaker. Inhibition of upstream regulators of Egfr, intracellular tyrosine kinase (Src) with PP2, and matrix metalloproteinase (MMP) with Ilomastat, abolished progestin-initiated sperm hypermotility by 17,20β,21-trihydroxy-4-pregnen-3-one (20β-S; 20 nM) and a specific mPRα agonist, Org OD 02-0 (20 nM). Pretreatment of croaker sperm with EGFR inhibitors, AG1478 (5 μM) and RG13022 (50 μM), the ErbB2 inhibitor, AG879 (5 nM), or the MEK1/2 inhibitor, U0126 (500 nM) blocked progestin stimulation of sperm motility. Levels of phosphorylated extracellular-related kinase 1 and 2 (P-Erk1/2) were increased after 20β-S treatment. These results demonstrate that progestin-mediated hypermotility via mPRα in croaker sperm involves activation of the Egfr, ErbB2 and Mapk pathways.

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1. Introduction

Rapid nongenomic progestin actions to increase sperm activity have been described in several vertebrate species including induction of hypermotility in fish (Tan and Thomas, 2014; Thomas, 2003; Tubbs and Thomas, 2009; Tubbs et al., 2011) and hyperactivation in mammals (Park et al., 2011; Uhler et al., 1992). Despite the importance of progestin signaling in regulating sperm physiology and fertility in vertebrates, the mechanisms through which progestins exert these effects remain poorly understood (Park et al., 2011; Thomas et al., 2009). Exposure of Atlantic croaker (*Micropogonias undulatus*) and Southern flounder (*Paralichthys lethostigma*) sperm to their endogenous progestin hormone, 17,20β,21-trihydroxy-4-pregnen-3-one (20β-S), causes an increase in sperm swimming speed (hypermotility) and percent motile sperm upon their activation in a high osmolality buffer (Thomas, 2003; Thomas et al., 2004, 2006) which has been shown to increase fertilization success of Atlantic croaker and Southern flounder sperm in strip spawning trials (Tubbs and Thomas, 2009; Tan et al., 2014).

Previous evidence suggests that rapid progestin stimulation of sperm hypermotility in several teleost species is mediated through the novel 7-transmembrane membrane progestin receptor-alpha (mPRα also known as Paqr7b) (Tan et al., 2014; Thomas, 2003; Tubbs and Thomas, 2009; Tubbs et al., 2011), which belongs to the progestin and adipoQ receptor (PAQR) family (Thomas et al., 2007). The mPRα was discovered in spotted seatrout ovaries and shown to be present on the oocyte plasma membrane where it acts as the intermediary in maturation-inducing steroid (MIS) induction of oocyte maturation in this species through a nongenomic mechanism (Zhu et al., 2003). The mPRα protein is also detected on plasma membranes of spotted seatrout sperm (Tubbs and Thomas, 2008; Zhu et al., 2003) as well as on the sperm of Atlantic croaker, red drum, and Southern flounder (Thomas et al., 2006; Tubbs and Thomas, 2009; Tubbs et al., 2011). Several lines of evidence suggest that progestin upregulation of sperm motility through mPRα in these species involves upregulation of sperm cAMP concentrations

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through increased activity of membrane-bound adenylyl cyclase (Acy) and is associated with activation of the alpha subunit of a stimulatory G protein (Thomas, 2003; Tubbs and Thomas, 2009; Tubbs et al., 2011). Co-immunoprecipitation studies have shown that mPRα is closely associated with a stimulatory olfactory G protein (G_{olf}) in Atlantic croaker sperm membranes (Tubbs and Thomas, 2009). Stimulatory G proteins, including G_{olf}, have also been detected on Southern flounder sperm membranes (Tan et al., 2014). Moreover, progestin-induced hypermotility of croaker and flounder sperm is associated with rapid increases in cAMP production, both of which are blocked by cotreatment with the Acy inhibitor, dd-Ado (Tan et al., 2014; Tubbs and Thomas, 2009; Tubbs et al., 2011). Collectively, these data suggest an important role for cAMP in inducing sperm hypermotility through this G protein α subunit-dependent signaling pathway. However, a recent study has shown that progestin initiation of Atlantic croaker sperm hypermotility through mPRα also involves activation of the Pi3k/Akt pathway and modulation of phosphodiesterase activity (Tan and Thomas, 2014), presumably through activation of the G protein βγ subunit. Activation of other signaling cascades through mPRα have been identified in various vertebrate cells (Dressing et al., 2010, 2012; Zuo et al., 2010), suggesting that additional signaling pathways may also mediate sperm hypermotility in Atlantic croaker.

The epidermal growth factor receptor/mitogen-activated protein kinase (Egfr/Mapk: abbreviation in fish for EGFR/MAPK) pathway is a plausible candidate for an additional signaling mechanism mediating progestin stimulation of sperm hypermotility in teleosts through mPRα. Both EGFR and MAPK pathways are activated by progesterone through mPRα in triple negative breast cancer cells (Dressing et al., 2012; Zuo et al., 2010). Moreover, components of this pathway have been identified in mammalian sperm. Activation of G proteins in bovine sperm has been shown to transactivate EGFR, which is localized on the head and midpiece, through protein kinase A (PKA), intracellular tyrosine kinase (Src), and matrix metalloproteinase (MMP) (Etkovitz et al., 2009). Src has also been detected on the head and flagellum of human sperm (Lawson et al., 2008). Interestingly, the mPRα protein has a similar localization to that of EGFR and Src on vertebrate sperm with a high concentration on the midpiece and lesser amounts also on the flagellum and head (Thomas et al., 2009; Tubbs and Thomas, 2009; Tubbs et al., 2011). Sperm motility in boars involves EGFR signaling (Oliva-Hernandez and Perez-Gutierrez, 2008) which is an important component of high fertility bovine sperm (Peddinti et al., 2008). Progestin binding to mPRα also induces phosphorylation of extracellular-related kinase 1 and 2 (Erk1/2, also known as Mapk1/3) in fish oocytes and ovarian follicle cells (Dressing et al., 2010; Pace and Thomas, 2005; Zhu et al., 2003), presumably through transactivation of Egfr. Recent results suggest the responsiveness of fish oocytes to progestins through mPRα is also dependent on signaling via ErbB2, another member of the Egfr family (Peyton and Thomas, 2011). Thus, transactivation of Egfr and ErbB2, as well as phosphorylation of Erk1/2 are all potential mechanisms involved in the progestin-mediated increase in sperm swimming velocity (hypermotility) in Atlantic croaker.

In the present study, we tested the hypothesis that progestin-stimulated sperm hypermotility in Atlantic croaker through mPRα is also mediated by transactivation of Egfr and ErbB2 and by activation of Mapk. The effects of upstream inhibitors of Egfr transactivation and inhibitors of Egfr, ErbB2 and Mapk activation on induction of croaker sperm hypermotility by the endogenous progestin in this species, 20β-S, and the specific mPRα agonist, 10-ethenyl-19-norprogesterone (Org OD 02-0) (Kelder et al., 2010) were investigated.

2. Materials and methods

2.1. Chemicals

The Atlantic croaker progestin hormone, 20β-S was purchased from Steraloids (Newport, RI). The synthetic progestin, Org OD 02-0 was a gift from Organon (Oss, the Netherlands). The Erk1/2 and phospho-Erk (P-Erk1/2) antibodies were purchased from Cell Signaling Technology (Danvers, MA). All inhibitors were purchased from Enzo Life Sciences (Farmington, NY). All other chemicals and reagents were purchased from Sigma unless otherwise noted.

2.2. Animals

Adult male Atlantic croaker were obtained from bait shops in the vicinity of Port Aransas, Texas in September, at the beginning of testicular recrudescence. Fish were transferred to fish holding facilities at the University of Texas Marine Science Institute and acclimated to laboratory conditions for two months before use in experiments in 12,000 L recirculating seawater tanks (salinity 30–32 ‰). Gonadal development in Atlantic croaker in the Gulf of Mexico occurs during the fall and spawning occurs in November and December (Gutherz, 1976). Croaker were maintained under fall environmental conditions of temperature (22–24 °C) and photoperiod (11 h light, 13 h dark) to stimulate testicular development and spermiation. All procedures used in this study were approved by the Institutional Animal Care and Use Committee of the University of Texas at Austin.

2.3. Milt collection

Milt was collected from fully mature male Atlantic croaker as described by Detweiler and Thomas (1998). The abdominal area surrounding the cloaca was dried with a towel to prevent premature activation of the sperm by exposure to seawater. Milt was expressed by applying gentle pressure to abdomen and the milt was collected with a syringe from the cloaca. Care was taken to prevent contamination of milt samples with urine, which would also cause premature activation of sperm. The milt was transferred to microcentrifuge tubes and kept on ice for up to 30 min prior to analysis.

2.4. Sperm motility analyses

Sperm motility experiments were performed as described previously (Detweiler and Thomas, 1998) with minor modifications. Croaker milt was diluted 200-fold in physiological saline and pre-incubated with progestins (20β-S or Org OD 02-0, 20 nM)), human epidermal growth factor (EGF, 100 nM), or vehicle (EtOH, 1%) for 1 min at room temperature. A 2.5 μL aliquot of each sperm suspension was added to 25 μL of hyperosmotic activator solution on a microscope slide (motility of marine fish sperm is triggered by the two-fold increase in external osmolality) and covered with a coverslip. Sperm motility was recorded for 1 min with a dark field microscope using a charge-coupled device camera (Cohu Electronics, San Diego, CA) connected to a computer capable of recording high quality videos with digital recording software (Pinnacle, Mountain View, CA). A video of croaker sperm hypermotility can be viewed in supplementary material in Tan and Thomas (2014). Each treatment trial was repeated three times in a single experiment and each study consisted of at least three experiments with sperm from different donors. Sperm swimming speed (average path velocity) was determined using CellTrak motion analysis software (Motion Analysis Corp., Santa Rosa, CA). The results shown in the graphs represent the composite of means of the average swimming speeds ± SEM.

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