Theriogenology 81 (2014) 613–624

Contents lists available at ScienceDirect

Theriogenology

journal homepage: www.theriojournal.com

Removal of GPI-anchored membrane proteins causes clustering of lipid microdomains in the apical head area of porcine sperm

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ARTICLE INFO

Article history:
Received 29 May 2013
Received in revised form 25 November 2013
Accepted 27 November 2013

Keywords:
Sperm capacitation
Membrane raft
Phosphatidyl inositol specific phospholipase C
Glycosylphosphatidylinositol-anchored protein release

ABSTRACT

The release of extracellular proteins is a part of the sperm capacitation process; this allows the sperm surface reorganization that enables the sperm to fertilize an oocyte. Some of the components released are 'decapacitation factors', an uncoordinated or early release of which may cause inappropriate surface destabilization and premature capacitation. We studied the involvement of glycosylphosphatidylinositol-anchored proteins (GPI-APs) in sperm capacitation, and reported that CD52 and CD55 exhibit bicarbonate-dependent release during *in vitro* sperm capacitation. Treating sperm with phosphatidylinositol-specific phospholipase C (PIPLC) resulted in the enzymatic cleavage of CD55, in both capacitating and noncapacitating conditions. Moreover, PIPLC treatment in noncapacitating conditions caused surface reorganization events that included exposure of the ganglioside GM1, aggregation of flotillin-1, and the swelling of the apical acrosome region; all of which have been reported to be associated with sperm capacitation. The acrosomal swelling was monitored using wet mount atomic force microscopy, a new imaging technique that allows nanometer-level sperm surface measurements in samples hydrated with physiological buffer rather than dried. Despite these surface changes, PIPLC treatment in identical incubation conditions did not stimulate hyperactive sperm motility or protein tyrosine phosphorylation (other hallmarks of sperm capacitation *in vitro*). In full capacitating conditions (i.e., the presence of bicarbonate and albumin), PIPLC treatment caused sperm deterioration. The possible role of GPI-APs removal from the sperm surface during sperm capacitation is discussed.

1. Introduction

The sperm cell surface is coated with extracellular glycoproteins that form a protective layer and stabilize the sperm cell during its transit through both the male and female genital tracts [1]. A number of these extracellular coating proteins become attached to the sperm surface during its transit through the epididymal duct, whereas other factors become associated to the sperm surface during ejaculation, when sperm cells are mixed with secretory fluids (seminal plasma) from diverse accessory sex glands [2]. Some of the extracellular proteins associate with the sperm surface via electrostatic interactions, whereas others are covalently linked to lipids by glycosylphosphatidylinositol (GPI) anchors or interact with hydrophobic moieties within the lipid bilayer (this phenomenon can be facilitated using posttranslational

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0093-691X/$ – see front matter © 2014 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.theriogenology.2013.11.014

acylation). With respect to sperm physiology, it has become clear that the extracellular coating process plays a dual role in protecting the integrity of the sperm membrane whereas also preventing premature sperm capacitation (the cumulative process in which sperm cells become competent to initiate fertilization of an oocyte; for review, see [1,3]). Sperm surface coating factors are believed to mask underlying proteins involved in (i) sperm–zona binding and (ii) docking of the acrosome required for the initiation of the acrosome reaction, in which both processes are inhibited until these so called decapacitation factors are released [4,5].

The removal of the decapacitation factors from the sperm surface allows the redistribution of membrane lipids and surface proteins [5]. During this process, an albumin-dependent efflux of nonlipid raft-associated sterols is induced [6]. One theory is that, in addition to this sterol efflux, the removal of decapacitation factors causes an aggregation of lipid-ordered microdomains in the apical ridge area of the sperm head. This would help to explain the temporally regulated generation of functional protein complexes at the sperm surface involved in zona binding and the acrosome reaction [7–9].

Isolated detergent-resistant sperm membranes (representing the lipid raft fraction of sperm cells) are enriched in cholesterol, desmosterol, and glycolipids, such as the ganglioside GM1 and seminolipid (a sulphated glycolipid specific for sperm) [4,10–12]. Detergent-resistant sperm membranes are also enriched in GPI-APs that are considered to be markers for membrane rafts (for instance, the protein CD55) [13]. During epididymal maturation, a number of proteins become GPI-anchored to the sperm surface using a poorly understood mechanism [14]. A number of these GPI-APs, such as CD52 and CD55, block the recognition of the sperm surface by phagocytes and also have an anticoagulant function. Interestingly, CD52 also interacts with the peripheral sperm surface decapacitation factor, seminogelin [15]. As a result, the action of a GPI-AP cleaving enzyme that removed CD52 could extend to concomitant removal of associated decapacitation factors from the sperm surface. Another GPI-AP, carbonyl reductase (or P34H), is reported to be involved in sperm–zona binding and originates from the epididymis [7,16]. Thus, selected GPI-APs need to be removed from the capacitating sperm surface whereas others remain or form complexes or association, for example at the area where sperm–zona binding take place. Interestingly, capacitation induces the release of GPI-APs [17], and a testis-specific angiotensin-converting enzyme (AcE) with GPI-AP releasing activity has been shown to be responsible for this release [18]. Once activated, this enzyme releases the entire protein part of a GPI-AP by cutting within the glycosidic GPI moiety. This AcE-mediated removal of GPI-APs appears to be important for capacitation, because AcE knockout mice are infertile [18]. An alternative method to cleave the GPI anchors is using phospholipase C or D enzymes, which can cleave the diacylglycerol or phosphatidic acid moiety from the GPI-AP, respectively. This also effectively results in the release of the entire protein domain of the GPI-AP, although the released protein contains the sugars from the GPI anchor (and in the case of phosphatidylinositol-specific phospholipase C (PIPLC; Sigma-Aldrich, Mannheim, Germany) also the phosphate group).

In the present study, we incubated sperm cells in *in vitro* capacitation media in the presence or absence of PIPLC to compare *in vitro* capacitation–driven removal of GPI-AP with PIPLC-induced removal of GPI-APs. Our aim was to investigate the putative role of GPI-AP removal on sperm surface organization, cell signaling and motility regulation.

2. Materials and methods

2.1. Sperm incubations

Ejaculates were collected from boars of proven fertility at a commercial boar semen collection and distribution centre (Varkens KI Nederland; Deventer, the Netherlands). Freshly ejaculated semen was filtered through gauze to remove gelatinous material, and then diluted and washed in Hepes buffered saline (137 mM NaCl, 2.5 mM KCl, 20 mM Hepes, pH 7.4). Next, the spermatozoa were washed by a two-step centrifugation at 300 and 750 × *g* for 5 and 15 minutes through a discontinuous 70%:35% (vol/vol) Percoll (GE Healthcare Bio-Science AB, Uppsala, Sweden) gradient, as described by van Gestel, et al. [7]. All solutions were iso-osmotic (290–310 mOsm/kg) with Hepes buffered saline at 23 °C. After centrifugation, the Percoll was discarded and the sperm pellet was resuspended at a final concentration of 100 million sperm cells/mL in Hepes buffered Tyrode's medium (HBT; 120 mM NaCl, 21.7 mM lactate, 20 mM Hepes, 5 mM glucose, 3.1 mM KCl, 2.0 mM $CaCl_2$, 1.0 mM pyruvate, 0.4 mM $MgSO_4$, 0.3 mM NaH_2PO_4, 300 mOsm/kg, pH 7.4). Hepes buffered Tyrode's medium was either not supplemented with bicarbonate (Bic) and BSA (further referred to as −Bic/−BSA) or supplemented with 15 mM $NaHCO_3$ and equilibrated with 5% CO_2 in a humidified atmosphere (further referred to as +Bic/−BSA). In addition, sperm were incubated in a medium supplemented with 0.3% (wt/vol) BSA (defatted fraction V: Boehringer Mannheim, Almere, the Netherlands) in the absence (−Bic/+BSA) or presence (+Bic/+BSA) of bicarbonate. The +Bic/+BSA medium is considered to be fully supportive of sperm capacitation, and is commonly used for porcine IVF. Media including bicarbonate were incubated with open vials in a 5% CO_2 atmosphere for 2 hours at 38.5 °C, whereas bicarbonate-free media were incubated in airtight vials for 2 hours at 38.5 °C in a water bath. Sperm were incubated in the presence or absence of the enzyme PIPLC at a final concentration of 0.1 IU/mL (equivalent to 0.1 μmol/min/mL diacylglycerol formation from phosphatidylinositol containing micelle suspensions).

2.2. Fluorescence-activated cell sorting

Sperm incubated in various media were washed in HBT and subsequently incubated for 5 minutes in 1 μg/mL fluorescein-conjugated peanut agglutinin to discriminate acrosome intact cells from acrosome-reacted cells. Next, sperm were incubated in 25 nM of the membrane impermeable vital stain propidium iodide to discriminate vital sperm from deteriorated sperm. Sperm suspensions were then analyzed on a fluorescence-activated cell sorting (FACS) Calibur flow cytometer equipped with a 100 mW argon laser (Becton Dickinson, San Jose, CA, USA). Excitation of both fluorescent probes was efficient at a