Theriogenology xxx (2014) 1–8

Contents lists available at ScienceDirect

Theriogenology

journal homepage: www.theriojournal.com

Expression of α-gustducin and α-transducin, G proteins coupled with taste receptors, in boar sperm

M. Spinaci*, D. Bucci, M. Mazzoni, E. Giaretta, C. Bernardini, C. Vallorani, C. Tamanini, P. Clavenzani, G. Galeati

Department of Veterinary Medical Sciences, University of Bologna, Bologna, Italy

ARTICLE INFO

Article history:
Received 19 September 2013
Received in revised form 12 March 2014
Accepted 13 March 2014

Keywords:
Pig
Sperm
G protein
Taste receptor

ABSTRACT

During the transit in the female genital tract, spermatozoa are exposed to an environment that varies in composition from the vagina to the oviduct. Because G proteins, α-gustducin and α-transducin, are accepted as markers of chemosensitive cells, this study was aimed at assessing whether these proteins are expressed in boar germ cells. Ejaculated sperm extracts were analyzed by Western blot, and indirect immunofluorescence was performed on testis sections, smears of epididymal and ejaculated spermatozoa, sperm cells after *in vitro* induction of capacitation and acrosome reaction (IVAR), and in sperm cells bound to zona pellucida during IVF. Based on immunoblot results, both G proteins are present in boar sperm. In the testicular tissue sections, α-gustducin and α-transducin positivity was recorded in the germinal cells near the tubular lumen, whereas no positive signal was evident in spermatogonia located in the outer region of the seminiferous tubules. α-Gustducin expression in epididymal and ejaculated spermatozoa was mainly detectable in both the acrosome and the principal piece of the tail, whereas α-transducin was confined to the acrosome and the midpiece. No changes after *in vitro* induction of capacitation and IVAR were observed, except for the disappearance of acrosomal positivity in reacted spermatozoa. In sperm bound to zona pellucida, the G protein signal was congruent with that observed in IVAR cells. To the best of our knowledge, this is the first description of α-transducin in mammalian sperm and the first description of α-gustducin in boar sperm. Further studies are needed to clarify the possible role of these G proteins in sperm physiology.

© 2014 Elsevier Inc. All rights reserved.

1. Introduction

During their journey along the female genital tract to meet the oocyte, spermatozoa are exposed to fluids, derived from specialized regions, that vary in composition from the vagina to the oviduct [1]. Even if the mechanisms are still largely unknown, it is generally supposed that spermatozoa possess chemoreceptive abilities allowing them to detect, in the female genital tract fluids, different chemical compounds and chemoattractive molecules that direct sperm toward the oocyte.

The expression of olfactory receptors in the sperm flagellum of human, dog, and rat [2–4] and the responsiveness of germ cells to floral ligands for olfactory receptors [5] have generated the hypothesis that olfactory receptors may have a role in the regulation of sperm motility and in the chemoreception during fertilization. However, physiologically relevant ligands for olfactory receptors have not been identified yet (for review, see [6]) and, moreover, olfactory receptors usually detect volatile, lipophilic substances [7], which are unlikely to be dissolved in appropriate concentrations in an aqueous environment such as the fluids of the female genital tract.

* Corresponding author. Tel.: +39 (0)51 2097909; fax: +39 (0)51 2097899.

E-mail address: marcella.spinaci@unibo.it (M. Spinaci).

0093-691X/$ – see front matter © 2014 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.theriogenology.2014.03.016

Taste receptor (TR) cells contain an array of proteins including ion channels, ligand-gated channels, transporters, and G protein-coupled receptors that serve as receptors for different food tastants [8]. Although ion channels are responsible for detecting salty and sour taste, chemosensory epithelial cells use G protein-coupled receptors to detect umami, sweet, and bitter taste [9]. A large family of TRs that mediate bitter gustatory signals, named T2Rs, and a small family of TRs that mediate sweet (T1R2 and T1R3) or L-amino acid signals including monosodium glutamate (umami taste) (T1R1and T1R3) have been discovered and cloned [10,11]. These TRs are expressed in the tongue taste buds in mammals and interact with specific Gα subunits, α-gustducin ($G_{\alpha gust}$) and α-transducin ($G_{\alpha tran}$), that mediate gustatory signaling [12,13]. In addition to the oral epithelia, the expression of taste signal transduction elements, including α-gustducin and α-transducin, has been reported in the gastric and intestinal mucosa and in the pancreas in humans, mammals, and fish and also in enteroendocrine cell lines in culture [14–18].

In addition to the previously mentioned expression of chemosensory receptors in digestive system, several studies have shown that bitter, sweet, and umami receptors are expressed in testis [19–23]. Moreover, Fehr, et al. [24] demonstrated the expression of α-gustducin during mouse spermatogenesis and a segmental distribution of this G protein along the flagellum of mouse, rat, bull, and human spermatozoa suggesting a functional role in processing intracellular signals controlling sperm motility.

Therefore, because G proteins α-gustducin and α-transducin are accepted as specific markers of chemosensitive cells, this study was aimed at assessing their presence and localization in boar germ cells during spermatogenesis and in spermatozoa in different functional moments. The expression of these G proteins in sperm cells bound to zona pellucida (ZP) during the fertilization process *in vitro* was also evaluated.

2. Methods

All the reagents were obtained from Sigma Chemical Co. (St. Louis, MO, USA) unless otherwise specified.

2.1. Sperm preparation and capacitation

To obtain epididymal spermatozoa, carefully dissected cauda epididymes, obtained from testes of mature boars collected at a local slaughterhouse, were excised and washed in modified PBS supplemented with 0.4% BSA. Cleaned tissues were transferred in fresh PBS supplemented with 0.4% BSA and cross-sectioned several times to allow the sperm to extrude into the medium.

Sperm-rich fraction of ejaculates were collected by gloved-hand technique from mature boars of proven fertility and extended in equal volume of Androhep. Boars were housed in the 'Inseme SPA' farm in single boxes, and semen was collected routinely once a week.

To induce capacitation, aliquots of ejaculated semen were washed twice with Brackett and Oliphant's medium [25] supplemented with 12% fetal calf serum (FCS; Gibco, Invitrogen, San Giuliano Milanese, Italy) and 10 μg/mL progesterone (IVF medium) at 800× *g* for 3 minutes. Sperm pellets were resuspended in 2 mL of IVF medium at a final concentration of 100×10^6 spermatozoa/mL and incubated for 2 hours at 39 °C in a humidified atmosphere of 5% CO_2/7% O_2. To induce acrosome reaction (AR), aliquots of capacitated semen were incubated in the presence of 10-μM calcium ionophore A23187 for 20 minutes.

The degree of capacitation was assessed by chlortetracycline (CTC) staining. Briefly, 50 μL of sperm suspension were mixed with the same amount of CTC solution (750 μM CTC in a buffer of 20 mM TRIS-HCl, 130 nM NaCl, 5 mM L-cysteine); after 30 seconds, 10 μL of 1% gluteraldeyde was added and then 10 μL of semen was placed onto a slide in a drop of Vectashield mounting medium (Vector Laboratories, Burlingame, CA, USA). After mounting, the slides were analyzed with an Eclipse E 600 (Nikon Europe BV, Badhoeverdop, The Netherlands) epifluorescence microscope using a UV set filter. Spermatozoa with fluorescence on the acrosome and the tail but with a fluorescence-free band on the postacrosomal region were considered as capacitated [26].

Acrosome integrity was measured with a Fluorescein isothiocyanate (FITC)-conjugated *Pisum sativum* agglutinin (FITC-PSA), which labels acrosomal matrix glycoproteins. Briefly, spermatozoa, fixed for at least 30 minutes at −20 °C in 95% ethanol, were dried onto heated microscope slides and then incubated with FITC-PSA solution (1 mg PSA-FITC/10 mL H_2O) for 15 minutes in the dark. After staining, samples were washed in PBS and mounted with Vectashield mounting medium with propidium iodide (PI; Vector Laboratories). The slides were then observed with the previously mentioned microscope. The presence of a green acrosomal fluorescence is indicative for an intact acrosome, whereas a partial or total absence of fluorescence is indicative of acrosome disruption or AR.

2.2. IVM and zona binding

Ovaries from prepubertal gilts were collected at a local slaughterhouse. Cumulus-oocyte complexes were aspirated from follicles 4 to 6 mm in diameter, selected under a stereomicroscope, and transferred into a petri dish (35 mm; Nunclon, InterMed, Roskilde, Denmark) prefilled with 2 mL of modified PBS supplemented with 0.4% BSA.

After three washes in NCSU-37 [27] supplemented with 5.0 μg/mL insulin, 0.57 mM cysteine, 10 ng/mL epidermal growth factor, 50 μM mercaptoethanol, and 10% porcine follicular fluid (IVM medium), groups of 50 cumulus-oocyte complexes were transferred to a Nunc four-well multidish containing 500 μL of the same medium per well and cultured at 39 °C in a humidified atmosphere of 5% CO_2/7% O_2. For the first 22 hours of IVM, the medium was supplemented with 1.0 mM dibutyryl-cAMP, 10 IU/mL eCG (Folligon; Intervet, The Netherlands), and 10 IU/mL hCG (Corulon; Intervet). Cumulus-oocyte complexes were then transferred to fresh IVM medium without supplementation and cultured for further 24 hours [28].

For the *in vitro* gamete binding experiments, boar semen was washed twice and finally resuspended in IVF medium. Sperm concentrations were evaluated, and 45 to 50 matured oocytes, freed from cumulus cells by gentle repeated pipetting, were transferred to 500 μL IVF medium

Download English Version:

https://daneshyari.com/en/article/10892025

Download Persian Version:

https://daneshyari.com/article/10892025

Daneshyari.com