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Involvement of cAMP-dependent unique signaling cascades in the decrease of serine/threonine–phosphorylated proteins in boar sperm head

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ABSTRACT

We previously suggested that protein phosphatase–dependent decrease of postacrosomal phosphorylated proteins may be necessary for the occurrence of acrosome reaction in livestock spermatozoa (Adachi et al., J Reprod Dev 54, 171–176, 2008; Mizuno et al., Mol Reprod Dev 82, 232–250, 2015). The aim of this study was to examine the involvement of the intracellular cAMP signaling cascades in the regulation of the decrease of post-acrosomal phosphorylated proteins in boar spermatozoa. Boar ejaculated spermatozoa were incubated with cAMP analogs and then used for the immunodetection of serine/threonine–phosphorylated proteins and assessment of acrosome morphology. The protein phosphatase–dependent decrease of postacrosomal phosphorylated proteins was greatly promoted by the incubation with a cAMP analog Sp-5,6-dichloro-1-β-D-ribofuranosyl-benzimidazole-3′,5′-monophosphorothioate (cBiMPS). This decrease was induced before the initiation of acrosome reaction and did not require the millimolar concentration of extracellular Ca^{2+} which was necessary for the initiation of acrosome reaction. Moreover, suppression of protein kinase A activity with an inhibitor (H89) had almost no influence on both decrease of phosphorylated proteins and occurrence of acrosome reaction in the spermatozoa incubated with cBiMPS. In addition, the prolonged incubation with a potentially exchange protein directly activated by cAMP-selective cAMP analog (8pM) could only partially mimic effects of cBiMPS on these events. These results indicate that the cAMP-dependent signaling cascades which are less dependent on protein kinase A may regulate the decrease of postacrosomal phosphorylated proteins in boar spermatozoa before the extracellular Ca^{2+}-triggered initiation of acrosome reaction.

1. Introduction

A number of reports [1–7] have reported that the intracellular cAMP signaling cascades play pivotal roles in the regulation of capacitation-associated changes in mammalian spermatozoa, and that the prime target molecule of cAMP is protein kinase A (PKA). In boar spermatozoa, the cAMP/PKA-dependent signaling cascades regulate the protein tyrosine phosphorylation (a hallmark of the capacitation) and the influx of extracellular Ca^{2+} leading to the occurrence of full-type flagellar hyperactivation [8–10]. Immunostaining experiments and proteomic analyses (boar [8], mouse [11], and human [12])

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reported PKA is localized mainly in the flagella including the connecting piece. In mouse spermatozoa; moreover, the PKA activity is detectable strongly in the flagella but scarcely in the head [13].

The cAMP signaling cascades of mammalian spermatozoa involve not merely PKA-dependent systems but also PKA-independent systems [6,14,15]. The prime candidate of the cAMP targets in the sperm PKA-independent system is the exchange protein directly activated by cAMP [EPAC1 and EPAC2 which are also called guanine-nucleotide exchange factor (RAPGEF) 3 and RAPGEF4, respectively] and localized mainly in the heads (human [16], mouse [17], and boar [18]). Tomes et al.[16,19,20] have shown that the acrosome reaction is initiated by the activation of cAMP/EPAC-dependent signaling cascades in human capacitated spermatozoa and proposed an interesting model for the extracellular Ca^{2+} influx-activated cAMP/EPAC-dependent signaling cascades leading to the acrosome reaction. For boar spermatozoa, Miro-Moran et al.[18] reported that the incubation with a potentially EPAC-selective cAMP analog (8-Br-2′-O-Me-cAMP) promoted the A23187-induced acrosome reaction in the capacitated spermatozoa, and that this incubation barely affected the capacitation-associated changes of plasma membrane phospholipids. According to these reports, it is likely that the cAMP/EPAC-dependent signaling cascades of mammalian sperm heads mediate the extracellular Ca^{2+}-triggered initiation of acrosome reaction after capacitation rather than capacitation-associated changes.

In livestock spermatozoa, the phosphorylation functions of the cAMP-dependent serine/threonine kinase PKA in the flagella are obviously indispensable to occurrence of cAMP-triggered events (including the capacitation-associated events) and subsequent extracellular Ca^{2+}-triggered hyperactivation [6,21]. By contrast, we previously found that the dephosphorylation functions of calyculin A–sensitive protein phosphatases (serine/threonine phosphatases) in the head are necessary for the occurrence of acrosome reaction [22,23]. Moreover, we observed that the intracellular cAMP signaling cascades promote the extracellular Ca^{2+} influx and subsequent occurrence of the acrosome reaction [24,25]. However, regulatory systems for these unique events in livestock sperm head are poorly understood. In this study, thus, we carried out experiments to examine involvement of the intracellular cAMP signaling cascades in the calyculin A–sensitive protein phosphatase–dependent decrease of serine/threonine–phosphorylated proteins in boar sperm head.

2. Materials and methods

2.1. An animal use ethics statement

The Institutional Animal Care and Use Committee of Kobe University (Permission number: 22–05–14 and 24–03–10) approved the procedures of this study which were carried out according to the guidelines of animal experiments.

2.2. Reagents and preparation of samples

All reagents were purchased from Wako Pure Chemical Industries (Osaka, Japan), unless otherwise specified.

Sperm-rich fractions from the ejaculates were obtained from mature boars (two Meishan pigs of Kobe University and two Large White pigs of the General Technological Center, >12 month old) by a manual method and then diluted with approximately two fold volume of the Tris-citric acid-glucose solution (111.0 -mM tris[hydroxymethyl]aminomethane, 34.7 -mM citric acid, and 185.0 -mM glucose) [26]. All of the samples were transported at 25°C to 30°C within 2 hours to our laboratory. The spermatozoa were immediately washed in an isotonic Percoll (2 mL 90% [v/v] and 5 mL 45% [v/v]; GE Healthcare UK Ltd., Buckinghamshire, UK) and then in a phosphate-buffered saline containing 0.1% [w/v] polyvinyl alcohol (PVA; Sigma–Aldrich Co., St. Louis, MO, USA) (PVA–phosphate-buffered saline) by centrifugation as described previously [24,27].

2.3. Experimental outlines

This study was composed of two sections. The first section included experiments to examine whether calyculin A–sensitive protein phosphatase–dependent decrease of postacrosomal serine/threonine–phosphorylated proteins in the sperm head is promoted by the activation of cAMP signaling cascades before the occurrence of the acrosome reaction. Moreover, additional experiments were done to examine possible relationship between serine/threonine–phosphorylated proteins and chlortetracycline (CTC)-binding state in the postacrosomal region. In the experiments of this section, we first observed time-related decrease of the serine/threonine–phosphorylated proteins and CTC-binding state in the postacrosomal region of spermatozoa during the incubation in the presence of cAMP analog "Sp-5,6-dichloro-1-β-D-ribofuranosyl-benzimidazole-3′,5'-monophosphorothioate (cBiMPS)" and $CaCl_2$ for induction of acrosome reaction (Fig. 1), next compared effects of the protein phosphatase inhibitor calyculin A on the cAMP analog–induced decrease between serine/threonine–phosphorylated proteins and CTC-binding state in the postacrosomal region (Fig. 2), and finally examined whether the cAMP analog-induced decrease of postacrosomal serine/threonine–phosphorylated proteins occurs during the incubation in the $CaCl_2$-deficienct medium (namely under the unfavorable condition for the acrosome reaction, Fig. 3). The second section included experiments to examine whether cAMP-dependent/PKA-less dependent signaling cascades are involved in the decrease of postacrosomal serine/threonine–phosphorylated proteins. In the experiments of the second section, we observed effects of the PKA inhibitor and potentially EPAC-specific cAMP analog on the decrease of postacrosomal serine/threonine–phosphorylated proteins (Figs. 4–7).

2.4. Incubation of spermatozoa for induction of acrosome reaction

A basic incubation medium was a modified Krebs–Ringer Hepes medium ([mKRH-PVA: 94.60 -mM NaCl, 4.78 -mM KCl, 1.19 -mM $MgSO_4$, 1.19 -mM KH_2PO_4, 1.71 -mM $CaCl_2$, 25.07 -mM Hepes [Dojindo Molecular Technologies, Inc., Kumamoto, Japan], 27.64 -mM glucose, 50 -μg/mL streptomycin sulfate [Nacalai Tesque, Inc., Kyoto, Japan], 100