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Signals of seminal vesicle autoantigen suppresses bovine serum albumin-induced capacitation in mouse sperm

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Abstract

Capacitation is the prerequisite process for sperm to gain the ability for successful fertilization. Unregulated capacitation will cause sperm to undergo a spontaneous acrosome reaction and then fail to fertilize an egg. Seminal plasma is thought to have the ability to suppress sperm capacitation. However, the mechanisms by which seminal proteins suppress capacitation have not been well understood. Recently, we demonstrated that a major seminal vesicle secretory protein, seminal vesicle autoantigen (SVA), is able to suppress bovine serum albumin (BSA)-induced mouse sperm capacitation. To further identify the mechanism of SVA action, we determine the molecular events associated with SVA suppression of BSA's activity. In this communication, we demonstrate that SVA suppresses the BSA-induced increase of intracellular calcium concentration ($[Ca^{2+}]_i$), intracellular pH (pH_i), the cAMP level, PKA activity, protein tyrosine phosphorylation, and capacitation in mouse sperm. Besides, we also found that the suppression ability of SVA against BSA-induced protein tyrosine phosphorylation and capacitation could be reversed by dbcAMP (a cAMP agonist).

Keywords: Capacitation; Seminal vesicle; Ca^{2+}; Protein tyrosine phosphorylation; cAMP-PKA pathway

During epididymal transit, sperm progressively acquire the ability to move, but they are still fertilization incompetent. Fertilization capacity is gained after residence in the female reproductive tract for a finite period of time, and the physiological changes in sperm during this period are collectively called "capacitation." Capacitation is a complex process first described and defined independently by Chang [1,2] and Austin [3,4]. The capacitation processes involve changes in membrane properties and dynamics, enzyme activities, elevation of $[Ca^{2+}]_i$, pH_i, and the cAMP level. It leads to energy consumption and hypermotility, and eventually an acrosome reaction by sperm [5,6].

Sperm capacitation occurs in the oviduct or uterus, depending on the species [6]. The process of sperm capacitation is tightly regulated by suppression factors (in the epididymis and seminal vesicles) and capacitation factors (in the female reproductive tract). Serum albumin is abundant in the female reproductive tract. It is thought to serve as a cholesterol-binding protein to remove sperm membrane cholesterol, by which to destabilize the sperm membrane and induce sperm capacitation [7–9]. Serum albumin has also been demonstrated to regulate the T-type Ca^{2+} channel of sperm, induce extracellular Ca^{2+} and bicarbonate ion influx [10], and elevate $[Ca^{2+}]_i$ and pH_i. The increase of $[Ca^{2+}]_i$ and pH_i upregulates the cAMP-dependent signaling and enhances the protein tyrosine phosphorylation, ultimately inducing hyperactivation and capacitation of sperm [5,11].

The suppressive effect of capacitation by suppression factors is referred to as "decapacitation" [12]. Without suppression regulation, most of the sperm would undergo a

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spontaneous acrosome reaction. An acrosome-reacted sperm lose its acrosome cap which is required for sperm binding to the zona pellucida of the egg. Thus, sperm ultimately lose their fertilization ability even though they still have hypermotility [6]. It has been reported that the epididymis and seminal plasma contain decapacitation activity [12–23]; the presence of suppression factors (decapacitation factors) may prevent the unfruitful capacitation of sperm and allow effective fertilization of an egg at the right time and place [17]. Currently, several decapacitation factors are known. Fraser et al. [18] suggested that the decapacitation mechanism involves fucose residues and a GPI-anchored receptor on sperm in the epididymis. A low molecular weight N-glycosidically linked oligomannosidic glycopeptide (MGp) isolated from the autoproteolysis products of human seminal plasma was reported to prevent premature sperm exocytosis [19]. Studies by Villemure et al. [21] revealed the gelatin-binding proteins from goat seminal plasma play a role in sperm decapacitation. A sperm adhesin family of boar accessory sex gland fluids is also supposed to consist of decapacitation factors [22]. In addition, the platelet-activation factor, acetylhydrolase (PAF-AH), was also suggested to play a role in decapacitation by hydrolysis of PAF to lyso-PAF [23]. However, the mechanisms of these potential factors in decapacitation have not been well defined.

Recently, we demonstrated that serum obtained from male and female mice immunized with seminal vesicle secretion (SVS) fluid is immunoreactive to an androgen-responsive glycoprotein [24,25], and it was designated seminal vesicle autoantigen (SVA). SVA is a 19-kDa protein secreted from luminal epithelium cells of seminal vesicles and contribute to the dominant component of seminal plasma (~300 μM) [26]. SVA binds Zn^{2+} [27] and choline-containing phospholipids, such as phosphatidylcholine and sphingomyelin [26]. SVA has been demonstrated to suppress BSA-induced zinc ion removal from the sperm membrane, sperm hypermotility, protein tyrosine phosphorylation, and capacitation [28]. In this communication, we further demonstrate that SVA suppresses BSA-induced $[Ca^{2+}]_i$, pH_i, the cAMP level, and PKA activity in mouse sperm. In addition, the suppressive effects of SVA on BSA-induced protein tyrosine phosphorylation and capacitation in mouse sperm can be reversed by a cAMP agonist.

Materials and methods

Materials. Fatty acid-free BSA, polyvinylalcohol, and Kemptide (Leu-Arg-Arg-Ala-Ser-Leu-Gly) were from Sigma (St. Louis, MO). Antiphosphotyrosine monoclonal antibody (clone 4G10) was from UBI (Lake Placid, NJ), horseradish peroxidase (HRP)-conjugated anti-mouse IgG was from Jackson ImmunoResearch Lab (West Grove, PA), Percoll, chemiluminescence detection ECL plus, [γ-^{32}P]ATP, and the cAMP assay kit (RPN 255) were from Amersham–Pharmacia Biotech (Buckinghamshire, UK), Fluo-3-AM and BCECF-AM were from Molecular Probes (Eugene, OR), dituylryl cAMP (dbcAMP), Rp-cAMPS, and IBMX were from Research Biochemicals International (Natick, MA), and H-89 was from LC Laboratories (Woburn, MA). Phosphocellulose Units SpinZyme Format for the radioactive kinase assay was from Pierce (Rockford, IL), and the scintillation counting cocktail was from Merck (Darmstadt, Germany). All other chemicals were of reagent grade.

Sperm preparation and cytological observations. Outbred CD-1 mice purchased from Charles River Laboratories (Wilmington, MA) were bred in the Animal Center at Taipei Medical University School of Medicine. Animals were handled in accordance with institutional guidelines on animal experimentations.

The culture medium used throughout these studies was modified Krebs–Ringer bicarbonate HEPES medium (HM) as described previously [28]. In brief, modified HM contains 120.0 mM NaCl, 2.0 mM KCl, 1.20 mM $MgSO_4{\cdot}7H_2O$, 0.36 mM NaH_2PO_4, 15 mM $NaHCO_3$, 10 mM Hepes, 5.60 mM glucose, 1.1 mM sodium pyruvate, and 1.7 mM $CaCl_2$. The pH of the medium was adjusted to 7.3–7.4 with humidified air/CO_2 (95:5) in an incubator at 37 °C for 48 h before use. Polyvinylalcohol (1 mg/ml) was added to serve as a sperm protectant. Mature mouse sperm were harvested by a swim-up procedure from the caudal epididymides and isolated with a 20–80% Percoll gradient. The viability and progressive motility of the sperm fraction used in the present study were more than 95%. The population of the capacitated stage in sperm was analyzed by the CTC staining method as described previously [29].

Flow cytometry. $[Ca^{2+}]_i$ of sperm was determined using fluo-3 AM by flow cytometry (FACScan, BD). In brief, Percoll-separated sperm were loaded with fluo-3 AM (10 μM) for 10 min. After 10 min incubation, sperm were washed twice with modified HM to remove any free fluo-3 AM. Fluo-3 AM-loaded sperm (10^6 cells/ml) were treated with SVA (0–66 μM) in the presence or absence of BSA (0.3%) at 37 °C for 90 min and then analyzed by epifluorescence microscope and flow cytometry.

pH_i of sperm was determined using BCECF-AM. In brief, Percoll-separated sperm were loaded with BCECF-AM (2 μM) for 10 min and then sperm were washed twice with modified HM to remove any free fluore. The fluore-loaded sperm were treated with BSA (0.3%) ± SVA (2, 20, and 66 μM) and then analyzed by flow cytometry. For pH_i calibration, a nigericin/high K^+ calibration protocol was used to derive the pH_i values as described previously [30]. The fluorescence of fluo-3 was excited at 488 nm and measured via a 515–540 nm filter, and the fluorescence of BCECF was excited at 510 nm and measured via a 564–606 nm filter. PMT voltages and gains were set to optimize the dynamic range of the signal. The fluorescence intensity of sperm was quantified for 10,000 individual cells.

cAMP assay. The amount of cAMP produced in living, intact sperm was determined using a nonradioactive enzyme immunoassay kit according to the manufacturer's instructions.

Assay of protein kinase A activity. Protein kinase A activity was measured using Kemptide (Leu-Arg-Arg-Ala-Ser-Leu-Gly) as the specific substrate. Sperm (10^7 cells/ml) were incubated under different experimental conditions, such as BSA (0.3%), SVA (66 μM) or BSA supplemented with SVA. DbcAMP (a cAMP agonist, 1 mM) plus IBMX (a phosphodiesterase inhibitor, 100 μM) were used to be a positive control, and H-89 (a PKA inhibitor, 30 μM) served as a negative control. After incubation at 37 °C for 90 min, the sperm suspension (10 μl) was mixed with an equal volume of 2× assay cocktail (10 μl) and incubated at 37 °C for additional 15 min. The final concentration of the assay components was 100 μM kemptide, [γ-^{32}P]ATP (6000 Ci/mmol) (2×10^6 cpm/assay), 100 μM ATP, 1% (v/v) Triton X-100, 1 mg/ml BSA, 10 mM $MgCl_2$, 40 mM β-glycerophosphate, 5 mM *p*-nitrophenyl phosphate, 10 mM Tris–HCl (pH 7.4), 10 μM aprotinin, and 10 μM leupeptin. The reactions were stopped by an equal volume of 20% TCA, and reaction mixtures were cooled on ice for 20 min and followed by centrifugation at 10,000*g* at room temperature for 3 min. Twenty-five microliter of the resultant mixture was applied onto an affinity support of the phosphocellulose unit and washed with 500 μl of 75 mM phosphoric acid for four times (10 min/each time). The washed-sample bucket was then transferred into a scintillation vial for counting.

Detection of protein tyrosine phosphorylation. Sperm (5×10^6 cells/ml) were incubated with BSA (0.3%) in the absence or presence of SVA (66 μM). In some experiments, BSA and SVA supplemented with dbcAMP (a cAMP agonist, 1 mM) plus IBMX (a phosphodiesterase inhibitor, 100 μM), or BSA and H-89 (a PKA inhibitor, 30 μM) or Rp-cAMP (a cAMP antagonist, 1 mM) were added. The reactions were incubated at 37 °C. After 90 min incubation, the cell lysate was prepared