Rod outer segment membrane guanylate cyclase type 1 (ROS-GC1) calcium-modulated transduction system in the sperm

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Objective: Evaluation of the presence of a Ca^{2+}-regulated membrane guanylate cyclase signal transudation system in the spermatozoa.
Design: Experimental study.
Setting: Research university laboratory.
Patient(s): Human sperm obtained from healthy donors who met the criteria of the World Health Organization for normozoospermia and bovine semen collected from bulls of proven fertility.
Intervention(s): Radioimmunoassay and immunohistochemistry of human and bovine spermatozoa.
Main Outcome Measure(s): The membrane guanylate cyclase activity and the presence of membrane guanylate cyclase transduction machinery components in the spermatozoa.
Result(s): The identity of a Ca^{2+}-modulated membrane guanylate cyclase transduction machinery in human and bovine spermatozoa has been documented. The machinery is both inhibited and stimulated within nanomolar to semimicromolar range of free Ca^{2+}. The transduction component of this machinery is the rod outer segment membrane guanylate cyclase type 1 (ROS-GC1). The enzyme coexists with three Ca^{2+}-dependent modulators: guanylate cyclase activating protein type 1 (GCAP1), S100B and neurocalcin δ. ROS-GC1 and its modulators are present in the heads and tails of both species' spermatozoa.
Conclusion(s): The coexpression of ROS-GC1 and its activators in spermatozoa suggests that the Ca^{2+}-modulated ROS-GC1 transduction system may be a part of the fertilization machinery. (Fertil Steril® 2010;93:904–12. ©2010 by American Society for Reproductive Medicine.)

Key Words: spermatozoa, cyclic GMP, membrane guanylate cyclase, ROS-GC1, calcium, GCAP1, S100B, neurocalcin δ

Spermatogenesis—the production of sperm in the testis—represents a complex process involving specific communications between the developing germ cell and supporting Sertoli cells under the control of androgen-producing Leydig cells (1). Mammalian sperm released from the seminiferous epithelium is immotile and not capable of fertilizing the oocyte. The acquisition of the fertilization ability involves a number of maturation processes, such as activation of motility in the epididymis, capacitation, hyperactivated motility, and acrosomal reaction in the female genital tract (2).

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In the sperm of vertebrates and invertebrates, the cellular processes of capacitation and acrosomal reactivity are regulated by cyclic nucleotides and calcium ions (3–6). Both of these molecules play crucial roles during various stages of fertilization, especially in facilitating the sperm motility (7–9). Although sperm motility is one of the most important predictors of fertilizing ability, the mechanisms controlling the swimming behavior remains poorly understood. It is well established that an increase in the intracellular calcium concentration results in increased sperm motility. However, the signals for the Ca^{2+} entrance into the sperm cell, the molecular identity of the calcium channels involved, and the Ca^{2+} conductance remain unknown.

Several ion channels contributing to the control of sperm membrane permeability have been identified. These channels include voltage-gated calcium channels such as N-, R-, and T-type, transient receptor potential (TRP) channel, and a unique, testes-specific, Ca^{2+} channel—CatSper (3, 10–13). In addition, a cyclic nucleotide-gated (CNG) channel

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has been identified in the mammalian sperm. By virtue of its high Ca^{2+} permeability, the CNG channel is the prime candidate for mediating Ca^{2+} entry into sperm. The homooligomeric α subunit of the CNG channel cloned from bovine testes is approximately 200-fold more sensitive to guanosine 3,5-cyclic monophosphate (cGMP) than to adenosine 3,5-cyclic monophosphate (cAMP), suggesting that the channel is a part of the cGMP-signaling pathway (14).

Cyclic GMP is synthesized by a group of enzymes termed *guanylate cyclases*. Depending on their cellular distribution guanylate cyclases have been classified into two forms: membrane bound and soluble (15, 16). On a biochemical basis, the membrane guanylate cyclase family is subdivided into two subfamilies: surface receptor and rod outer segment guanylate cyclase (ROS-GC). A striking functional feature separating these two subfamilies is that the former is designed to transduce the extracellularly generated signals of peptide hormones, and the latter is designed to transduce the intracellularly generated Ca^{2+} signals. The first subfamily consists of the atrial natriuretic factor (ANF) receptor guanylate cyclase (ANF-RGC; also known as GC-A), C-type natriuretic peptide (CNP) receptor guanylate cyclase (CNP-RGC; also known as GC-B), and heat-stable enterotoxin (STa) receptor guanylate cyclase (STa-RGC or GC-C). To the second subfamily belong the rod outer segment guanylate cyclase type 1 (ROS-GC1) and type 2 (ROS-GC2; also termed RetGC1 and RetGC2) and the olfactory neuroepithelium guanylate cyclase (ONE-GC, also known as GC-D) (16).

To date, functional activity of the soluble guanylate cyclase and of the ANF-RGC and CNP-RGC have been shown in testes and in spermatozoa (17–23). Recently we have demonstrated the presence of Ca^{2+}-modulated membrane guanylate cyclase transduction machinery—ROS-GC1 in bovine testes (24). The machinery is both inhibited and stimulated by nanomolar- to semicromolar-free Ca^{2+} levels. The Ca^{2+} sensor component of the inhibitory mode of the machinery is GCAP1, and for the stimulatory mode it is S100B. ROS-GC1 is predominantly expressed in spermatogenic cells. GCAP1 expression is restricted to a small population of spermatogonia, whereas S100B is present in the majority of spermatocytes and spermatids. The expression of GCAP1 and S100B in spermatocytes and spermatids is mutually exclusive (24).

Guided by the observations that both cyclic nucleotides and calcium ions play a key role in fertilization and spermatogenesis, and that ROS-GC1 transduction machinery is present especially in the primary spermatocytes and spermatids of testis, we reasoned that the ROS-GC1 signal transduction system regulated by intracellular Ca^{2+} may be present also in the spermatozoa. This hypothesis has been validated experimentally in the present studies.

MATERIALS AND METHODS

Human sperm samples were obtained from healthy donors who met the criteria of the World Health Organization for normozospermia, by masturbation after 3–5 days of abstinence. The study was approved by the ethics review board of Poznan University of Medical Sciences. Informed patients' consent was obtained for this study.

Semen was diluted 1:5 in Ham's F-10 medium (Invitrogen, Paisley, United Kingdom) and washed by centrifugation (170 $\times$ *g*, 10 minutes) and fixed on glass slides in acetone (5 minutes at room temperature).

Preparation of the membrane fraction of spermatozoa

The membrane fraction of spermatozoa was isolated according to the protocol described previously (25) with some modifications. Briefly, the sperm were centrifuged for 10 minutes at 500 $\times$ *g* at room temperature. The pellet was homogenized in a buffer containing 250 mM sucrose, 5 mM Tris-HCl (pH 7.5), 1 mM ethylenediamine tetraacetic acid (EDTA) and 1mM phenylmethylsulfphonyl fluoride (PMSF). The suspension was sonicated three times for 15 seconds each. The membrane fraction was separated by three-step centrifugation (4,000 $\times$ *g* for 15 minutes, 10,000 $\times$ *g* for 10 minutes, and 100,000 $\times$ *g* for 60 min, at 4°C). The pellet was designated as the membrane fraction.

Guanylate cyclase activity

The particulate fraction of the spermatozoa was assayed for guanylate cyclase activity as described previously (24, 26). The membranes were preincubated in an ice bath with or without GCAP1, S100B, or neurocalcin δ in an assay system containing 10 mM theophylline, 15 mM phosphocreatine, 20 μg creatine kinase, and 50 mM Tris-HCl (pH 7.5), adjusted to the appropriate free Ca^{2+} concentration with precalibrated Ca^{2}/EGTA (Ethylene glycol-bis[2-aminoethylether]-N,N,N′,N′-tetraacetic acid) solutions (Molecular Probes, Leiden, The Netherlands). The reaction was initiated by the addition of a substrate solution (4mM $MgCl_2$ and 1mM GTP-guanosine triphosphate, final concentration) and continued by incubation at 37°C for 10 minutes. The reaction was terminated by the addition of 50 mM sodium acetate (pH 6.2), followed by heating in a boiling water bath for 3 minutes. The amount of cGMP formed was determined by radioimmunoassay (27).

Immunohistochemistry

After nonspecific binding had been blocked in blocking solution (tris-buffered saline [TBS], pH 7.5, containing 100 mM tris[hydroxymethyl]aminomethane [TRIS-HCl], 0.9% NaCl, 0.05% Tween-20 [TBS-T] and 1% bovine serum albumin [BSA]) fixed spermatozoa were incubated with the rabbit primary antibodies against ROS-GC1, GCAP1, S100B and neurocalcin δ for 60 minutes at 37°C in a humidified chamber and washed four times (15 minutes each) in TBS-T. The antibodies were used in the dilution of 1:200 for ROS-GC1, and 1:100 for GCAP1, S100B and neurocalcin δ. Characterization of highly specific antibodies raised against GCAP1, S100B, neurocalcin δ, and ROS-GC1 has been described previously (28–30). The antibodies were enriched by precipitating the immunoglobulin fraction using ammonium sulphate. Western blotting and ELISA were used to test the specificity and to determine the titer of purified antibodies. The primary antibodies were detected using Cy3-conjugated sheep anti-rabbit

immunoglobulin (Ig) G, diluted 1:200 (Sigma-Aldrich, St. Louis, MO). Incubation and washing conditions were as described for primary antibodies. A Carl Zeiss LSM 510 confocal microscope was with a 63x objective used for localization of the antigen-antibodies complex. Controls included detection reactions performed under identical conditions, except that the primary antibodies were replaced by preimmune serum.

The use of computer-assisted image transformation allowed for assessment of immunohistochemical results. Images generated in .lsm true color file format from Zeiss LSM Image Browser Version 3.1.0.99 for Carl Zeiss Laser Scanning System LSM 510 (Carl Zeiss GmbH, Jena, Germany) were exported to Microsoft Windows bitmap file format and saved. In Adobe Photoshop 5.5 CEEA (Adobe Systems Inc., San Jose, CA), the color mode was changed to grayscale format (8 bits per channel, 256 gray levels) and stored as a tagged image file format (TIFF). Using Corel Photo Paint 9 (Trumatch Inc, New York, NY), the grayscale was supplement into negative photo format and stored as a final TIFF file.

RESULTS

Functional Ca^{2+}-dependent membrane guanylate cyclase is present in the sperm

It has been shown previously that Ca^{2+}-modulated membrane guanylate cyclase transduction system is present in bovine testes (24). The membrane guanylate cyclase identified was the ROS-GC1 and its Ca^{2+}-dependent modulators were GCAP1 and S100B. The study, however, did not localize precisely the two transduction systems. To fill this gap, the present study was undertaken to determine whether the ROS-GC1 transduction system is present in the sperm.

Membrane fraction of bovine and human sperm was isolated and tested for guanylate cyclase activity. A cumulative value representing the specific activity of all putative guanylate cyclases present in the fraction was approximately 8.5–10 pmol of cGMP per milligram of protein per minute for human and bovine sperm, respectively. To assess whether the activity is Ca^{2+}-modulated, the membrane fraction was exposed to increasing (0.01–10 μM) concentrations of free Ca^{2+}, and the guanylate cyclase activity was measured. The results are presented in Figure 1. Within the Ca^{2+} concentrations ranging 10–800 nM, the cyclase activity progressively decreased and the minimum activity was observed at 800 nM Ca^{2+}. Further increase in Ca^{2+} concentrations resulted in the dose-dependent increase in cyclase activity. The inhibitory IC_{50} value for Ca^{2+} was approximately 200 nM, and the stimulatory EC_{50} value was approximately 2 μM (Fig. 1). Thus it was concluded that sperm contains membrane guanylate cyclase that is both inhibited and stimulated by free Ca^{2+} concentrations ranging from nanomolar to micromolar.

Spermatozoa express a guanylate cyclase with the biochemical attributes of ROS-GC

There are three known Ca^{2+}-modulated membrane guanylate cyclases: ROS-GC1, ROS-GC2 and ONE-GC. These cyclases can be biochemically differentiated. The ROS-GCs are stimulated by GCAPs at low nanomolar Ca^{2+} concentrations and the activity is progressively inhibited by increasing concentrations of Ca^{2+} (31–33). In the semimicromolar to micromolar Ca^{2+} concentrations, both ROS-GCs are stimulated by S100B (34, 35) and ROS-GC1 is also stimulated by neurocalcin δ (36). ONE-GC is stimulated only by Ca^{2+}-bound neurocalcin δ (37). It is noted that ONE-GC is not expressed in human and bovine tissues; it is a rodent- specific protein. There is, however, ONE-GC–like membrane guanylate cyclase expressed in bovine hippocampus (40).

To determine which one (or more) of Ca^{2+}-modulated membrane guanylate cyclases is expressed in the sperm, the membrane fraction of bovine and human sperm was incubated with GCAP1 or GCAP2 in the presence of 10 nM Ca^{2+} and S100B or neuroclacin δ in the presence of 100 μM Ca^{2+}. The stimulation by GCAP3 was not tested because its expression was not detected in bovine testes tissue (38). Both GCAPs stimulated the cyclase activity in a dose-dependent manner with an EC_{50} of approximately 1 μM for GCAP1 (Fig. 2A) and of approximately 6 μM for GCAP2 (Fig. 2B). The maximal stimulation of the cyclase was more then two-fold above the basal value (Fig. 2A, B). The Ca^{2+}-bound S100B also stimulated the cyclase activity in a dose-dependent fashion. The half-maximal stimulation was achieved at

FIGURE 1

Ca^{2+} regulation of guanylate cyclase activity in sperm. Membrane fraction of human and bovine spermatozoa was prepared and assayed for guanylate cyclase activity as described in Materials and Methods. Membranes were incubated within the presence of the indicated concentration of Ca^{2+}.

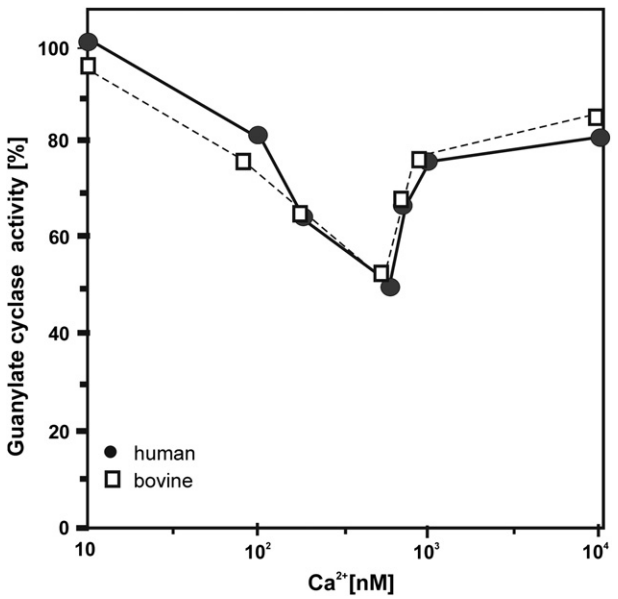

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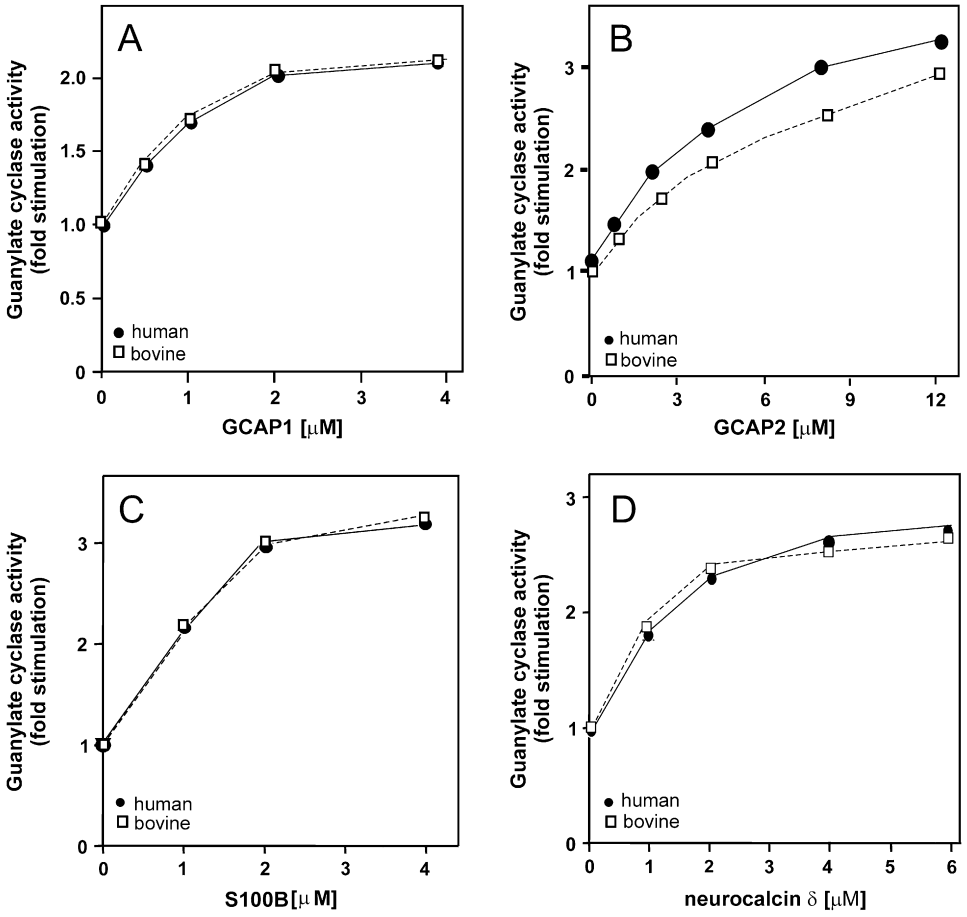
FIGURE 2

Effect of calcium-binding proteins on spermatozoa membrane guanylate cyclase activity. Membranes of spermatozoa were incubated with increasing concentrations of (**A**) GCAP1, (**B**) GCAP2 in the presence of 10 nM free Ca^{2+} or with increasing concentrations of (**C**) S100B, (**D**) neurocalcin δ in the presence of 100 μM Ca^{2+}. Each experiment was done in triplicate, and repeated two times. The results shown are from one representative experiment. Error bars are within the size of the symbols.



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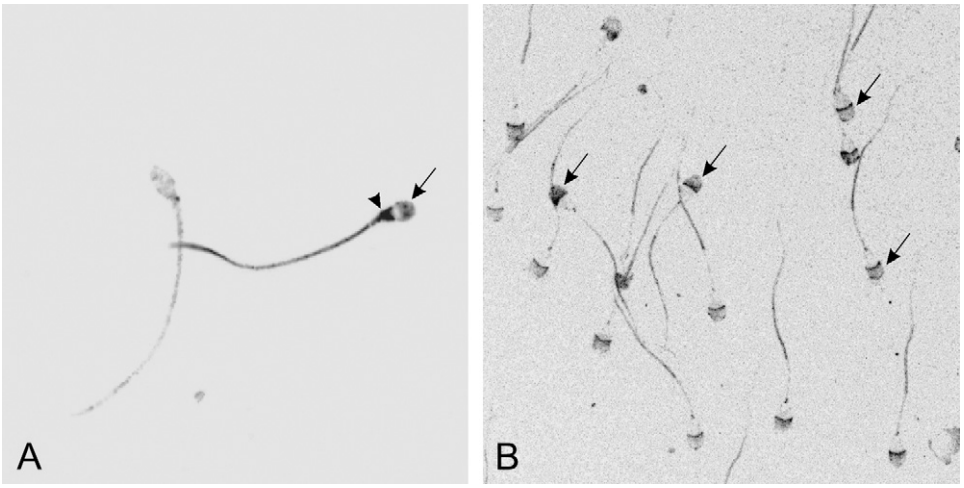
0.8 μM S100B, and the maximal stimulation was approximately threefold (Fig. 2C). In addition, neurocalcin δ stimulated the cyclase activity in a dose-dependent fashion; the EC_{50} value was approximately 1 μM, and the maximal stimulation of approximately 2.5-fold was at 6 μM neurocalcin δ (Fig. 2D). These stimulatory profiles allowed the conclusion that the membrane fraction of sperm contains a Ca^{2+}-modulated guanylate cyclase that functionally mimics ROS-GC1.

ROS-GC1 is expressed in bovine and human sperm

To verify the preceding conclusion at the protein level, immunohistochemical analyses were performed. Bovine and human sperm fixed in acetone were probed with ROS-GC1 monospecific antibodies followed by detection with Cy3-conjugated sheep anti-rabbit antibody. Positive reaction after computer-assisted image transformation is indicated in black (Figs. 3 and 4). The results show that ROS-GC1 protein is present in both human and bovine spermatozoa. The staining pattern for these two species' sperm was very similar; however, it varied among individual spermatozoa. ROS-GC1 was localized in both heads and tails of the analyzed sperm. The highest accumulation of the cyclase was observed in the acrosomal cap of human (Fig. 3A) as well as bovine spermatozoa's head (Fig. 3B), although strong staining of the postacrosomal region was also observed. In the majority of cells, the positive staining of the entire flagellar membrane was detected (Fig. 3); it is evident, however, that the staining is not uniform among the spermatozoa: some bovine spermatozoa lacked the midpiece staining, indicating varying levels of ROS-GC1 expression among these cells (Fig. 3B). No labeling was observed in the controls, where the primary antibodies were omitted. These results show that the spermatozoa contain ROS-GC1.

FIGURE 3

Immunolocalization of ROS-GC1 in spermatozoa. Immunohistochemistry was performed using antibodies against ROS-GC1 on acetone-fixed human (**A**) and bovine sperm (**B**). The cyclase was expressed in the acrosomal cap (indicated by *arrows*) and postacrosomal region of spermatozoa (*arrowheads*). In most cells, the positive staining of the entire flagella was observed; however, some bovine spermatozoa lack the midpiece staining. Original magnification ×630.



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ROS-GC1 modulators: GCAP1, S100B and neurocalcin δ are present in sperm

In contrast to the peptide hormone receptor guanylate cyclases, ROS-GC1 receives Ca^{2+} signals indirectly through its sensor proteins, which belong to two classes: GCAPs and Ca^{2+}-dependent GCAPs (CD-GCAPs). Upon binding Ca^{2+}, GCAPs undergo conformational changes and inhibit ROS-GC. CD-GCAPs also undergo conformational changes, but they stimulate ROS-GC (16, 39). Across species, GCAP exists in two forms: GCAP1 and GCAP2. In addition, a third form of GCAP, GCAP3, has been detected in humans and zebra fish (31, 32, 38). CD-GCAP exists also in three forms: S100B, neurocalcin δ, and frequenin (31, 33, 36, 40).

To determine whether GCAP1, S100B and/or neuroclacin δ are the Ca^{2+} sensors of ROS-GC1 present in the sperm membranes (Fig. 1), their expression in spermatozoa was investigated by immunohistochemistry. The presence of GCAP2, GCAP3, and frequenin was not tested because the expression of these proteins was not detected in bovine testes (24, 38).

All three proteins—GCAP1, S100B, and neurocalcin δ—were detected in principal segment of acrosomal cap of human (Fig. 4A, C, E) and bovine spermatozoa (Fig. 4B, D, F). Their higher accumulation was observed in the apical segment of the bovine sperm (Fig. 4 B, D, F). Strong labeling of the flagellar membrane of both human and bovine spermatozoa was also observed (Fig. 4A-F). In addition, neurocalcin δ immunoreactivity was also observed in the postacrosomal region of some spermatozoa (Fig. 4F). No labeling was observed in the controls, where the primary antibodies were omitted (data not shown).

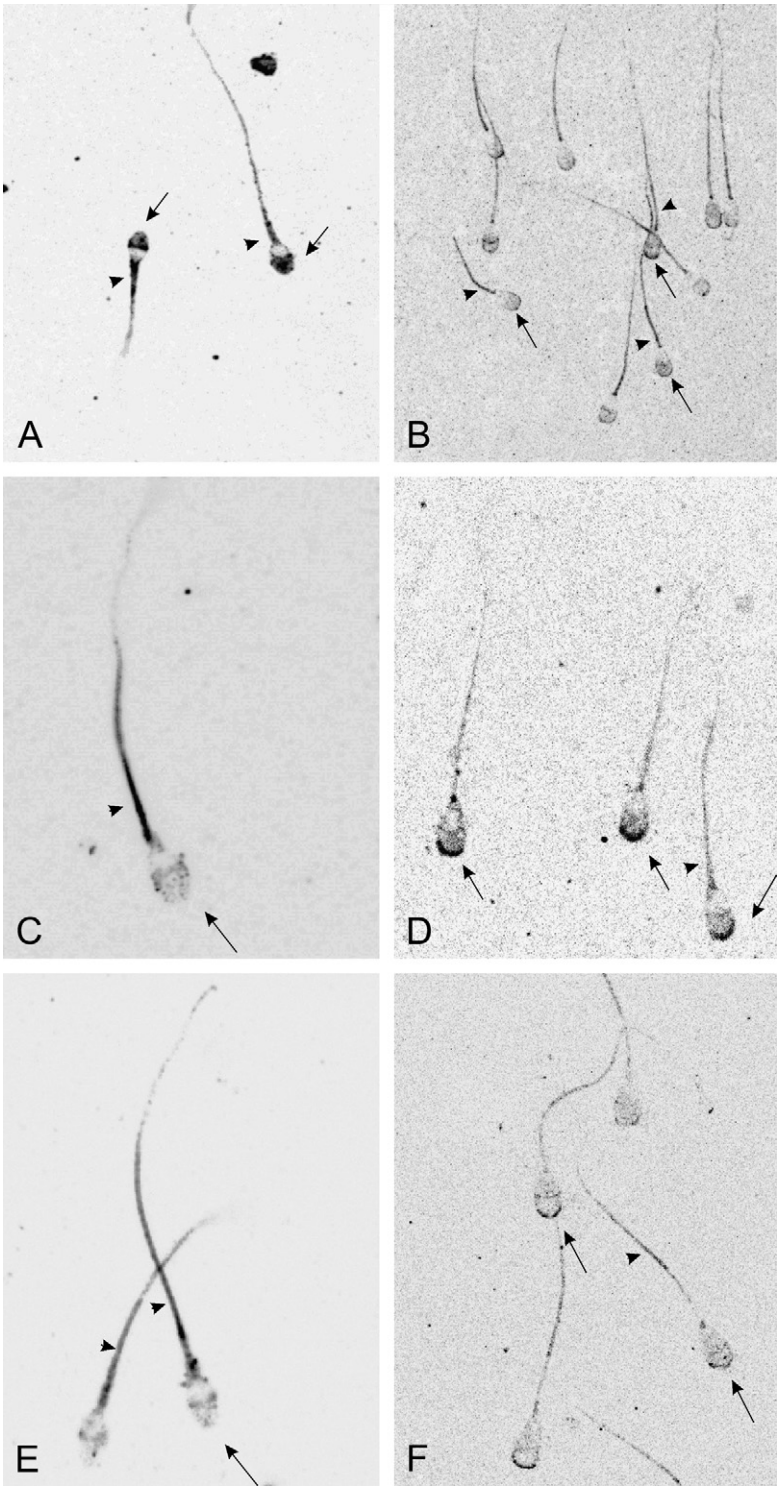
Thus Ca^{2+} sensor proteins regulating guanylate cyclase activity colocalise with ROS-GC1 in the bovine and human spermatozoa.

DISCUSSION

With the discovery of Ca^{2+}-modulated ROS-GC signal transduction system, the belief arose that it is exclusively present in the retinal rod and cone outer segments where it functions as the key component of the recovery phase of phototransduction. Upon sensing progressive rises in free Ca^{2+} (from low nanomolar to semimicromolar), GCAPs proportionately decelerate the ROS-GC activity. This concept was modified with the discovery of ROS-GC modulators that stimulated its activity in semimolar to micromolar Ca^{2+} concentrations and with the demonstration that ROS-GC transduction systems are also present outside photoreceptor outer segments. Accordingly, ROS-GC1/GCAP1 and ROS-GC1/S100B transduction systems were subsequently shown to exist in the mutually exclusive subpopulations of pinealocytes (41, 42). The ROS-GC1/GCAP1 system has been localized to the mitral cells of the olfactory bulb (31) and the presynaptic region of the pedicles in cone photoreceptors (44). The ROS-GC1/S100B system is present in the photoreceptor-bipolar synaptic layer of the retina (43) and in the gustatory epithelium of tongue (29). Recently, we have shown that the ROS-GC1/GCAP1 system also exists outside the central nervous system, in spermatogenic cells of the seminiferous epithelium of testes (24). These findings imply that the ROS-GC transduction system has a more widespread role in

FIGURE 4

Immunolocalization of ROS-GC1 modulators in spermatoza. All three proteins: GCAP1 (**A**, **B**), S100B (**C**, **D**) and neurocalcin δ (**E**, **F**) were detected in principal segment of acrosomal cap (*arrows*) of human (left panel: **A**, **C**, **D**) and bovine sperms (right panel: **B**, **E**, **F**). Particularly strong signal of flagellar membrane was detected when anti GCAP1 antibodies were used. Neurocalcin δ was expressed in sperm tails, especially in its principal piece as well as in postacrosomal region. Original magnification $\times$630.



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general Ca^{2+}-dependent signaling. With the background information that both cyclic GMP and Ca^{2+} play an important role in the spermatogenesis and fertilization processes and the ROS-GC1 transduction machinery is present in spermatogenic cells of testis, the present study was aimed at assessing the presence of a ROS-GC transduction system in the sperm.

The results of the study provide a functional and immunological description of the Ca^{2+}-modulated membrane guanylate cyclase transduction machinery present in the spermatozoa. They show that the type of this machinery is ROS-GC1, and that it is present with its three Ca^{2+} sensors GCAP1, S100B, and neurocalcin δ. Therefore, it is also possibly modulated by these Ca^{2+} sensors.

The interesting aspect of this machinery is the composition of its Ca^{2+} sensors. They are both GCAPs and CD-GCAPs. In nanomolar and semimicromolar to micromolar Ca^{2+}, ROS-GC1 is respectively stimulated and inhibited by GCAP1. On the other hand, the cyclase is stimulated by the S100B and neurocalcin δ in the semimicromolar to micromolar Ca^{2+} concentrations. All the stimulatory profiles observed during experiments were in accord with those established earlier for the reconstituted systems consisting exclusively of recombinant ROS-GC1 and individual Ca^{2+} sensor proteins, GCAPs, S100B, or neurocalcin δ (16, 28, 32, 33, 35, 36, 45).

Immunohistochemical experiments revealed that ROS-GC1 is expressed in the acrosomal cap and postacrosmal region of heads as well as tails of both human and bovine spermatozoa. ROS-GC1 colocalizes with calcium-binding proteins, which are expressed both in the acrosomal cap and tail of human and bovine spermatozoa. Some differences in accumulation of the cyclase activators among individual spermatozoa were observed. Particularly strong staining of the middle piece of flagella with anti-GCAP1 antibodies was detected. In addition, high expression level of neurocalcin δ in the flagella and postacrosomal regions of particular spermatozoa was observed.

Thus these results document, for the first time, the functional identity of a Ca^{2+}-modulated membrane guanylate cyclase transduction machinery in human and bovine spermatozoa. The transduction component is the guanylate cyclase ROS-GC1. The enzyme coexists with its three Ca^{2+}-dependent modulators: GCAP1, S100B, and neurocalcin δ. In response to localized changes in free calcium concentration GCAP1, S100B and neurocalcin δ bind to the specific domains, residing in the intracellular region of the cyclase, and inhibit or stimulate ROS-GC1. In this manner Ca^{2+} pulses precisely regulate the levels of cGMP generated in the cell.

What are the targets of the cyclic GMP that is synthesized by ROS-GC1 in response to GCAP1, S100B, or neurocalcin δ stimulation? There is no definite answer to this question; however, some possibilities can be envisioned. The simplest one is based on the presumption that the function of cGMP produced by ROS-GC1 in testes is similar to that in rod outer segments, olfactory, and gustatory epithelium where it regulates the activity of the cyclic nucleotide-gated channels (CNG). In this prediction, the testes contain a CNG channel, and the channel is linked to ROS-GC1.

In fact, a CNG channel is expressed in bovine sperm and precursors cells (14). It was shown that the motility of sperm can be regulated by Ca^{2+} entry through CNG channels. The α and β subunits of the channel are expressed in sperm and precursor cells. The CNG channels serve as a Ca^{2+} entry pathway that respond more sensitively to cGMP than to cAMP. In mature sperm, the α subunit is observed along the entire flagellum, whereas the short β subunit is restricted to the principal piece of the flagellum. The CNG channels subtypes differ in their calcium ions' permeability, and dissimilar location may give rise to a pattern of Ca^{2+} microdomains along the flagellum, providing the structural basis for the control of flagellar bending waves (14). The sequence of a novel cGMP gated K^+ channel was discovered in sea urchin lately (46). It was already shown that the hyperpolarization evoked by chemoattractants is caused by the opening of K^+-selective CNG channels in flagellum and calcium ions influx starts at the onset of recovery from hyperpolarization (47). The results of this study established a model of chemosensory transduction in sperm, whereby a cGMP-induced hyperpolarization opens calcium channels by a "recovery-from-inactivation" mechanism and unveils an evolutionary relationship between transduction mechanisms in sperm and photoreceptors (47). Therefore, our results demonstrating the presence of ROS-GC transduction machinery in sperm are in agreement with the data published previously (24).

Another possibility is that the target of the cyclic GMP produced via the GCAP1, S100B, and neurocalcin δ–modulated ROS-GC1 transduction system is cyclic GMP-dependent kinase (PKG). Indeed, it was shown that sea urchin sperm contain low levels of PKG (48), and the PKG inhibitors are able to block the sodium nitropruside–induced acrosome reaction (AR) in human sperm, suggesting that the NO/cGMP pathway needs the activation of a PKG to trigger the AR (49). This pathway is also beneficial to pig and human sperm capacitation and acrosomal reaction (50, 51). Furthermore, it was shown recently that the effect of ANP and of ginsenosides (the biologically effective components of ginseng) on acrosome reaction could be blocked by cGMP-dependent proteine kinase inhibitors KT5823 and/or Rp-8-pCPT-cGMPS (50, 51).

At present, there is no exact answer to the question about the role of ROS-GC1, given the complete lack of precedence on which a working model can be based. However, expression of ROS-GC1 in testes and in spermatozoa suggests that the guanylate cyclase is a part of fertilization machinery. Colocalization of the cyclase and its activators in the acrosomal cap provides the evidence that acrosomal reaction may be regulated by calcium-modulated membrane guanylate cyclase transduction machinery. Alternatively, the flagellar localization of the enzyme also suggests that motility of sperm can be controlled by the cGMP that is produced by ROS-GC1.

Future experiments are needed to show that this pattern provides the molecular basis for modulating sperm behavior.

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