Guanylate Cyclase Activity and Sperm Function

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In species with external fertilization, the guanylate cyclase family is responsible for the long-distance interaction between gametes, as its activation allows sperm chemotaxis toward egg-derived substances, gamete encounter, and fertilization. In species with internal fertilization, guanylate cyclase-activating substances, which are secreted by several tissues in the genital tracts of both sexes, deeply affect sperm motility, capacitation, and acrosomal reactivity, stimulating sperm metabolism and promoting the ability of the sperm to approach the oocyte, interact with it, and finally fertilize it. A complex system of intracellular pathways is activated by guanylate cyclase agonists in spermatozoa. Sperm motility appears to be affected mainly through an increase in intracellular cAMP, whereas the acrosome reaction depends more directly on cyclic GMP synthesis. Both cyclic nucleotides activate specific kinases and ion signals. A complex cross-talk between cAMP- and cyclic GMP-generating systems occurs, resulting in an upward shift in sperm function. Excessive amounts of certain guanylate cyclase activators might exert opposite, antireproductive effects, increasing the oxidative stress on sperm membranes. In view of the marked influence exerted by guanylate cyclase-activating substances on sperm function, it seems likely that guanylate cyclase activation or inhibition may represent a new approach for the diagnosis and treatment of male and/or female infertility. (Endocrine Reviews 23: 484–494, 2002)

I. Introduction: The Guanylate Cyclase Family

Cyclic GMP (cGMP) was described as a biological product in 1963, but for many years it was not considered as a potential second messenger. There are several reasons for this, including its relatively low concentration in the tissues (1). It is now clear, however, that cGMP is a key signaling molecule in many tissue functions, such as retinal phototransduction, intestinal secretion, smooth muscle relaxation, platelet activation, and neurotransmission (2).

Guanylate cyclases, the ubiquitous enzymes that catalyze the conversion of GTP to cGMP, are expressed in both soluble (sGC) and particulate, membrane-bound (mGC) isoforms (Fig. 1). These isoforms coexist in most cells, where their relative amount depends on the type and physiological state of the tissue (1).

The known guanylate cyclase catalytic domains are homologous to the mammalian adenylate cyclase C1 and C2 catalytic regions (2). The recent resolution of the crystal structure of adenylate cyclases provided important clues to the structure of guanylate cyclases (3). An amphipathic α-helix (hinge region) divides the intracellular part of guanylate cyclase into 1) a protein kinase homology domain in mGC or a putative heme-binding region in sGC, and 2) a cyclase homology domain in both mGC and sGC. In addition, mGC contains an extracellular ligand-binding domain, which recognizes a variety of different peptides (3).

The mGC is a cell surface receptor enzyme that contains an extracellular receptor domain and an intracellular catalytic domain separated by a single transmembrane domain (4). Eight subclasses of mGC have been identified so far in vertebrates: they are homodimeric glycoproteins (3, 5), which may be associated with the plasma membrane, the endoplasmic reticulum, the Golgi bodies, and the nuclear membrane (1). Some mGC subclasses (GC-A, GC-B) appear to represent the receptors for three structurally similar peptides termed natriuretic peptides, which contain 22–32 amino acids and are characterized by a 17-amino acid disulfide ring (6). Atrial natriuretic peptide (ANP) and the B-type natriuretic peptide appear to be synthesized principally in the heart and to circulate in the bloodstream, whereas the C-type natriuretic peptide is distributed in a variety of tissues, is not...
found in appreciable amounts in the blood, and may therefore act locally (7). The mGC subclass GC-A binds with higher affinity to ANP and the B-type natriuretic peptide, whereas GC-B binds with higher affinity to the C-type natriuretic peptide (3). The mGC subclasses GC-C (cloned from rat intestine) and OK-GC (cloned from opossum kidney) bind the heat-stable enterotoxin of *Escherichia coli* (18–19 amino acids). Guanylin, uroguanylin, and lymphoguanylin, three 15-residue peptides isolated from rat intestinal mucosa, opossum urine, and opossum lymphoid tissues, respectively, were identified as physiological ligands of GC-C and OK-GC (3, 8). Molecular cloning of cDNAs led to the identification of the mGC subclasses GC-D (olfactory epithelium), GC-E, GC-F (retina, pineal gland), and GC-G (lung, intestine, skeletal muscle), but no ligands have been identified for these receptors (termed “orphan” mGCs) (3, 4). The mGC is regulated by phosphorylation and by Ca\(^{2+}\)-binding proteins (6).

The soluble isoform of guanylate cyclase (sGC) includes a group of heterodimeric hemoproteins composed of α- and β-subunits (5). Four distinct sGC subunits (α1, α2, β1, and β2) have been cloned, but the only sGCs shown to exist as a protein in animal tissues are α1/β1 and α2/β1: the existence of other sGCs *in vivo* is still a matter of debate (3, 4). The β2-subunit, which contains a consensus isoprenylation site, was shown to inhibit the NO-stimulated activity of the α1/β1 heterodimer (9). The guanylate cyclase sGC isoform contains a prosthetic heme group on each heterodimer at the level of the β1-subunit (4). The heme moiety can bind diffusible gases such as nitric oxide (NO) and carbon monoxide (3). After such binding, the enzyme’s catalytic activity is enhanced from 5-fold (with carbon monoxide) to 400-fold (with NO) (4).

NO is a short-lived, free radical gas synthesized in many mammalian cell types (10) by a class of reduced nicotinamide adenine dinucleotide phosphate-dependent NO synthases (NOSs) (Fig. 2), which catalyze the conversion of l-arginine to l-citrulline and NO with a stoichiometry of 1:1 and are

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**Fig. 1.** Schematic representation of the guanylate cyclase isoforms. COOH, Carboxy-terminal end; NH\(_2\), amino-terminal end. [Modified from J. W. Denninger and M. A. Marletta: *Biochim Biophys Acta* 1411:344–350, 1999 (4) with permission from Elsevier Science.]

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**Fig. 2.** Diagram of NO synthase (NOS) activity. COOH, Carboxy-terminal end; NH\(_2\), amino-terminal end. [Modified from J. W. Denninger and M. A. Marletta: *Biochim Biophys Acta* 1411:344–350, 1999 (4) with permission from Elsevier Science.]
competitively inhibited by L-arginine analogs (11). Three different NOS isoenzymes have been characterized to date (12, 13). Two of them are constitutive, Ca\(^{2+} /\text{calmodulin}\)-activated isoforms (endothelial NOS and neuronal NOS), and one is an inducible, Ca\(^{2+}\)-independent isoform detected in macrophages. NO, an ubiquitous mediator of cell-to-cell interaction, is produced in response to a large number of stimuli and displays a wide spectrum of activities, such as smooth muscle relaxation, inhibition of platelet aggregation and adhesion, neurotransmission, and cytotoxicity (14). The activation of sGC and the subsequent induction of cGMP synthesis mediate the effects of NO on vascular smooth muscle cell relaxation and growth, as well as on platelet aggregation and on the adherence of neutrophils to endothelial cells (15).

II. Activators of Guanylate Cyclase in Spermatozoa

The spermatozoon of invertebrate species and of mammals possesses both the soluble and the membrane-bound isoforms of guanylate cyclase (16). The mGC isoform is the most intensively studied in invertebrates, because it participates in the complex mechanisms of gamete recognition over relatively long distances in the open environment and is therefore important for the reproduction of species with extracorporeal fertilization. The mGC isoform acts as a cell-surface receptor on spermatozoa for species-specific chemotactic substances released by the eggs of some species, such as sea urchins. This isoform exists in the sperm cells of echinoderms as two different subclasses (GC-A and GC-B) with similar ligand specificities (17).

The mGC molecule is a large glycoprotein consisting of 986 amino acids, with an oligosaccharidic component containing N-acetylgalcosamine, mannosamine, galactose, and 2-amino-erythritol (18). Effector substances acting on sperm mGC in echinoderms are small, species-specific peptides produced by the jelly layer that coats the egg. Overall, 75 sperm-activating peptides have been purified from the solubilized jelly layer of 17 different species of sea urchins. Their biological effects are practically the same in all sea urchin spermatozoa but appear to be species-specific according to the taxonomic ordinal level (19). The best known mGC-activating substances in spermatozoa are the following:

1. Resact. This 14-amino-acid peptide, associated with the eggs of the echinoderm Arbacia punctulata, has sperm-specific and species-specific chemotactic properties and stimulates oxygen consumption, motility, and cGMP synthesis in A. punctulata spermatozoa (20).

2. Speract. In two species of sea urchins, Strongylocentrotus purpuratus and Lytechinus pictus, this decapeptide, which is associated with the egg jelly, binds to specific sperm surface receptors inducing an increase in sperm motility and respiration rate (21, 22). The spermatozoa of L. pictus possess a surface receptor that responds to picomolar concentrations of the jelly coat-secreted guanylate cyclase activator speract (23). The association rate of speract to the receptor is markedly reduced in seawater that is free of Na\(^+\)/H\(^+\), rich in K\(^+\)/H\(^+\), and contains no divalent cations, but is increased by substances that raise the intracellular pH (23). In turn, binding of speract to its receptor elicits an abrupt increase in intracellular pH (23).

3. Sperm-activating peptide-I (SAP-I). This decapeptide has been isolated from the solubilized jelly layer of Hemicentrotus pulcherrimus, which stimulates the respiration, motility, and acrosomal reactivity of H. pulcherrimus spermatozoa. SAP-I increases cAMP and cGMP and intracellular pH and Ca\(^{2+}\) and activates the Na\(^+\)/H\(^+\) antiporter in H. pulcherrimus spermatozoa (19). It was postulated that two classes of receptors for SAP-I exist on the sperm plasma membrane: high-affinity receptors, which mediate the respiration-stimulating activity
and intracellular pH elevation, and low-affinity receptors that might be responsible for the rise in cGMP and intracellular Ca\(^{2+}\) (19).

4. **Natriuretic peptides.** Specific binding sites for ANP have been detected and localized in viable human spermatozoa (24).

NO is thought to be the main activator of the soluble isofrom of guanylate cyclase in spermatozoa. It has significant effects on the function of the male genitourinary system of rodents (25) and humans (26). Because NO-producing tissues have been also identified in the adult human female upper genital tract (27, 28), it is likely that NO affects sperm function as the sperm approaches the oocyte. Spermatozoa themselves express a NO activity and are able to synthesize NO (29–33); their NO production can be enhanced by specific chemical stimuli (31, 33). Studies have clearly demonstrated the presence of endothelial (29, 32, 33) and neuronal (29, 30) NOS isoforms in human spermatozoa. The NO donor sodium nitroprusside increases the intracellular cGMP levels of the spermatozoa of bulls (34) and humans (35, 36); another NO donor, spermine-NONOate, raises the cGMP concentration in murine spermatozoa (37). A mechanism of negative feedback regulation seems to be active, because NO released from sodium nitroprusside has been shown to desensitize sGC (38), leading to a progressive decrease of cGMP synthesis.

III. Guanylate Cyclase Activity and Sperm Functions

A. **Spermatogenesis and sperm transport**

The soluble isofrom of guanylate cyclase and its product cGMP have been detected by immunohistochemistry in the peritubular lamina of seminiferous tubules and in the blood vessels of human testis (39). The concentration of nitrates and nitrates, the final stable metabolites of NO, is inversely correlated with the pulsatility index of the transmediastinal artery, a variable that expresses vascular resistance in intra-testicular blood vessels (40). The endothelial and neuronal isoforms of NOS were localized in the seminiferous tubules, where NOS activity was demonstrated by decreased cGMP synthesis upon incubation with the NOS inhibitor N\(^{G}\)-nitro-\(L\)-arginine methyl ester (39). Endothelial NOS is expressed in Sertoli and Leydig cells in the human (41, 42) and rat (43) testis; in the germ cell line, endothelial NOS is not detectable in viable cells, but only in the cytoplasm of degenerating, apoptotic cells (41, 43). Furthermore, germ cell apoptosis in the rat testis is reduced by administration of NOS inhibitors (44), suggesting a role for NO in spermatogenesis and germ cell apoptosis. Interestingly, the expression of the inducible NOS isoform within the rat testis appears to be enhanced by intratesticular ischemia (45) and inflammation (46), suggesting that the seminiferous epithelium damage caused by some pathological events (e.g., testicular torsion or orchitis) may be linked to a huge local NO production.

In adult rats, NOS immunostaining revealed the presence of nitrinergic nerve fibers along the length of the vas deferens, possibly involved in the regulation of unidirectional sperm transport (47). Even in the human, endothelial NOS was immunohistochemically localized in the epithelium of epididymis and vas deferens (42). According to some authors (40), the concentration of nitrates and nitrates in seminal plasma is higher in normozoospermic subjects than in oligozoospermic or azoospermic patients. This observation suggests that local NO synthesis may be involved in determining the difference in sperm transport between normozoospermic and oligozoospermic men in response to sequential ejaculations within 1–4 h and 24 h (48, 49). Sildenafil, a potent cGMP-dependent phosphodiesterase (type V) blocker that elicits an increase of cGMP levels, may also affect sperm transport, especially in men with erectile disorders (50). Taken together, these data suggest that NO may be part of the regulatory mechanisms of sperm output by human testicles and of sperm transport along the male genital tract.

B. **Sperm motility**

The motility of human spermatozoa is inhibited by the NOS inhibitor N\(^{G}\)-nitro-\(L\)-arginine methyl ester (29) and by the NO scavenger methylene blue (31). These findings suggest that the NO endogenously synthesized by the spermatozoon is necessary to support motility. Even exogenous NO released by sodium nitroprusside can induce an *in vitro* hyperactivated motility of mouse spermatozoa (51) and is able to maintain the motility and viability of frozen-thawed human spermatozoa (52). The NOS-dependent phosphodiesterase inhibitor sildenafil was reported by some authors (53), but not by others (54–56), to increase the velocity and amplitude of lateral head displacement in human spermatozoa. Low concentrations of NO enhance the *in vitro* motility of hamster (57) and human spermatozoa (29, 35), whereas at higher concentrations NO inhibits the motility of mouse (51) and human spermatozoa (58). High concentrations of NO are also able to affect sperm viability (56, 59, 60), displaying a cytotoxic effect that is probably mediated by oxidative stress and lipid peroxidation of sperm membranes (61).

Seminal plasma concentration of nitrates and nitrates, which reflects the local *in vivo* NO release, was found to be higher in infertile than in fertile men (60) and to correlate with the percentage of immotile spermatozoa (59), leading to the hypothesis that infection and/or flogosis in semen can affect sperm function via induction of excessive NO synthesis by leukocytes or reproductive epithelia. It was even claimed that the dysfunction in sperm motility observed in patients with varicocele depends on excessive local NO synthesis, as nitrite concentration in the dilated varicocele vein is significantly higher than in the peripheral circulation (62), and NO concentration in the seminal plasma of patients with varicocele is higher than that of the controls (63). In other studies, however, the concentration of nitrates and nitrates in seminal plasma was higher in normozoospermic subjects than in oligozoospermic or azoospermic patients (40), and no correlation between nitrates in seminal plasma and the presence of leukocytes or azoospermic or azoospermic patients was observed (64). A possible explanation of these contradictory findings is that seminal plasma nitrates could not clearly correspond to the actual NO synthesis in the male genital tract, as nitrogen oxides could be locally transformed into some other compounds not detectable as nitrite/nitrate. The sensitivity of nitrite/nitrate concentration in seminal
plasma as a marker of local NO formation and sperm oxidative stress needs further investigation.

C. Acrosome reaction (AR)

Human follicular fluid contains variable amounts of ANP, with maximal concentrations found in follicles containing oocytes that are subsequently fertilized in vitro (65). The ANP from several species is able to induce the AR in humans, but the greatest response is elicited by human ANP (65). The AR-inducing effect of ANP can be observed in both capacitated and noncapacitated spermatozoa. In the latter, it is independent of extracellular Ca^{2+}, as mediated by cGMP synthesis (66), and may be completely inhibited by the addition of 1 μM LY83583, a guanylate cyclase inhibitor (65). The possibility of a complete or partial AR in noncapacitated spermatozoa, observed by some (67) and denied by others (68), may be linked to the activation of alternative pathways that bypass the classical membrane-mediated events leading to acrosomal exocytosis. It was also shown that different AR inducers or the use of different probes to measure AR may explain some of the discrepancies in the findings of different researchers (69). ANP markedly stimulates the AR of capacitated bull spermatozoa in a Ca^{2+}-dependent way. This effect is a result of interaction with GC-A, whose activation elicits cGMP synthesis (34). The AR-inducing effect of ANP in this experimental model may be abolished by the competitive GC-A antagonist anantin and restored by addition of the cGMP analog 8-bromo-cGMP (34).

Uncapacitated human spermatozoa produce low levels of NO, whereas under capacitating conditions, a time-dependent increase in NO synthesis has been observed (70). Studies in vitro have shown that low concentrations of NO enhance the AR of mouse (71) and bull (34) spermatozoa, as well as the zona pellucida-binding ability of human spermatozoa (72). Activation of endothelial NO synthase is implicated in the follicular fluid-induced AR of human spermatozoa (33). Moreover, NO donors such as sodium nitroprusside, 3,3-bis(aminomethyl)-1-hydroxy-2-oxo-1-triazene (DETA-NONOate), and N-nitroso-N-acetylpenicillamine are able to stimulate the AR of human spermatozoa in a specific and dose-dependent way (33, 73). The AR-inducing effect of sodium nitroprusside is abolished by the guanylate cyclase inhibitors LY83583 and 1H-[1,2,4]oxadiazolo-[4,3-a]quinoxalin-1-one (ODQ) and can be recovered by the addition of 8-bromo-cGMP (36).

D. Sperm chemotaxis

Sperm chemotaxis is defined as an oriented movement in response to a chemical gradient, resulting in an approach toward the chemical attractant, or in retreat from a chemical repellent. Sperm chemotaxis in humans was demonstrated in vitro. Recent studies suggest that its role is to select the fertilizing capacitated spermatozoa and to ensure their continuous availability for an extended period of time (for a comprehensive review see Ref. 74). In some invertebrate species, whose gametes are spawned into water before fertilization, chemotaxis is a key event in reproduction as the spermatozoa must be guided toward the eggs over relatively long distances. Egg-derived, species-specific peptides able to attract spermatozoa have been identified and purified in echinoderms, whose spermatozoa have specific surface receptors for these chemotactic substances (75). Activation of such receptors, which belong to the mGC family, causes an increase in intracellular cGMP (76) and mediates ion fluxes across the sperm membrane (77); in turn, this affects flagellar motion and finally determines the direction of movement (78). In species with internal fertilization, and particularly in mammals, chemotaxis may appear to be less crucial, as millions of spermatozoa are ejaculated directly into the female genital tract, and many of them can be stored in the cervix and/or in the oviduct (79), not far from the fertilization site. It was calculated, however, that only about 1 in 25,000 spermatozoa inseminated into the vagina reaches the fallopian tubes and, considering the overall surface of the tubal epithelium as well as the oocyte’s volume, the chance of a chaotic successful collision between gametes is statistically minimal (74, 80). In humans, the existence of some communication at distance between the gametes could positively affect their chance of meeting and, finally, the chance of fertilization. Interestingly, the tubal ampulla in which the human egg resides contains a significantly higher number of sperm cells than the contralateral tube (81).

A chemical attraction to spermatozoa could be caused by a substance (or substances) whose concentration increases from the lower to the upper part of the salpinx. Such substance could be present in the fluid surrounding the oocyte or secreted by cells surrounding the egg. The attention of researchers in this area has been focused mainly on follicular fluid (74, 82). In mammals, sperm chemotaxis and chemokinesis to a follicular factor(s) has been established and has been distinguished from other processes that might cause sperm accumulation (83–85). Human follicular fluid contains ANP (86, 87), and specific receptors for ANP have been identified on the surface of human spermatozoa (24). Experiments accomplished by accumulation (66) and choice assays (88) led to the hypothesis of sperm chemotaxis exerted by ANP toward human spermatozoa. However, the role of ANP as sperm attractant is questionable because no correlation was found between the chemotactic activity of a given follicular fluid and its ANP content (66). Moreover, sperm chemotaxis to ANP at physiological concentrations is observed only when phosphoramidon, a neutral endopeptidase inhibitor probably absent in vivo, is added to the system (88). Thus, ANP might simply be a substance capable of activating mGC in vitro in a way similar to that caused by the physiological attractant in vivo. As to other putative sperm chemotacticants contained in follicular fluid, such as progesterone (89) and N-formylated peptides (90), there is no evidence for their involvement in mGC in their signaling.

In a recent study, an accumulation assay was used to examine the possible role of NO in sperm chemotaxis (91). Mouse sperm was allowed to migrate in an experimental plate to medium containing sodium nitroprusside (50 nm) or to a control medium: after 3 or 5 h of incubation, sperm concentration was significantly higher in the medium containing the NO donor. This finding, however, could not be attributed unequivocally to chemotaxis, as in accumulation assays a “trapping” phenomenon caused by the onset of nonlinear, hyperactivated sperm motility cannot be ex-
cluded. The role of NO in mammalian sperm chemotaxis still awaits clarification.

E. Sperm-egg interaction

NO is claimed to play a role in sperm-egg interaction (zona pellucida binding, sperm-oocyte fusion), as well as in the egg activation after sperm penetration. Incubation of human spermatozoa with the NOS inhibitor N\textsuperscript{-}nitro-L-arginine methyl ester was found to inhibit their ability to penetrate zona-free hamster eggs in vitro, but not their ability to bind to the human zona pellucida in the hemizona assay (92).

The freshly laid oocytes of the fathead minnow Pimephales promelas express NOS protein near the site of sperm entry and transiently produce NO a few seconds before fertilization (93). An abrupt increase in nitrosylation within oocytes was recorded a few seconds after insemination, just before the onset of intracellular Ca\textsuperscript{2+} oscillations, a phenomenon that represents a typical egg response to fertilization in several species and is known to influence early embryo development (94). Microinjection of NO donors or recombinant NOS can mimic egg activation after fertilization, whereas an oocyte preload with oxyhemoglobin, a physiological NO scavenger, prevents Ca\textsuperscript{2+} pulses in fertilized eggs (94). On the contrary, in chordate eggs, the sperm-induced Ca\textsuperscript{2+} rise is not associated with any change in intracellular NO and is not affected by the presence of the NOS inhibitor N\textsuperscript{-}nitro-L-arginine methyl ester (95). In conclusion, the role played by NO in egg activation and fertilization is still unclear, and further studies are needed to clarify it.

IV. Signaling Pathways Following Guanylate Cyclase Activation in Spermatozoa

A. Changes in guanylate cyclase phosphorylation state

After the binding of chemotactic peptides, the phosphorylation level of mGC changes, affecting the extent of its activation (16, 76). In sperm cells of the sea urchin A. punctulata, mGC is a major glycoprotein of the flagellar plasma membrane; when the jelly layer peptide resact binds to mGC, it triggers dephosphorylation of the enzyme. Each mGC molecule loses approximately 15 phosphate groups, resulting in a sudden decrease (within 1 min) in the enzyme activity (96).

Similarly, when intact S. purpuratus spermatozoa are incubated with the egg-derived peptide speract, the activity of mGC decreases, its apparent molecular weight shifts, and there is a detectable loss of \textsuperscript{32}P label from the enzyme (97).

The phosphorylation state of mGC modulates the absolute activity of the enzyme and the extent of interaction between the catalytic site and the GTP-binding site (98). If a preparation containing protein phosphatase from E. coli is used to catalyze the dephosphorylation of the enzyme, there is a sudden decrease in mGC activity, together with a rapid decrease in the molecular mass (from 160 to 150 kDa) (98). The regulatory site for phosphorylation is likely to be the mGC carboxy-terminal 95-amino acid portion, which contains 20% serine (98, 99).

Extracellular pH is an important factor in determining the mGC phosphorylation state. Sea urchin spermatozoa incubated in seawater containing ammonia (pH 8.8) undergo sudden mGC dephosphorylation, with accompanying changes in molecular mass and enzymatic activity. Transfer of the cells into ammonia-free seawater (pH 7.4) results in rephosphorylation, reversion to a 160-kDa mass, and recovery of the initial enzymatic activity (100).

B. Increase in cGMP and cAMP

Early biochemical responses of spermatozoa to resact (A. punctulata) or speract (S. purpuratus) include H\textsuperscript{+} efflux and an increase in cAMP (other than cGMP) concentration (20). Human spermatozoa subjected to capacitating conditions increase their endogenous NO synthesis and their intracellular cAMP content; the latter is further increased by NO-releasing compounds and conversely decreased by incubation with NOS inhibitors (70). Furthermore, the cGMP-specific phosphodiesterase (type V) inhibitor sildenafil causes a dose-dependent cAMP increase in human spermatozoa (53), and human spermatozoa incubated with NO releasers under capacitating conditions show a marked increase in intracellular cAMP concentrations (70). Activation of adenylate cyclase requires an increase in intracellular pH, which appears to be critical in determining whether cGMP-mediated or cAMP-mediated pathways predominate in speract signal transduction (101). Speract-induced accumulation of cGMP and cAMP is enhanced by phosphodiesterase inhibitors (53). In addition, the sperm-activating peptide SAP-V, isolated from the egg jelly of Brissus agassizii, stimulates sperm respiration and thereby increases intracellular cAMP and cGMP levels in a concentration-dependent manner (102). The concomitant increase of both cyclic nucleotides is suggestive of a cross-talk between the cAMP and cGMP signaling pathways, a phenomenon that is facilitated in many tissues by the presence of cGMP-regulated phosphodiesterase isoforms. Of the seven known phosphodiesterase isoforms, three (PDE-II, PDE-V, and PDE-VI) are allosterically regulated by cGMP, and one (PDE-III) is inhibited by the binding of cGMP to its active site (4). Thus, an increase in cGMP could evoke a concomitant increase in cAMP by inhibiting its PDE-III-catalyzed hydrolysis to AMP, as observed in human platelets and vascular smooth muscle cells (103, 104) (Fig. 3). An alternative way of increasing intracellular cAMP levels occurs in rat liver and other tissues, where sGC, stimulated by NO, undergoes striking modifications in its activity, becoming able to synthesize cAMP too (105).

Speract and resact markedly stimulate the incorporation of \textsuperscript{32}P into various proteins of isolated sperm membranes in the presence, but not in the absence, of GTP, the substrate of guanylate cyclases. The addition of cAMP and cGMP further stimulates protein phosphorylation in the same domains as those phosphorylated by egg peptides plus GTP, indicating that a peptide-induced increase in intracellular cyclic nucleotides is responsible for the observed changes in the phosphorylation state of proteins on the plasma membrane (106). In human spermatozoa, the potent cGMP-dependent phosphodiesterase blocker sildenafil markedly increases the tyrosine phosphorylation of two proteins belonging to the fibrous sheet (p105 and p81), increasing sperm velocity, lateral head displacement, and sperm capacitation (53). When ca-
it should be mentioned that high concentrations of ANP elicit the AR even in noncapacitated human spermatozoa and/or in the absence of Ca\(^{2+}\) (65). The NO donor sodium nitroprusside appears to be ineffective in inducing the AR when spermatozoa are incubated in a Ca\(^{2+}\)-free, EDTA-containing medium (36).

The role of cyclic ADP-ribose in mammalian spermatozoa activation still awaits clarification. It is now known that sea urchin sperm cells can synthesize cyclic ADP-ribose and that they contain this compound in amounts comparable to those measured in other tissues (114). In several cell types (gut interstitial cells, macrophages, pancreatic \(\beta\)-cells) NO increases intracellular Ca\(^{2+}\) via activation of a ryanodine receptor, which allows ion efflux from inositol-1,4,5-trisphosphate-insensitive Ca\(^{2+}\) pools (115). In sea urchin eggs, NO and cGMP seem to mobilize Ca\(^{2+}\) from intracellular stores by inducing synthesis of cyclic ADP-ribose, a putative agonist of the ryanodine receptor (115). In mammalian spermatozoa, the induction of AR is accomplished by an influx of external Ca\(^{2+}\) via opening of voltage-dependent calcium channels (116), possibly as a result of NO-induced activation of plasma membrane Ca\(^{2+}\) channels or inhibition of a Ca\(^{2+}\) pump. NO and cGMP are known to inhibit voltage-dependent Ca\(^{2+}\) channels in other cell types (117), but cyclic nucleotide-gated channels were found to be expressed in mammalian sperm (118), where they can regulate a Ca\(^{2+}\) entry pathway that responds more sensitively to cGMP than to cAMP (119). On the other hand, NO inhibits the sarcoplasmic reticulum Ca\(^{2+}\)-ATPase (120, 121), and the sarcoplasmic reticulum Ca\(^{2+}\)-ATPase antagonist thapsigargin is able to induce a rise in intracellular Ca\(^{2+}\) and AR in human spermatozoa (122), and to induce the AR in mouse spermatozoa (123). Interestingly, the increase in intracellular Ca\(^{2+}\) in human sperm by the use of the internal Ca\(^{2+}\)-ATPase inhibitor 2,5-di(tert-butyl) hydroquinone initiates the AR, but only in the presence of extracellular calcium (124). Because it has been shown that human spermatozoa, despite their requirement for extracellular Ca\(^{2+}\), require also the movement of Ca\(^{2+}\) through internal stores before the AR occurs (122), it is conceivable that the NO/cGMP-elicited AR needs extracellular Ca\(^{2+}\) to restore the Ca\(^{2+}\) content of intracellular pools after a stimulus has caused their emptying.

D. Activation of cGMP-dependent protein kinases

Among the molecular targets of cGMP are cGMP-dependent protein kinases (Fig. 3). Two different cGMP-dependent protein kinases [protein kinase GI (PKGI) and protein kinase GII (PKGII)] have been identified in mammals (125, 126). The PKG inhibitors 8-bromoguanosine-3’,5’-monophosphorothioate Rp-isomer (Rp-8-Br-cGMPS) and 8-(4-chlorophenylthio)guanosine-3’,5’-monophosphorothioate Rp-isomer (Rp-8-pCPT-cGMPS) are able to block the sodium nitroprusside-induced AR in human sperm, suggesting that the NO/cGMP pathway, which is activated by sodium nitroprusside stimulation, needs the activation of a PKG to trigger the AR in human spermatozoa (36). On the other hand, in mice lacking PKGI, spermatozoa can normally undergo the AR and fertilize oocytes (127).

C. Changes in intracellular ions concentration

Experimental data indicate that a specific Ca\(^{2+}\)-activated K\(^+\) channel participates in an early phase of speract signal transduction in sea urchin spermatozoa (107). This hyperpolarizing effect of speract on the membrane potential involves intracellular GTP (108). Activation of K\(^+\) channels is indeed mediated by cGMP; the speract-induced increase in cGMP content seems to directly mediate K\(^+\) channel opening, which in turn causes hyperpolarization of the sperm membrane (109) (Fig. 3). The resulting increase in intracellular pH leads to mGC inactivation and K\(^+\) channel closure; however, K\(^+\) permeability may be restored upon subsequent increase in intracellular Ca\(^{2+}\) (107).

An increase in intracellular cGMP precedes the major increase in cytoplasmic free Ca\(^{2+}\) (78, 110) (Fig. 3). In sperm, this increase appears to depend on the influx of extracellular Ca\(^{2+}\), as no detectable Ca\(^{2+}\) response to 8-bromo-cGMP is observed in Ca\(^{2+}\)-free medium or in the presence of the Ca\(^{2+}\) channel blocker pimozide (111). The need for extracellular Ca\(^{2+}\) in mammalian sperm AR has often been reported (reviewed in Ref. 112). Ca\(^{2+}\)-dependence was observed during the induction of human sperm AR by ANP (113), although it should be mentioned that high concentrations of ANP elicit the AR even in noncapacitated human spermatozoa and/or in the absence of Ca\(^{2+}\) (65). The NO donor sodium nitroprusside appears to be ineffective in inducing the AR when spermatozoa are incubated in a Ca\(^{2+}\)-free, EDTA-containing medium (36).

The role of cyclic ADP-ribose in mammalian spermatozoa activation still awaits clarification. It is now known that sea urchin sperm cells can synthesize cyclic ADP-ribose and that they contain this compound in amounts comparable to those measured in other tissues (114). In several cell types (gut interstitial cells, macrophages, pancreatic \(\beta\)-cells) NO increases intracellular Ca\(^{2+}\) via activation of a ryanodine receptor, which allows ion efflux from inositol-1,4,5-trisphosphate-insensitive Ca\(^{2+}\) pools (115). In sea urchin eggs, NO and cGMP seem to mobilize Ca\(^{2+}\) from intracellular stores by inducing synthesis of cyclic ADP-ribose, a putative agonist of the ryanodine receptor (115). In mammalian spermatozoa, the induction of AR is accomplished by an influx of external Ca\(^{2+}\) via opening of voltage-dependent calcium channels (116), possibly as a result of NO-induced activation of plasma membrane Ca\(^{2+}\) channels or inhibition of a Ca\(^{2+}\) pump. NO and cGMP are known to inhibit voltage-dependent Ca\(^{2+}\) channels in other cell types (117), but cyclic nucleotide-gated channels were found to be expressed in mammalian sperm (118), where they can regulate a Ca\(^{2+}\) entry pathway that responds more sensitively to cGMP than to cAMP (119). On the other hand, NO inhibits the sarcoplasmic reticulum Ca\(^{2+}\)-ATPase (120, 121), and the sarcoplasmic reticulum Ca\(^{2+}\)-ATPase antagonist thapsigargin is able to induce a rise in intracellular Ca\(^{2+}\) and AR in human spermatozoa (122), and to induce the AR in mouse spermatozoa (123). Interestingly, the increase in intracellular Ca\(^{2+}\) in human sperm by the use of the internal Ca\(^{2+}\)-ATPase inhibitor 2,5-di(tert-butyl) hydroquinone initiates the AR, but only in the presence of extracellular calcium (124). Because it has been shown that human spermatozoa, despite their requirement for extracellular Ca\(^{2+}\), require also the movement of Ca\(^{2+}\) through internal stores before the AR occurs (122), it is conceivable that the NO/cGMP-elicited AR needs extracellular Ca\(^{2+}\) to restore the Ca\(^{2+}\) content of intracellular pools after a stimulus has caused their emptying.

D. Activation of cGMP-dependent protein kinases

Among the molecular targets of cGMP are cGMP-dependent protein kinases (Fig. 3). Two different cGMP-dependent protein kinases [protein kinase GI (PKGI) and protein kinase GII (PKGII)] have been identified in mammals (125, 126). The PKG inhibitors 8-bromoguanosine-3’,5’-monophosphorothioate Rp-isomer (Rp-8-Br-cGMPS) and 8-(4-chlorophenylthio)guanosine-3’,5’-monophosphorothioate Rp-isomer (Rp-8-pCPT-cGMPS) are able to block the sodium nitroprusside-induced AR in human sperm, suggesting that the NO/cGMP pathway, which is activated by sodium nitroprusside stimulation, needs the activation of a PKG to trigger the AR in human spermatozoa (36). On the other hand, in mice lacking PKGI, spermatozoa can normally undergo the AR and fertilize oocytes (127).
E. Protein kinase C (PKC) activation

The PKC inhibitor calphostin can block the AR-inducing effect of sodium nitroprusside, pointing to a role for PKC in the transduction mechanism of NO-dependent AR (36). Studies have demonstrated the involvement of PKC in the AR of human spermatozoa (112), as well as the effectiveness of calphostin in blocking the PKC-mediated AR of these cells (128). Both the phorbol ester 12-O-tetradecanoylphorbol-13-acetate and the diacylglycerol analog 1-oleoyl-2-acetylglycerol are powerful inducers of the AR in capacitated human spermatozoa (129). A recent study showed that the ANP-induced AR of human spermatozoa involves PKC, because it can be inhibited by the PKC inhibitors staurosporine and GF-109203X (113). The mechanism by which cGMP (the natural product of ANP receptor activation) can lead to an increase in PKC activity is still unknown.

V. Conclusions

A large body of experimental evidence indicates that the activation of guanylate cyclase strongly influences sperm function both in species with external fertilization and in mammals. In the former, the guanylate cyclase enzyme family appears to be mainly responsible for the long-distance interaction between gametes, as sperm chemotaxis toward egg-derived substances is crucial for reproduction, allowing sperm-egg encounter in open spaces. Echinoderms, fishes, and amphibians have served as useful models for studying the effects of egg-secreted, species-specific peptides on sperm movement. The intracellular mechanisms elicited by guanylate cyclase activation, leading ultimately to an oriented flagellar movement, have been thoroughly elucidated in these species.

In species with internal fertilization, the need for such precise chemotactic mechanisms seems to be less vital, as gamete encounter is apparently easier in a “closed” system such as the female internal genital apparatus. However, sperm chemotaxis evidently exists in mammals, and the acquisition of chemotactic responsiveness is likely to represent a part of the activation process that the spermatozoa of mammals undergo in their trip through the female genital tract. The concept of chemotaxis as part of the capacitation process in mammals is supported by experimental findings, and although the substance(s) responsible for sperm chemotaxis in mammals has not yet been identified, it seems likely that guanylate cyclase activation is an essential part of the process.

Guanylate cyclase-activating substances (in particular ANP and NO) strongly affect sperm motility, capacitation, and acrosomal reactivity. They therefore stimulate sperm metabolism and promote the ability of the sperm to approach the oocyte, interact with it, and finally fertilize it. The guanylate cyclase-activating system seems to be an important regulatory feature in mammalian reproduction. Several tissues in the genital tract of both sexes may produce guanylate cyclase agonists capable of interacting with gametes, and furthermore, the spermatozoa itself can produce the powerful sGC activator NO in response to substances physiologically present in the female genital tract. Thus, NO may influence sperm function via both endocrine and autocrine mechanisms.

Important sperm characteristics are affected by guanylate cyclase activation, and a complex system of intracellular pathways is activated by its agonists. Sperm motility appears to be affected by guanylate cyclase activation mainly through an increase in intracellular cAMP, whereas the acrosome reaction depends more directly on cGMP synthesis. Both cyclic nucleotides activate specific kinases and ion signals. A complex cross-talk between cAMP- and cGMP-generating systems occurs in response to guanylate cyclase activation, resulting in an upward shift in sperm function. Recent data suggest that guanylate cyclase activation could also affect the two extremes of sperm existence, spermatogenesis, on the one hand, and sperm-egg interaction. In addition, experimental observations indicate that excessive amounts of certain guanylate cyclase activators might exert opposite, antireproductive effects, increasing the oxidative stress on sperm membranes.

In view of the marked influence exerted by guanylate cyclase-activating substances on sperm function, it seems likely that sperm production and transport in vivo, and sperm motility, capacitation, and acrosomal reactivity in vitro will be amenable to pharmacological modulation by interaction with the sperm guanylate cyclases. Future exploration of the therapeutic potential of such a tool will require a more complete knowledge of the effects of guanylate cyclase-modulating drugs on human spermatozoa. Such drugs may serve as a new contraception modality. On the other hand, faulty precontact sperm-egg communication may be one of the causes of infertility, and activation or inhibition of guanylate cyclase may represent an exciting new approach for the diagnosis and treatment of male and/or female infertility.

Acknowledgments

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