Sperm capacitation and the acrosome reaction

Lynn R. Fraser

Anatomy and Human Biology Group, King’s College London, Strand, London WC2R 2LS, UK

To achieve successful fertilization under normal circumstances in vivo, mammalian spermatozoa must first undergo capacitation and then the acrosome reaction, an exocytotic event that allows cells to penetrate the zona pellucida and fuse with the oocyte plasma membrane. These complex events permit spermatozoa to achieve fertilizing ability at the right time in the right place, important considerations since relatively few sperm cells actually reach the site of fertilization in vivo. Several mechanisms that may be involved in regulating the acquisition of fertilizing ability are considered. In vivo, selective pressures placed on the initial population of spermatozoa help ensure that the ‘fittest’ spermatozoa are able to fertilize. Since intracytoplasmic sperm injection bypasses this selection process, it is best used only in cases where spermatozoa are judged to be incapable of achieving normal fertilization in vitro.

Key words: acrosome reaction/Ca²⁺ channels/capacitation/decapacitation factor/diacylglycerol/fertilization-promoting peptide

Introduction

Spermatozoa have two roles to play during fertilization: (i) contribution of a haploid set of chromosomes with the paternal pattern of gene imprinting required for normal development and (ii) triggering oocyte activation and subsequent initiation of metabolic events required to support embryonic development. In vivo, the acquisition of fertilizing ability by mammalian spermatozoa is a relatively lengthy process, necessitated by the need for the cells to travel a considerable distance from the site of deposition, in the lower part of the female reproductive tract, to the site of fertilization, in the upper ampullary region of the oviduct. Since relatively few sperm cells actually reach the ampulla, it is very important that they do not become fertile too quickly and thus lose the ability to interact successfully with oocytes to effect fertilization. During the past several decades, evidence has been obtained that provides at least some information about mechanisms that control sperm function and thus help to ensure that potentially fertile spermatozoa reach the site of fertilization. These mechanisms provide the focus for this article.

Since 1951 (Austin, 1951; Chang, 1951) it has been known that mammalian spermatozoa are not fertile when they leave the male reproductive tract, even though they are immediately highly motile. They require a species-dependent period of time to undergo complex changes, collectively referred to as ‘capacitation’ (Austin, 1952), that transform them from non-fertilizing to potentially fertilizing cells. In the capacitated state, spermatozoa can then respond to molecular signals provided by the oocyte and its associated investments (cumulus cells, zona pellucida) and undergo the acrosome reaction. The latter is an exocytotic event leading to the release of enzymes that aid penetration of the zona pellucida and to the acquisition of properties by the sperm head plasma membrane that permit fusion with the oocyte (Yanagimachi, 1994). In addition to capacitation-related changes in the sperm head, there are also changes in the sperm flagellum. These involve an alteration in the pattern of motility from progressive, with relatively low amplitude flagellar bending, to hyperactivated, with
large amplitude flagellar bending. Hyperactivated motility appears to aid progression up the oviduct, allowing spermatozoa to move away from the oviductal epithelium rather than remaining in association with these cells (DeMott and Suarez, 1992), and to provide the motive thrust needed for penetration of the zona pellucida (Katz et al., 1978).

**Ionic regulation of capacitation**

The extracellular environment plays a crucial role in promoting or inhibiting functional changes in mammalian spermatozoa. In vivo, of course, the female reproductive tract is the site where capacitation and fertilization take place, but it is difficult to manipulate the environment and then evaluate the effects on sperm function in a precise manner. The development of culture systems that support capacitation and fertilization in vitro has made it possible to study in detail the requirements for the acquisition of fertilizing ability. Particular attention has been paid to the ionic composition of the culture media; by varying the concentration and the temporal availability of individual ions, it has been possible to determine which ions are required specifically for capacitation and for the acrosome reaction. Cations, e.g. Ca$^{2+}$, Na$^+$, have been evaluated more extensively than anions, e.g. Cl$^-$, HCO$_3$.

Of these ions, the most important appears to be Ca$^{2+}$. Extracellular Ca$^{2+}$ is required for both complete capacitation and the acrosome reaction, although there are species- and stage-dependent differences. Relatively little Ca$^{2+}$, i.e. low micromolar concentrations, is required for capacitation in at least some species (e.g. guinea pig, mouse), but millimolar concentrations are required for maximal hyperactivation, acrosome loss and fertilization in all species evaluated (for review see Fraser, 1994, 1995). The requirement for extracellular Ca$^{2+}$ reflects a need for internalization of the ion to promote a rise in the intracellular Ca$^{2+}$ concentration ([Ca$^{2+}]_i$).

Capacitation is slow, occurring over a period of hours, while the acrosome reaction is rapid, requiring only minutes. It is therefore not surprising that different mechanisms appear to control changes in [Ca$^{2+}]_i$ during these two stages. Indeed, the magnitude of change also differs. Studies using both populations of spermatozoa (e.g. White and Aitken, 1989; Adeoya-Osiguwa and Fraser, 1993) and individual cells (e.g. Florman, 1994; Suarez and Dai, 1995) have revealed that modest and gradual rises in [Ca$^{2+}]_i$ occur during capacitation. In contrast, the acrosome reaction involves a rapid and large influx of Ca$^{2+}$ (e.g. Brucker and Lipford, 1995). Since these changes are stage-dependent, differing in both temporal nature and magnitude, it is plausible that the mechanisms controlling the Ca$^{2+}$ fluxes may differ. To date, three mechanisms have been identified in mammalian spermatozoa as potential modulators of [Ca$^{2+}]_i$: (i) a Ca$^{2+}$-ATPase acting as a Ca$^{2+}$ extrusion pump; (ii) a Na$^+$/$Ca^{2+}$ exchanger promoting a Na$^+$ out/Ca$^{2+}$ in exchange; (iii) Ca$^{2+}$ channels allowing Ca$^{2+}$ influx.

Of these three, evidence most strongly supports the involvement of Ca$^{2+}$-ATPase during capacitation (Table I). Drugs such as quercetin and ethacrynic acid, known to inhibit somatic cell Ca$^{2+}$-ATPase, have been shown to accelerate capacitation in bull (Fraser et al., 1995), guinea pig (Roldan and Fleming, 1989), human (DasGupta et al., 1994) and mouse (Fraser and McDermott, 1992) spermatozoa. Almost all somatic cell Ca$^{2+}$-ATPases are calmodulin-stimulated (Carafoli, 1992) and treatment of mammalian sperm suspensions with calmodulin inhibitors such as trifluoperazine and W-7 has been shown to stimulate accumulation of $^{45}$Ca$^{2+}$ in uncapacitated mouse spermatozoa (Adeoya-Osiguwa and Fraser, 1993), capacitation in bull (Fraser et al., 1995), human (DasGupta et al., 1994) and mouse (Fraser and McDermott, 1992) spermatozoa and in-vitro fertilizing ability in mouse spermatozoa (Adeoya-Osiguwa and Fraser, 1996). These responses are consistent with the presence in mammalian sperm cells of a calmodulin-stimulated Ca$^{2+}$-ATPase. When active, the enzyme would help maintain a low [Ca$^{2+}]_i$, by

| Table I. Temporal differences in capacitation and the acrosome reaction necessitate different mechanisms to regulate the intracellular Ca$^{2+}$ concentration |
|---------------------------------|---------------------------------|
| Capacitation:                   | Acrosome reaction:              |
| slow (hours)                    | rapid (minutes)                 |
| modest rise in [Ca$^{2+}]_i$    | large rise in [Ca$^{2+}]_i$     |
| — Ca$^{2+}$-ATPase              | — Ca$^{2+}$ channels            |

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extruding Ca^{2+}; a decline in activity during capacitation would result in the gradual and modest rise in [Ca^{2+}] \_i that has been observed.

There is evidence that a Na^{+}/Ca^{2+} exchanger is present in mammalian spermatozoa (Fraser, 1994), but little is known about its possible role in capacitation. Caltrin, a low molecular weight protein that associates with bull spermatozoa at the time of ejaculation, has been proposed to modulate Na^{+}/Ca^{2+} exchanger activity, initially inhibiting it but then stimulating activity to promote Na^{+} out/Ca^{2+} in changes (Lardy and San Agustin, 1989). Little evidence exists for such a mechanism operating in other species.

Ca^{2+} channels have been identified in many mammalian spermatozoa and they do appear to play an important role, but during the acrosome reaction rather than during capacitation. The opening of Ca^{2+} channels results in a large, rapid influx of Ca^{2+}, consistent with the changes in [Ca^{2+}] \_i observed during the acrosome reaction (Table I). These are discussed further below.

Na^{+} is the most abundant cation in the normal extracellular environment \textit{in vivo} and in culture media used \textit{in vitro}. Although extracellular Na^{+} is required for capacitation and the acrosome reaction, current evidence indicates that relatively little will suffice for capacitation (~25 mM for mouse spermatozoa; Fraser \textit{et al}, 1993), but much higher concentrations are required for the acrosome reaction and fertilization (>135 mM for the mouse; Fraser \textit{et al}, 1993). As with Ca^{2+}, the requirement for extracellular Na^{+} reflects a need for internalization of Na^{+}. Adding the Na^{+} ionophore monensin to uncapacitated suspensions promotes both capacitation and the acrosome reaction in mouse (Fraser \textit{et al}, 1993) and human (O’Toole \textit{et al}, 1996b) spermatozoa.

Two mechanisms have been identified as possible regulators of [Na^{+}] \_i, namely a Na^{+}/K^{+}-ATPase and a Na^{+}/Ca^{2+} exchanger. Relatively little experimental evidence has been accumulated to favour either of these. Although ouabain-inhibitable ATPase activity, presumed to be a Na^{+},K^{+}-ATPase, was reported to rise during hamster sperm capacitation (Mrsny \textit{et al}, 1984), ouabain treatment of uncapacitated mouse sperm suspensions stimulated capacitation (Fraser \textit{et al}, 1993). These two sets of results are contradictory, the first suggesting that an increase in enzyme activity promotes capacitation, but the second suggesting that a decrease in activity has the same effect. Given that spermatozoa appear to need a rise in [Na^{+}] \_i, it is difficult to reconcile a rise in Na^{+},K^{+}-ATPase activity (Na^{+} out, K^{+} in) with a rise in [Na^{+}] \_i. Essentially, there is no evidence for the Na^{+}/Ca^{2+} exchanger regulating [Na^{+}] \_i.

One component of capacitation is a rise in intracellular pH (pH_i; e.g. Working and Meizel, 1983; Vredenburgh-Wilberg and Parrish, 1995). The requirement for extracellular Na^{+} during capacitation may reflect its involvement in a Na^{+}-, Cl^- and HCO_3^- dependent acid efflux pathway recently proposed to be important in the observed rise in pH_i during capacitation and the acrosome reaction in mouse spermatozoa (Zeng \textit{et al}, 1996). When active, this Na^{+}-dependent pathway would produce HCO_3^-/Cl^- exchange. In contrast to the requirement for extracellular Na^{+} during pH_i regulation, there was no obvious requirement for extracellular K^{+}. This is consistent with earlier studies indicating that K^{+} is not needed during capacitation (reviewed by Fraser, 1994).

Current evidence indicates that HCO_3^- is required for mammalian sperm capacitation (Neill and Olds-Clarke, 1987; Boatman and Robbins, 1991; Shi and Roldan, 1995; Visconti \textit{et al}, 1995a; Forsdike and Fraser, 1996), although one study suggested a role only during the acrosome reaction (Lee and Storey, 1986). As with Na^{+}, this may reflect involvement in regulation of pH_i. HCO_3^- is also known to stimulate mammalian sperm adenylate cyclase (e.g. Okamura \textit{et al}, 1985) which would promote the generation of cyclic AMP (cAMP). Adenylate cyclase activity increases during capacitation and exogenous analogues of cAMP and/or inhibitors of cyclic nucleotide phosphodiesterases have been shown to accelerate capacitation \textit{in vitro} (reviewed by Fraser and Monks, 1990). The rise in [Ca^{2+}] \_i during capacitation would also produce a stimulation of adenylate cyclase (Fraser and Monks, 1990). Consistent with these observations, recent studies on capacitation \textit{in vitro} of mouse spermatozoa have indicated a time-dependent increase in protein tyrosine phosphorylation during capacitation (Visconti...
et al., 1995a), this phosphorylation and thus capacitation apparently being regulated by cAMP and protein kinase A (Visconti et al., 1995b).

Decapacitation factors
Capacitation involves considerable alteration in the sperm surface, with various molecules being lost, rearranged or unmasked (e.g. Oliphant et al., 1985). Those belonging to the first category are often referred to as ‘decapacitation factors’ (DF) because their addition to capacitated suspensions will rapidly inhibit fertilizing ability (Bedford and Chang, 1962). This inhibition is reversible, such that further incubation of cells under capacitating conditions will result in restored fertility. Thus, capacitation itself appears to be reversible, whereas the acrosome reaction, involving membrane vesiculation and loss, is irreversible. DF may be of either epididymal or seminal plasma origin and represent a mechanism for preventing the spermatozoa from becoming fertile too quickly.

In the mouse, we have been investigating a DF of epididymal origin. Since epididymal and ejaculated mouse spermatozoa exhibit similar capacitation kinetics, i.e. there appears to be no detectable additional inhibition resulting from contact with seminal plasma, this DF may be a major regulator of capacitation in the mouse. Removal of the DF from uncapacitated cells by centrifugation stimulates fertilizing ability in vitro and its readdition to capacitated suspensions causes a significant reversal from the capacitated to the uncapacitated state (assessed using the chlortetracycline fluorescence assay) and inhibits fertilizing ability (Fraser, 1984; Fraser et al., 1990).

Based on indirect evidence that a Ca2⁺-ATPase is involved in controlling [Ca2⁺], during capacitation (see above), we have postulated that the DF modulates capacitation by regulating Ca2⁺-ATPase activity associated with the plasma membrane. Using a [γ-32P]ATP hydrolysis assay to determine ATPase activity and membranes prepared from isolated mouse sperm heads and tails, we now have obtained direct evidence for the presence of a Ca2⁺-dependent ATPase in head membranes that is further stimulated by calmodulin and partially purified DF, used individually and in combination (Adeoya-Osiguwa and Fraser, 1996). Furthermore, the addition of exogenous DF to cells depleted of their endogenous DF significantly inhibited the accumulation of 45Ca2⁺ by intact cells and caused a temporary reversal from the capacitated to the uncapacitated state as determined using chlortetracycline fluorescence analysis (Adeoya-Osiguwa and Fraser, 1996).

Taking into account both indirect and direct experimental evidence, we have proposed that the association of this epididymally derived DF with the mouse sperm plasma membrane stimulates the endogenous activity of a calmodulin-stimulated Ca2⁺-ATPase, resulting in a low [Ca2⁺]. Following ejaculation into the female reproductive tract or release into culture medium, the DF begins to dissociate or to undergo inactivation and Ca2⁺-ATPase activity declines, allowing [Ca2⁺] to rise modestly and promote capacitation. Inhibitors known to affect somatic cell Ca2⁺-ATPase can stimulate capacitation in several mammalian species (see above), and the addition of mouse DF to capacitated human sperm suspensions can cause a reversal of capacitation (shown using chlortetracycline; DasGupta et al., 1994), suggesting that modulation of Ca2⁺-ATPase may be a mechanism for regulating [Ca2⁺] during capacitation in many, or possibly all, mammalian spermatozoa.

Fertilization promoting peptide
While DF present on spermatozoa at ejaculation provide an inhibitory mechanism to prevent premature acquisition of fertilizing ability, there is evidence that stimulatory mechanisms also come into play at the time of ejaculation. Under normal circumstances, the interplay of these opposing mechanisms could optimize the timing of the acquisition of fertilizing potential within the female tract.

A tripeptide (pyroglutamylglutamylprolineamide) that is structurally similar to thyrotrophin-releasing hormone (TRH; pyroglutamylhistidylprolineamide) has been identified in a number of mammalian tissues, including the prostate and the pituitary gland (Ashworth, 1994). It is also found in seminal plasma (e.g. mean ± SEM concentration in human, 49.5 ± 10.3 nM; Cockle et al., 1994), suggestive of a possible biological function relating to spermatozoa. In recent studies we have provided
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Table II. FPP regulates mammalian sperm function

<table>
<thead>
<tr>
<th>Stimulatory effects on uncapacitated spermatozoa</th>
<th>Inhibitory effects on capacitated spermatozoa</th>
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<tr>
<td>Capacitation (CTC)</td>
<td>Spontaneous acrosome reaction (CTC)</td>
</tr>
<tr>
<td>Hyperactivation (CASA)</td>
<td>Not agonist-induced acrosome reaction (CTC)</td>
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<tr>
<td>Fertilizing ability (IVF, HOPT)</td>
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FPP = fertilization promoting peptide; CTC = chlortetracycline; CASA = computer-assisted sperm analysis; IVF = in-vitro fertilization; HOPT = zona-free hamster oocyte penetration test.

the first evidence for a physiologically relevant action for this tripeptide, demonstrating that its addition to uncapacitated mouse and human sperm suspensions promotes both capacitation (chlortetracycline fluorescence assay) and fertilizing ability in vitro (Green et al., 1994, 1996b). Using computer-assisted sperm analysis, we have also demonstrated that the peptide stimulates hyperactivated motility in mouse spermatozoa (Green et al., 1996a; Table II). Consequently, we have proposed that the peptide should be known as fertilization promoting peptide (FPP).

In our most recent studies (Green et al., 1996c), we have sought clues to the possible mechanism of action involved in FPP-elicited responses. We now have demonstrated that FPP stimulation of capacitation requires extracellular Ca\(^{2+}\). Interestingly, although FPP stimulates uncapacitated cells, we also observed that addition of FPP to capacitated suspensions inhibited spontaneous acrosome loss in mouse spermatozoa. Because earlier studies (e.g. Monks et al., 1986) had identified similar biphasic, capacitation-related changes in responses to adenosine, i.e. stimulatory early in capacitation and inhibitory later, we investigated the possibility that both FPP and adenosine might act via the same pathway. When FPP and adenosine were used in combination with uncapacitated mouse sperm suspensions, both at low non-stimulatory and high stimulatory concentrations, the response was greater than with either compound alone, suggesting that they are affecting a common pathway but initiating responses independently. We also observed that adenosine, like FPP, inhibited acrosome loss in capacitated cells (Green et al., 1996c).

One of the most interesting and relevant experimental observations was that while FPP and adenosine each inhibited spontaneous acrosome loss in capacitated suspensions, the cells were still able to undergo an acrosome reaction in response to progesterone, a physiological agonist of the exocytotic response (Table II). Since adenosine, acting via specific cell surface receptors, has been shown to stimulate adenylate cyclase activity (Fraser and Duncan, 1993) and fertilizing ability (Fraser, 1990) in uncapacitated mouse spermatozoa, yet inhibit adenylate cyclase activity in capacitated cells (Monks et al., 1986), we have proposed that FPP also acts via the adenylate cyclase/cAMP signal transduction pathway. This is consistent with the recent demonstration of a time-dependent, cAMP-regulated increase in tyrosine phosphorylation of several proteins during capacitation in vitro of mouse spermatozoa (Visconti et al., 1995a,b). Furthermore, Fénichel et al. (1996) have reported that an adenosine analogue stimulated protein tyrosine phosphorylation in uncapacitated human sperm suspensions and inhibited it in capacitated suspensions.

We have proposed (Green et al., 1996b,c) that FPP binds to sperm cells at ejaculation and then remains bound, exerting a stimulatory effect on capacitation as spermatozoa ascend the female tract. Adenosine is also present in seminal plasma (Fabiani and Ronquist, 1995) and body fluids (Fredholm et al., 1983). This availability of adenosine would allow it to augment FPP action and even to replace it if FPP is lost from the cell surface. We therefore have suggested (Green et al., 1996c) that FPP and adenosine, by modulating adenylate cyclase activity, provide an endogenous mechanism to promote capacitation but inhibit spontaneous acrosome loss and thus help to optimize the fertilizing potential of the few spermatozoa that reach the site of fertilization in vivo.

Ionic regulation of the acrosome reaction

The acrosome reaction during normal fertilization is Ca\(^{2+}\)-dependent, with millimolar concentrations in the extracellular environment being required for a maximal response (Fraser, 1994; Brucker and
Lipford, 1995). Molecules associated with oocytes and their investments act as agonists to trigger the acrosome reaction, one of the early responses in the complicated sequence of events being a rise in \([Ca^{2+}]_i\). There is now considerable evidence that zona glycoproteins (ZP) provide the signal, with ZP3 being the relevant molecule in the mouse (Ward and Kopf, 1993); related molecules appear able to initiate similar responses in other species. Progesterone, a component of follicular fluid produced by granulosa and cumulus cells, has also been shown to stimulate a rise in \([Ca^{2+}]_i\) and the acrosome reaction, first in human spermatozoa (Thomas and Meizel, 1989; Blackmore et al., 1990) and more recently in other species, including the pig (Melendrez et al., 1994) and mouse (Roldan et al., 1994). This response appears to involve progesterone interacting with a non-genomic receptor located on the sperm head (reviewed by Revelli et al., 1994).

Considerable evidence obtained during the past decade indicates that the rise in \([Ca^{2+}]_i\) during the acrosome reaction is due to Ca\(^{2+}\) influx via Ca\(^{2+}\) channels with properties similar to the voltage-sensitive L-type channels identified in vertebrate skeletal and cardiac muscle (e.g. Florman et al., 1992; Fraser, 1993; Florman, 1994; O'Toole et al., 1996b). Binding sites for Ca\(^{2+}\) channel antagonists such as dihydropyridines and phenylalkylamines have been identified (Florman et al., 1992). Acrosome reactions in response to solubilized zona proteins (Florman et al., 1992) and progesterone (Shi and Roldan, 1995; O'Toole et al., 1996b) can be inhibited significantly by similar Ca\(^{2+}\) channel antagonists. A few studies on mammalian spermatozoa have also detected the presence of voltage-sensitive Ca\(^{2+}\)-conducting channels (e.g. Cox et al., 1991; Beltran et al., 1994).

Another ion that is important during the acrosome reaction is Na\(^+\), with a large influx being associated with the exocytotic response. The Na\(^+\) ionophore monensin can trigger the acrosome reaction readily in both mouse (Fraser et al., 1993) and human spermatozoa (O'Toole et al., 1996b) in medium with a high millimolar Na\(^+\) concentration but not in mouse spermatozoa in a medium with a low Na\(^+\) concentration. Consistent with these observations, fertilization in vitro in the mouse required a high Na\(^+\) concentration (Fraser et al., 1993), and the removal of extracellular Na\(^+\) from capacitated human sperm suspensions significantly inhibited progesterone-induced acrosome loss (Garcia and Meizel, 1994).

This requirement for extracellular Na\(^+\) during the acrosome reaction could reflect its involvement in the rise in pH\(_i\) known to occur during agonist-induced acrosome loss. It has been suggested that Na\(^+\) again is important for the functioning of \(Na^+\)-, Cl\(^-\)- and HCO\(_3^-\)-dependent acid efflux pathway, where all ions are required to be present, that has been detected in mouse spermatozoa (Zeng et al., 1996). However, two studies (Shi and Roldan, 1995; L.Fraser et al., unpublished results) have provided evidence that HCO\(_3^-\) is not required for the progesterone-stimulated acrosome reaction in mouse spermatozoa. The alternative possibility that Na\(^+\) might be important in Na\(^+\)/H\(^+\) exchange as a mechanism for promoting a rise in pH\(_i\) has been suggested (Fraser et al., 1993). Even though the work of Zeng et al. (1996) found little evidence for the functioning of this exchange mechanism during regulation of pH\(_i\), it would be consistent with experimental evidence indicating a requirement for Na\(^+\) but not for HCO\(_3^-\) during the acrosome reaction.

Following the initial interaction between spermatozoa and ZP3 or progesterone, there are many individual steps in the sequence that culminate in the actual exocytosis of the acrosome. Knowing that the opening of Ca\(^{2+}\) channels is one of these steps, it is possible to determine whether other events occur before or after the activation of Ca\(^{2+}\) channels. Current evidence indicates that Na\(^+\) influx precedes the Ca\(^{2+}\) influx, since the acrosome reaction stimulated by monensin can be blocked by the prior inclusion of nanomolar concentrations of dihydropyridine Ca\(^{2+}\) channel antagonists such as nifedipine (human: O‘Toole et al., 1996b; mouse: Fraser, 1993).

An important consequence of Ca\(^{2+}\) channel opening and the resulting rise in \([Ca^{2+}]_i\) is the activation of polyphosphoinositide-specific phosphoinositidase C (PIC). Although PIC is also a phospholipase C (PLC), it is less confusing if PLC is used to denote the enzyme that hydrolyses phospholipids other than polyphosphoinositides.
Activation of PIC leads to hydrolysis of phosphatidylinositol 4,5-bisphosphate to produce inositol 1,4,5-trisphosphate (InsP$_3$), which stimulates release of stored intracellular Ca$^{2+}$, and diacylglycerol (DAG), which generally activates protein kinase C (Berridge, 1993). DAG can also be generated following the activation of other phospholipases, including PLC and the phospholipase D (PLD)/phosphatidic acid phosphohydrolase pathway (Exton, 1990, 1994).

Studies on mammalian spermatozoa have shown that PIC-mediated hydrolysis of membrane polyphosphoinositides is an important early event in the acrosome reaction (Roldan and Harrison, 1989; Thomas and Meizel, 1989). Current evidence suggests that InsP$_3$ does not appear to play a role in the acrosome reaction (Harrison et al., 1990), but that DAG does (Roldan and Harrison, 1992; O’Toole et al., 1996a). In human spermatozoa, although PIC activity is required to initiate DAG production, much of the DAG formed in response to either progesterone or the Ca$^{2+}$ ionophore A23187 is generated by the PLC pathway, with little evidence for PLD pathway involvement (O’Toole et al., 1996a). This DAG production occurs downstream of Ca$^{2+}$ channel opening, since the inclusion of nifedipine not only blocks the endpoint of exocytosis but also the generation of DAG (O’Toole et al., 1996b). Exogenous DAG will induce the acrosome reaction, but only in capacitated cells (determined using chlorotetracycline); the fact that inclusion of nifedipine does not block this response is consistent with DAG formation and action during the acrosome reaction occurring after Ca$^{2+}$ channel opening (O’Toole, 1996b).

In somatic cells, DAG activates protein kinase C (PKC; Berridge, 1993), but an unequivocal role for PKC during the acrosome reaction has yet to be demonstrated. PKC activity in sperm cells is much lower than in somatic cells (Rotem et al., 1990). Indirect evidence, obtained using various activators and inhibitors of PKC, has suggested that the enzyme may function during the acrosome reaction (e.g. DeJonge et al., 1991; Breitbart et al., 1992; Rotem et al., 1992). More direct evidence is needed before firm conclusions can be drawn regarding PKC participation in events culminating in acrosomal exocytosis.

Conclusions

Both capacitation and the acrosome reaction involve a very complicated sequence of events (Table III), with the ionic composition of the external environment playing a crucial role in controlling the acquisition and expression of fertilizing potential. The requirements for extracellular ions such as Ca$^{2+}$ and Na$^+$ reflect a need for changes in the intracellular concentration of these ions during the acquisition of fertilizing ability. In the absence of sufficient extracellular ions, sperm function will be inhibited. However, considerable evidence indicates that some men produce spermatozoa that are defective and hence unable to respond appropriately, even in an optimal environment. In some instances, these defective cells may be unable to undergo capacitation and, consequently, unable to undergo the acrosome reaction and interact successfully with the oocyte. In others, the defects may affect only the events associated with the acrosome reaction; for example, some spermatozoa cannot undergo an acrosome reaction in response to the influx of Ca$^{2+}$ stimulated by the Ca$^{2+}$ ionophore A23187, a treatment which bypasses the normal signal transduction pathways that trigger a rapid rise in [Ca$^{2+}$]; (Cummins, 1994).

Where they function correctly, the mechanisms that control both capacitation and the acrosome reaction help to ensure that potentially fertilizing spermatozoa reach the site of fertilization in vivo. These cells have lost their DF, acquired hyperactivated motility and, although apparently being inhibited from undergoing spontaneous acrosome loss by FPP and/or adenosine, are able to undergo the acrosome reaction in response to the oocyte and its associated investments. Thus, all of these complex steps can be seen to provide a delivery mechanism for depositing the spermatozoa’s genetic payload in the oocyte cytoplasm and, at the same time, activating the oocyte’s metabolism, both events being crucial to normal development. The fact that intracytoplasmic sperm injection (ICSI), using uncapacitated and acrosome-intact spermatozoa, is successful proves that the genetic information carried by these cells is fully competent to support complete development once it has reached the oocyte’s cytoplasm. However, it is important to remember that during capacitation and the acro-
some reaction in vivo, only a very small proportion of the spermatozoa in the ejaculate will reach the site of fertilization in a physiologically appropriate state to be able to fertilize an oocyte, if present. Thus, in vivo there are a number of selective pressures placed on the initial population of spermatozoa and these may be important in helping to select the ‘fittest’ spermatozoa for fertilization. No such selection is imposed on the spermatozoa used for ICSI. Therefore, it is of utmost importance to monitor carefully the development, throughout childhood and into adulthood, of individuals born as a result of ICSI. Indeed, since at present we have no way of identifying ‘fit’ spermatozoa, it would be best to use ICSI only in cases where the spermatozoa are judged to be incapable of achieving normal fertilization in vitro, rather than using ICSI as the first choice of treatment in all or most cases.

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References


Table III. Mechanisms regulating capacitation and the acrosome reaction

<table>
<thead>
<tr>
<th>Mechanism</th>
<th>Modulator</th>
<th>Capacitation</th>
<th>Acrosome reaction</th>
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<tbody>
<tr>
<td>Ca$^{2+}$-ATPase$\downarrow$</td>
<td>DF</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Adenylate cyclase$\uparrow/\downarrow$</td>
<td>FPP/adenosine</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>$pH$,$\uparrow$</td>
<td>Na$^+$, Cl$^-$, HCO$_3^-$</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Ca$^{2+}$ channels$\uparrow$</td>
<td>ZP3 plus Na$^+$, Cl$^-$, HCO$_3^-$</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>PIC$\uparrow \rightarrow$ DAG$\uparrow$</td>
<td>ZP3/progesterone (indirect)</td>
<td>-</td>
<td>+</td>
</tr>
</tbody>
</table>

$\uparrow$, $\downarrow$ = increase or decrease in activity or concentration respectively.

DF = decapacitation factor; FPP = fertilization promoting peptide; ZP3 = zona glycoprotein 3; PIC = phosphoinositidase C; DAG = diacylglycerol.


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