ZP3-dependent Activation of Sperm Cation Channels Regulates Acrosomal Secretion During Mammalian Fertilization

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Abstract. The sperm acrosome reaction is a Ca2+-dependent secretory event required for fertilization. Adhesion of the egg's zona pellucida promotes Ca2+ influx through voltage-sensitive channels, thereby initiating secretion. We used potentiometric fluorescent probes to determine the role of sperm membrane potential in regulating Ca2+ entry. ZP3, the glycoprotein agonist of the zona pellucida, depolarizes sperm membranes by activating a pertussis toxin-insensitive mechanism with the characteristics of a poorly selective cation channel. ZP3 also activates a pertussis toxin-sensitive pathway that produces a transient rise in internal pH. The concerted effects of depolarization and alkalinization open voltage-sensitive Ca2+ channels. These observations suggest that mammalian sperm utilize membrane potential-dependent signal transduction mechanisms and that a depolarization pathway is an upstream transducing element coupling adhesion to secretion during fertilization.

The acrosome reaction is a Ca2+-dependent secretory event in sperm that is an obligatory early step in the fertilization process. It results in the release of acrosomal granule contents, the extensive reorganization of sperm surface proteins, and the display of new membrane domains at the surface. Only sperm that have completed the acrosome reaction are capable of fusing with eggs (reviewed by 67).

In mammals, acrosome reactions are triggered by contact with the zona pellucida (ZP),1 the egg's extracellular matrix, or by treatment with soluble ZP extracts (reviewed by 60). Such extracts contain three glycoproteins, designated ZP1, ZP2, and ZP3 (reviewed by 63 and 64). ZP3 completely accounts for the acrosome reaction-inducing agonist activity, as demonstrated by studies with highly purified oocyte ZP3 (10, 45) and with recombinant ZP3 expressed by mammalian cells (5, 35). The mechanism of ZP3 action is presently a central, unresolved aspect of fertilization models.

Intracellular Ca2+ ([Ca2+]i) mediates stimulus-secretion coupling in many cellular systems, including the ZP3-activated pathway in sperm. ZP glycoproteins produce sustained elevations of sperm [Ca2+]i, as reported by ion-selective fluorescent probes (4, 14, 21, 25, 38). Several lines of evidence indicate that ZP signals promote Ca2+ influx through voltage-sensitive channels during initiation of acrosome reactions: a) ZP-dependent acrosome reactions and [Ca2+]i elevations are inhibited by several different structural classes of voltage-sensitive Ca2+ channel antagonists, including 1,4-dihydropyridines (21, 25). b) Depolarization of sperm membrane potential with [K+]o or with gramicidin D produces [Ca2+]i elevations as well as acrosome reactions in the absence of ZP3. c) Depolarization-dependent responses are also attenuated by antagonists of voltage-sensitive Ca2+ channels (2, 25). This channel mediates an essential component of the ZP-dependent [Ca2+]i elevation leading to acrosome reactions and is a likely site of action for the reported human contraceptive effects of 1,4-dihydropyridines (6, 31).

Present understanding of the mechanism of ZP3 signal transduction includes the identification of candidate sperm surface receptors (12, 39, 42) and the demonstration that both G protein (19, 27, 62) and tyrosine kinase (40, 57) signaling pathways are stimulated. Yet the coupling between receptor activation and Ca2+ channel opening is presently not well understood. In somatic cells, extracellular signals activate voltage-sensitive Ca2+ channels by depolarizing membrane potential as well as by modulating channel function through phosphorylation of channel proteins or by a rapid, membrane-delimited mechanism that may represent a direct interaction with G protein subunits (reviewed by 32 and 66).

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The Journal of Cell Biology, Volume 134, Number 3, August 1996 637-645

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1. Abbreviations used in this paper: BCECF, 2,7-bis(carboxyethyl)-5(6)-carboxyfluorescein; [Ca2+]i, intracellular Ca2+; DiSBAC2(3), bis(1,3-diethylthiobarbituric acid) trimethine oxonol; DISC3(5), 3,3'-dipropylthiodi-carbocyanine iodide; pI-Ij, internal pH; PTx, pertussis toxin; ZP, zona pellucida, mZP and bZP, mouse and bovine zona pellucida.
Here, the role of sperm membrane potential as an effector of ZP3 signals was examined using potentiometric fluorescent indicators. We find that ZP and ZP3 depolarize mouse and bovine sperm membrane potential by activating a pertussis toxin (PTx)-insensitive pathway that has the characteristics of a poorly selective cation channel. ZP signals also activate a second PTx-sensitive pathway that produces transient elevations of sperm internal pH (pHi). Voltage-sensitive Ca\(^{2+}\) channels function as a coincidence detector by integrating these two ZP-derived signals. These results suggest that sperm membrane potential is an important effector of egg-induced sperm activation.

### Materials and Methods

#### Solutions and Chemicals

Bovine and mouse sperm were incubated in dTALP and in Hepes-buffered CM media, respectively (68). Medium HNKG contains (mM) 25 Hepes, 120 Na\(^+\)-gluconate, and 5 K\(^+\)-gluconate. Medium NT contains MgCl\(_2\), KHEPO\(_4\), Hepes, and metabolic substrates appropriate for bovine HCl) and 22.6 Tris-carbonate, as well as concentrations of KCl, CaCl\(_2\), MgCl\(_2\), KH\(_2\)PO\(_4\), Hepes, and metabolic substrates appropriate for bovine and mouse sperm (68). Additional modifications of these media, described under Results, are obtained by compensatory adjustment of [NaCl] or of [NMDG\(^+\)-Cl\(^-\)]. PO\(_4^{2-}\) was removed during experiments with Cd\(^{2+}\), Cu\(^{2+}\), and La\(^{3+}\). Polyvinylpyrrolidone (M\(_w\) 40,000) was substituted protein before membrane potential determinations, as well as the characteristics of a poorly selective cation channel. ZP signals also activate a second PTx-sensitive pathway that has

#### Biological Preparations

Sperm were isolated from bovine seminal secretions and from mouse cauda epididymides, capacitated in vitro, and assayed for the ability to undergo ZP-initiated acrosome reactions and to fertilize eggs in vitro as described previously (25). Mouse and bovine ZPs were obtained from ovarian homogenates and soluble extracts were obtained as described (25). ZP glycoproteins were purified from mouse by SDS-PAGE under nonreducing conditions (8) and from bovine by two-dimensional PAGE. SDS and other electrophoretic reagents were removed by sequential dialysis in 8 M urea and in a 25 mM Hepes/125 mM NaCl, pH 7.4, medium (8). Acrosome reactions were assayed by the Coomassie blue dye-binding method (43, 45) and, in fluorescence microscopic experiments, by differential interference contrast microscopy (11).

Experiments determining the zona pellucida-dependent alteration of Ca\(^{2+}\), pH\(_i\), and acrosomal secretion used 100 \(\mu\)g/ml zona pellucida glycoprotein concentration, similar to dose–response relationships described previously (10, 23). In contrast, membrane depolarization occurs at \(\approx 1 \mu\)g/ml zona pellucida glycoprotein (see Fig. 2). Such differences may reflect distinct experimental conditions, such as the removal of media protein before membrane potential determinations, as well as the characteristics of a bifurcated signal transduction mechanism (see Discussion).

#### Membrane Potential Determinations

Sperm population membrane potentials were determined from the fluorescence emission of the anionic oxonol, DiSBAC\(_2(3)\), and confirmed in parallel experiments with the cationic carbocyanine, DiSC\(_3(5)\). Following correction for the contributions of mitochondrial potentials to probe signals, the membrane potentials of mouse sperm populations reported by these two probes were \(-54 \pm 4 \text{ and } -61 \pm 7 \text{ mV, respectively}\), whereas values for bovine sperm were \(-51 \pm 3 \text{ and } -57 \pm 8 \text{ mV, respectively}\). These values are similar to those obtained previously (25, 68) and are stable during several minutes in protein-free media (Fig. 1 A and B; before protein addition).

Solvulibilized ZPs depolarize mouse and bovine sperm membrane potentials. Fig. 1 A shows the effects of bovine ZPs (bZP, 100 \(\mu\)g/ml; 1 bZP \(\sim 27 \text{ ng protein}\); 23) on the membrane potential of homologous sperm as reported by DiSBAC\(_2(3)\). In this experiment, the initial depolarization rate was 14.5 mV/min (Fig. 1 A, dashed line). Initial rates in both species are dose-dependent, as shown in Fig. 2. Mouse ZPs (mZP, 4 ng protein/mZP; 9) produce a half-maximal response at 44 \(\pm 9 \text{ ng/ml and a maximal rate of } 19.7 \pm 3.7 \text{ mV/min at } \geq 250 \text{ ng/ml (Fig. 2 A)}, \text{ while bovine sperm exhibit a half-maximal response with } 83 \pm 19 \text{ ng/ml bZP and a maximal response of } 17.8 \pm 2.9 \text{ mV/min at } \geq 1 \mu\text{g/ml (Fig. 2 B). Similar conclusions were drawn from parallel experiments in both species using the DiSC\(_3(5)\) probe.}

The specificity of this response is indicated by several control experiments. First, comparable concentrations of an unrelated glycoprotein (fetuin) produced only minor effects on membrane potential (\(<0.7 \text{ mV/min}\) that probably reflect direct binding of extracellular protein to probe. Similar effects were observed in sperm-free solutions. Second, depolarization occurred only when sperm were treated with homologous ZP solutions. Fig. 1 B shows that mZPs failed to depolarize bovine sperm membrane potential, and similar species selectivity was found when mouse sperm were treated with bZP. Finally, ZP-dependent responses require a polarized membrane potential in sperm. When membrane potentials were dissipated with gramicidin D, the subsequent addition of ZPs had only minor effects on probe fluorescence. These effects were similar to those observed in intact sperm treated with heterologous ZPs (not shown). ZP-dependent depolarization precedes acrosome reac-
Figure 1. Effects of homologous and heterologous zonae pellucidae on the membrane potential and acrosome reaction of bovine sperm populations. Membrane potentials (---) were calculated from DiSBAC2(3) fluorescence emission and the occurrence of acrosome reactions (O) were determined as described in Materials and Methods. Shown are the calculated membrane potentials and incidence of acrosome reactions in bovine sperm populations before and after addition of: (A) soluble preparations of bovine zonae pellucidae (100 µg/ml; arrow), which produces a maximal dye response (see Fig. 2); and (B) soluble preparations of mouse zonae pellucidae (100 µg/ml; arrow). Fluorescence emission acquisition rate was 1 Hz and the initial rate of depolarization was estimated from linear fit of data obtained in the first 30 s (A, dashed line) following zona pellucida addition. Initial rates in the experiments shown here were (A) 14.5 mV/min, and (B) 0.4 mV/min. Acrosome reactions data represent the mean (± SD) of triplicate slides obtained from a sample at the indicated time point, with ~200 sperm assayed per slide.

Figure 2. Initial rate of sperm membrane depolarization. Membrane potential was calculated from DiSBAC2(3) fluorescence emission and used to determine initial depolarization rates following addition of unfractionated zonae pellucidae, enriched fractions of zona pellucida glycoproteins, and control proteins to mouse (A, open symbols) and bovine sperm (B, closed symbols). Initial rates were determined as indicated in Fig. 1, and data represent the mean (± SD) of three to seven separate determinations. The effects of unfractionated zonae pellucidae and ZP3-enriched fractions were fit to the relationship: \( R_c = \left[ \frac{R_{\text{max}} + C}{K + C} \right] + N \), where \( R_c \) and \( R_{\text{max}} \) are initial rates at each concentration of zona pellucida glycoproteins and the maximal initial rate, respectively; \( C \) is the concentration of zona pellucida glycoprotein; \( K \) is the apparent equilibrium constant for this reaction; and \( N \) is the nonspecific background depolarization rate following buffer addition. Values of \( R_{\text{max}} \) (mV/min), \( K \) (ng/ml), and \( N \) for fit curves (---) were: mouse ZP, 19.7, 44, and 0.03; mouse ZP3, 14.8, 1.2, and 0.9; bovine ZP, 16.7, 83, 3.4; bovine class III glycoproteins, 21.1, 1.1, 1.4. Symbols: total ZP, (O, ●); ZP3-enriched fractions, (Δ, ▲); ZP1 + ZP2, (△, ▼); fetuin, (<, •).
Unlike the case in the mouse, bZP3 has not previously been identified. Fig. 3 shows that the bZP consists of three distinct classes of glycoproteins (Fig. 3, inset). To identify the group containing bZP3, individual groups were eluted from gel slices, dialyzed, and assayed for the ability to initiate acrosome reactions in bovine sperm. Fig. 3 shows that class III glycoproteins exclusively account for induction of acrosome reactions, whereas classes I + II lack this activity. Therefore, class III is enriched in bZP3.

Class III glycoproteins also account for the ability of bovine ZPs to depolarize sperm membrane, as shown in Fig. 2 B (half-maximal response ~1.1 ± 0.4 ng/ml; maximal response ~21.1 ± 3.1 mV/min). In contrast, classes I + II lack apparent stimulatory activity. These results demonstrate that in two mammalian species, fractions enriched in ZP3 depolarize sperm membrane potentials.

**Permeation Characteristics of a Zona Pellucida-activated Depolarization Pathway**

In order to deduce the ion selectivity of a ZP3-activated depolarization mechanism, we examined the effects of medium ion composition on the initial rates of response. Capacitated sperm were suspended in test media containing DiSBAC2(3). After fluorescence signals stabilized, ZP agonists were added (mZP, 250 ng/ml; bZP, 1 μg/ml) and the initial response rates were estimated from time courses similar to that in Fig. 1 A.

Fig. 4 illustrates the results obtained in a complete gamete culture medium. In this series of experiments, the initial depolarization rate following buffer addition was <1.5 mV/min (mouse, 1.4 ± 1.1 mV/min; bovine, 0.7 ± 0.5 mV/min) and was enhanced by 1.5–2-fold following addition of heterologous ZPs (Fig. 4) or fetuin (not shown). In contrast, homologous ZPs stimulated the initial rate by an additional 8–15-fold relative to control glycoproteins (mouse, 19.7 ± 3.7 mV/min; bovine, 17.8 ± 2.9 mV/min). In complete culture media, the ZP-dependent depolarization was not substantially reduced by either chelation of Ca2+ or of all divalent cations but was inhibited by 10 mM Cd2+ (not shown) or by 0.1 mM La3+ (Fig. 4). Cd2+ also inhibits the voltage-sensitive Ca2+ channel, but at 10–100-fold lower concentration (21), thereby differentiating that pathway from the depolarization mechanism described here. In the following series of experiments, La3+ and Cd2+ sensitivity provides a signature for the ZP3-activated pathway.

Anion composition did not significantly influence ZP-
evoked depolarizations, as shown in experiments in HNKG medium (Fig. 4). Robust responses occur following ZP treatment in a Na+/K+-based media containing either monovalent- or divalent anions (gluconate, Fig. 4; aspartate, Br⁻, Cl⁻, SCN⁻, and SO₄²⁻, data not shown). La³⁺ (Fig. 4) and Cd²⁺ (data not shown) inhibited responses in this simplified medium with potencies similar to those observed in complete medium.

In contrast, the ZP-induced sperm membrane depolarization was dependent on the cation composition of the medium. Responses were reduced by >90% in medium NT (Fig. 4), where Na⁺ and K⁺ are replaced by the larger cation, N-methyl-d-glucamine⁺ (NMDG⁺). Subsequent substitution of NMDG⁺ in NT medium with smaller mono- and divalent cations, such as Na⁺ (Fig. 4), K⁺, Ba²⁺, Ca²⁺, Mn²⁺, or Ni²⁺ (not shown), restored ZP-dependent depolarization. The responses that are restored following Na⁺ or Ca²⁺ readdition are inhibited by 0.1 mM La³⁺ and by 10 mM Cd²⁺, reflecting the activation of that same depolarization mechanism as was observed in complete medium (Na⁺, Fig. 4; Ca²⁺, data not shown). Alternatively, ZP-evoked depolarizations could not be restored to sperm in medium NT by alterations of anion composition, as shown when Cl⁻ was replaced with other aspartate, Br⁻, SCN⁻, or SO₄²⁻. Comparable results were obtained in a less extensive series of experiments with the bovine sperm using DiSC₃(5). These observations suggest that ZP-dependent depolarization activates a mechanism with the anticipated characteristics of a poorly selective cation channel.

**Role of Membrane Depolarization in Zona Pellucida-controlled Acrosome Reactions**

Previous studies showed that pertussis toxin (PTx) inhibits an early component of ZP3 signal transduction and mitigates the induced elevations of [Ca²⁺]ᵢ and pHᵢ (4, 21, 24) as well as the ZP-dependent acrosome reaction (19, 20). These effects most likely reflect the activation of sperm G₁₁ and/or G₂ by ZP3 (62). Yet PTx treatment of mouse and bovine sperm had only minor effects on ZP-induced depolarization. Treatment with 100 ng/ml PTx, which inhibits the induced acrosome reaction by >80% (19, 24), reduced the ZP-dependent initial depolarization rate of mouse sperm from 15.9 ± 2.0 to 13.4 ± 2.7 mV/min and of bovine sperm from 14.3 ± 2.6 to 11.1 ± 1.8 mV/min (n = 3, nonspecific background subtracted). We examined the coupling between membrane depolarization and Ca²⁺ channel activation in a series of single cell experiments. Stereotypic cells are illustrated in Fig. 5 and the results of these experiments are summarized in Fig. 6.

Fig. 5 A shows an example of a bovine sperm labeled with the fura 2 and BCECF fluorescent probes, permitting simultaneous determination of relative [Ca²⁺]ᵢ and pHᵢ levels, respectively. Addition of bZPs produces a sustained [Ca²⁺]ᵢ elevation, as reported previously (21, 24), as well as an increase in pHᵢ. This cell completes the acrosome reaction within 12–13 min (Fig. 5 A, dashed line). As shown in Fig. 6, addition of homologous ZPs elevated [Ca²⁺]ᵢ by three- to fourfold and also increased the fraction of acrosome reacted sperm by threefold, relative to buffer-treated controls.

As shown in Fig. 5 B, the ZP-dependent [Ca²⁺]ᵢ elevation is inhibited by PN200-110 (21), the 1,4-dihydropyridine antagonist of some classes of voltage-sensitive Ca²⁺ channels. In these experiments, 10 μM PN200-110 reduced the peak [Ca²⁺]ᵢ response to ZP stimuli from a 3.15 ± 0.13-fold increase to a 0.69 ± 0.15-fold elevation (n = 78 and 61
Discussion

There are two central observations in this study. First, sperm membrane potential is depolarized during contact with the ZP as a result of ZP3-dependent activation of a pathway with the anticipated characteristics of a cation channel. Second, depolarization mediates a component of ZP3 signaling leading to [Ca^{2+}]_i elevations and acrosome reactions.

Fluorescent probes report that capacitated sperm have a resting membrane potential of ~−50 to −60 mV and that ZP3 depolarizes this potential by ~30 mV. The application of both anionic and cationic probes in two species lends confidence that depolarization is a general aspect of ZP3 signal transduction. Depolarization is produced by activating a mechanism with the anticipated characteristics of a cation channel, and the selectivity of this pathway was determined from the effects of ion depletion on the initial rate of probe response. This approach permits qualitative discrimination between ionic species that support the operation of this pathway and species that are not permissive, thereby establishing cation dependence. The more
A typical method of establishing ion selectivity from the current reversal potential (32) was not feasible with sperm, where cytoplasmic dialysis using the whole-cell patch clamp has not been reported successfully.

Previously, we used fura 2 fluorescence and fluorescence-quenching methods to describe a cation transport mechanism in the bovine sperm head that is activated by ZPs (21). Divalent cation transport through that pathway was demonstrated, strongly supporting the notion that it is a channel. That cation transporter shares several functional features with the depolarization mechanism described here, including: cation selectivity (both are permeable to Ca\(^{2+}\), Mn\(^{2+}\), and Ni\(^{2+}\)), anion rejection, inhibition by high concentrations of Cd\(^{2+}\), and activation by ZP/ZP3 through a PTx-insensitive mechanism. These two experimental approaches most likely reveal a single ZP3-regulated mechanism in the sperm head. The relationship of this depolarization mechanism to the other cation-selective channels that have been described in mammalian (16, 26, 56, 65) and echinoderm sperm (29) must be established.

ZP3-evoked depolarization is slow compared to the time course of activation of ion channels. The protracted kinetics may be due to several factors. First, mammalian sperm response to ZPs is unsynchronized and heterogeneous. Extensive cell-to-cell variation is observed in the lag time that precedes ZP-induced responses. In addition, only a subpopulation (25–50%) of sperm complete the capacitation process and respond to ZPs, further adding to population heterogeneity (23, 61). This functional heterogeneity dominates the population kinetics of ZP-dependent [Ca\(^{2+}\)]\(_i\), and acrosome reaction responses (21, 24, 38). The redistribution-type probes used here provide spatially averaged membrane potentials for populations and thus do not directly reflect the time course of either membrane depolarization in individual cells or the activation kinetics of a cation channel. Single cell determinations of membrane potential are required to assess the contribution of population heterogeneity in these responses. Second, cation channel density has not been determined and may contribute to the protracted kinetics. Finally, certain other secretory systems also exhibit slow depolarizations that are a component of the signal transduction mechanism (for example, 44).

The most likely function of ZP3-dependent depolarization during sperm-egg interaction is to participate in the control of Ca\(^{2+}\) channels. A model for ZP3-activated [Ca\(^{2+}\)]\(_i\) responses during mammalian fertilization can be proposed based on the results of these and other experiments (Fig. 7). A central feature of this model is the ZP3-dependent activation of a sperm cation channel which, in the presence of an inwardly negative membrane potential (present study, 25, 68), mediates a depolarizing current. Insensitivity to PTx inhibition is a signature of this portion of the signaling pathway. Plausible mechanisms of ZP3-depolarization coupling include the direct activation of a ligand-gated cation channel as well as the mediation of PTx-insensitive transduction elements. In this regard, sperm possess both a ZP3-stimulated tyrosine kinase activity (39, 40) as well as the PTx-insensitive G proteins, G\(_1\) and G\(_2\) (60).

It is known that ZP3 also activates sperm pHi regulatory mechanisms (20, 24, 25, 38) and that the resultant alkalinization modulates membrane potential–dependent regulation of sperm Ca\(^{2+}\) channels and acrosome reactions (2, 25). Our data demonstrate that the induced alterations of pHi constitute the PTx-sensitive step of ZP signal transduction. This is illustrated most directly by the effects of 10 mM NH\(_4\)\(^+\). At this concentration, this permeant base does not induce [Ca\(^{2+}\)]\(_i\) elevations (Figs. 5, C and D) or acrosome reactions (Fig. 6; ref. 25) in the absence of ZP stimulation, yet it is sufficient to restore both the ionic and secretory response to ZPs in PTx-treated sperm (see Fig. 5 C). These observations strongly suggest that a pHi-regulatory pathway is the major PTx-sensitive step in ZP3 signal transduction. Functional studies demonstrate that the principal regulators of sperm pHi include a Na\(^+\)-dependent Cl\(^{-}\)/HCO\(_3\)\(^{-}\) exchanger and an anion-independent, arylinonbenzoate-sensitive regulator (69), while immuno-fluorescence and northern hybridization suggest that a Na\(^{+}\)-H\(^+\) exchanger and a Na\(^{+}\)-independent Cl\(^{-}\)/HCO\(_3\)\(^{-}\) exchanger may also be present (48, 49). The pathway that mediates PTx-sensitive alkalinization in response to ZP3 stimuli has not been identified. In this regard, ZP3 activation of PTx-sensitive G\(_1\) and G\(_2\) has been demonstrated in detergent-permeabilized sperm (62) and may control a sperm pHi-regulatory pathway.
Thus, these observations suggest that ZP3 activates a bifurcated signaling cascade consisting of the opening of a PTX-insensitive cation channel and the stimulation of a PTX-sensitive pH regulator (Fig. 7). Concerted depolarization and alkalization are more effective in promoting [Ca^{2+}]_{i} elevations than either signal alone. A branched signal transduction structure, with high- and low-affinity limbs, may account for the observed differences in potency of zona pellucida-dependent depolarizations and pH responses.

The [Ca^{2+}]_{i} response mechanism functions operationally as the integrator of a coincidence detecting system. Similarly, voltage-sensitive Ca^{2+} channels in somatic cells (18, 34, 36, 41, 51, 59; reviewed in 46), as well as other ligand- and voltage-regulated channels (for example, 7, 13, 15, 17, 28, 30, 37, 58; reviewed in 41), are also modulated by pH. Frequently, such Ca^{2+} channel modulation is observed at values that are more acidic than the typical range of somatic cell pH and may function as a health sensor, curtailling cellular activity during internal acidification. In contrast, pH_{i} in mammalian sperm is relatively acidic, as determined by fluorescent probes (2, 3, 24, 50, 53, 59, 69) and by 31P-NMR (54). Under these conditions, this mechanism of Ca^{2+} channel modulation may function within the physiological range of pH values and operate as a behavioral switch.

Given that sperm have only a single secretory granule and that the fertilizing ability of this cell is compromised when acrosome reactions are either induced prematurely or inhibited (22), it is anticipated that secretion may be regulated stringently. Dual modulation by membrane potential and pH provides a means of suppressing Ca^{2+} entry until ZP contact. The utility of a channel-based coincidence detector is illustrated by considering the storage state of sperm within the cauda epididymis. The luminal fluid of that compartment has a Na:K ratio of 1:2 (33), sufficient to depolarize sperm membrane potential (23, 68). The relatively acidic conditions within epididymidal plasma (pH < 7; ref. 33) may limit Ca^{2+} entry through voltage-sensitive channels and may account for the low levels of spontaneous exocytosis during storage.

This work was supported by grants from the Philippe Foundation to C. Arnoult and from NIH to Y. Zeng (HD32177 and HD28627). The Journal of Cell Biology, Volume 134, 1996 644

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