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Cation and anion channels in rat and human spermatozoa

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Abstract

Ionic fluxes are thought to be important in the initiating process of gamete interaction such as acrosome reaction. Different populations of ion channels in rat and human spermatozoa were investigated using the planar lipid bilayer technique. Membrane proteins were isolated from rat and human sperm and inserted into lipid bilayer via fusion. We observed K^+-selective and Na^+-selective channels, as well as divalent permeable cation channels in membrane preparations from rat sperm. K^+ channels, which were sensitive to the K^+ channel blocker, tetraethylammonium (0.1 mM), exhibited a mean single channel conductance of 24 pS. Whereas, larger conductance, 109 pS, was found to be associated with Na^+ channels. Low conductance anion channel, 15 pS, was also observed when permeant cations in the bathing solutions were substituted with *N*-methyl-D-glucamine leaving Cl^- as the major permeant ion species. This channel exhibited a slower channel open and closed kinetics when compared to other cation channels. Both cation and anion channels with characteristics similar to that found in rat sperm were also observed in preparations from human sperm. The variety in the types of ion channels observed in rat and human spermatozoa suggests that ion channels may play different roles in sperm physiology and gamete interaction.

Keywords: Spermatozoon; Cation channel; Anion channel; (Rat); (Human)

1. Introduction

It has been shown that sperm motility initiation is associated with a complex ionic event [1–3]. It was found that when the cauda epididymal spermatozoa were flushed out with oil, they were immotile, or when flushed out in a sodium-free medium, they exhibited only transient motility. However, if these immotile spermatozoa were resuspended in a sodium-containing medium, they initiated or regained forward motility [1]. Later, it was also found that an efflux of K^+, measured by $^{86}Rb^+$, was associated with such sodium-induced motility and that tetraethylammonium (TEA), a K^+ channel blocker, inhibited the motility initiation in a dose-dependent manner [4]. Hyperpolarization of the sperm membrane was also observed during motility initiation, presumably due to outward movement of K^+. Ca^{2+} has also shown to be important in sperm motility initiation [2]. These observations have led to the speculation that ion channels are the mediators of the ionic events involved in sperm motility initiation.

It is also believed that ion fluxes are fundamental in the response of spermatozoa to the egg. The sperm

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acrosome reaction, a regulated exocytosis initiated in mammalian sperm by adhesive contacts with the egg's extracellular matrix, is shown to be mediated by elevation of intracellular Ca^{2+} (see review, [5]). This process is required in the preparation of the sperm cell for fertilization of the egg. In addition to Ca^{2+} influx, a sequence of ionic changes such as Na^+ influx and efflux of H^+ and K^+ has also been observed in association with the acrosome reaction [6]. Though not directly, these early fluorometric and pharmacological studies have implicated the involvement of ion channels in the acrosome reaction.

Electrophysiological studies on intact sperm have been precluded by their small size. Up to date, only a few studies have been made to investigate ion channels in plasma membranes of spermatozoa using the planar lipid bilayer technique [7–11]. Only Ca^{2+}-conducting non-selective cation channels and Ca^{2+} channels have been characterized by these studies, while other types of channels, especially anion channels, have not been described although a role of anion channels in the mechanism of acrosome reaction has also been implicated in bull and porcine spermatozoa [12,13]. In order to fully understand the role of ion channels in sperm motility initiation as well as in gamete interaction, we undertook this study to examine different ion channel populations using the planar lipid bilayer technique. We also report here for the first time different ion channels observed in human sperm, which may shed light to the understanding of the ionic basis underlying gamete interaction and some cases of male infertility.

2. Materials and methods

2.1. Materials and solutions

Tetraethylammonium (TEA), KCl, NaCl, $CaCl_2$, Tris, Na_2HPO_4, NaH_2PO_4, decane, hexane, chloroform, ethanol were from Merck (Darmstadt, Germany). EDTA, phenylmethylsulfonyl fluoride (PMSF), Triton X-100, Hepes, *N*-methyl-D-glucamine (NMDG) and cholesterol were from Sigma (St. Louis, USA). Phospholipids, phosphatidylcholine (PC) were purchased from Avanti Polar Lipids (Alabaster, USA).

2.2. Collection of epididymal sperm and isolation of sperm plasma membranes

Rat sperm samples were collected from dissected cauda epididymidis. Epididymides were removed from rats (400–600 g, $n = 20$) and cauda region was dissected and placed in a Petri dish. The intraluminal contents of the cauda segment were flushed out through an incision in the distal cauda by back-flushing of the vas deferens with 0.01 M phosphate-buffered saline. Human sperm were collected from 7 healthy subjects of proven fertility by masturbation.

The preparation of sperm proteins was based on the method described by Yan et al. [14]. Both rat and human spermatozoa were separated from seminal plasma by centrifugation at $1500 \times g$ for 10 min. The sperm pellet was resuspended and washed three times with 0.01 M phosphate buffered saline, pH 7.4. Spermatozoa were then collected and counted. Sperm were resuspended ($5 \cdot 10^9$/ml) in 0.01 M Tris-HCl buffer containing 0.2 mM the antiproteinase, PMSF, 1 mM EDTA and 0.5 M NaCl, pH 8.3. A final concentration of 1% of Triton X-100 was added to the suspension of sperm. The homogenate was stirred at 4°C for 12 h and stored in the cold room overnight. Protein content was determined by modified Lowry method, ranging from 3 to 23 mg/ml. Insoluble material was removed by centrifugation at $6000 \times g$ for 30 min. The supernatant was stored at -70°C.

2.3. Planar bilayer experiments and data acquisition

Planar lipid bilayers of phosphatidylcholine (PC) (Avanti Polar) in n-decane (20 mg/ml) and cholesterol(5 mg/ml) were painted with brush onto the 300 μm orifice of the teflon membrane (Yellow Springs, OH, USA) in a two-compartment chamber. When a bilayer membrane was formed, currents across the membrane elicited by different voltage pulses (-100 to 100 mV) were measured and membrane resistance could be determined. A bilayer membrane was considered to be satisfactory for further experiment if it exhibited a resistance ranging from 50 to 100 GΩ. The volumes of the *cis* and *trans* compartments were 3 ml. Isolated sperm plasma membranes were added to the *cis* compartment and continuously stirred with a small magnetic bar in either KCl, NaCl, NMDG-Cl,

or $CaCl_2$ buffer solutions containing 5 mM Hepes and 2 mM Tris-HCl (pH 7.4). An osmotic gradient of 300 mM KCl or NaCl (*cis*) to 100 mM KCl or NaCl (*trans*) was used to promote sperm protein fusion. After application of osmotic gradient across the bilayer membrane, current–voltage relationship was examined again to ensure high resistance of the membrane. Current noise level was also monitored, and isolated plasma membrane was only added to bilayers exhibiting low noise level.

Current and voltage measurements were made by using patch-clamp system (Axopatch 200). Both sides were connected to the electrodes via salt bridges (3 M KCl) in series with Ag/AgCl electrodes. All measurements were made at room temperature (20°C). Unfiltered current and voltage signals were digitized with an audio PCM-C processor and recorded on videotape. The current signal (at 10 Hz) was monitored simultaneously in an oscilloscope. Stored records were transferred, using a TL-1 interface and *p*clamp 6.0.2. software (Axon instruments), to an IBM-AT computer for analysis.

3. Results

Adding isolated plasma membranes from rat sperm to planar lipid bilayers yielded channel activity with a successful rate of about 30% (number of observation with channel activity divided by total number of bilayer preparations with sperm plasma membrane added). A complete experiment required recording currents at a series of desired voltages (mostly between −100 and 100 mV), which would largely depend on the stability of bilayers. Sometime, it was only possible to record channel activity at a few holding potentials and in a very short period of time, making further analysis and characterization of channel events impossible. The following analysis were based on the results of 51 relatively completed experiments (out of total 300 experiments in which channel activities were observed).

We initially used a 300/100 mM *cis* to *trans* KCl gradient. Usually 3–10 minutes after addition of isolated rat sperm membrane, incorporation of two or three channels was frequently observed. Single channel or as many as up to 5 channels could also be observed in one bilayer. Fig. 1A shows the channel events recorded at different holding potentials in a bilayer bathed in KCl solutions. In this case, one prominent channel was identified (Fig. 1C) with a reversal potential close to the equilibrium potential of K^+ (Fig. 1B). According to the Goldman–Hodgkin–Katz equation, a permeability ratio of P_K/P_{Cl} of 15 could be derived, suggesting a cation selective nature of the channel. When KCl solutions were used, the prominent channels in all 10 bilayers examined exhibited reversal potentials ranging from -7 to -30 mV, indicating selectivity for cation over anion (permeability ratio of K^+ to Cl^- averaged >10). However, the channel characteristics could vary from one bilayer to another. The channel events shown in Fig. 2A exhibited higher number of opened channels at most of the holding voltages as compared to that shown in Fig. 1A. This bilayer had at least two channels present (Fig. 2C) as compared to one channel present in the bilayer shown in Fig. 1C. The voltage dependence of channel open time was also analyzed. No apparent voltage dependence was found in the two bilayers mentioned above as illustrated in Fig. 3. However, the percentage of open time for the channel events shown in Fig. 2 was much higher than that in Fig. 1. throughout the voltage range examined. The single unit conductance observed also ranged from 6 to 51 pS with a mean of 24 ± 4.5 pS ($n = 13$). The apparent differences observed could be due to the presence of different types of cation channels, e.g., K^+ channels and non-selective cation channels which were permeable to other cations as well as K^+. Further characterization of channel events was carried out in order to identify specific ion channels in rat spermatozoa.

3.1. K^+ channels

Initially we tried to do ion substitution experiments by perfusing different solutions through the bilayer chamber, but failed in most cases to maintain a stable membrane while doing perfusion. Later experiments were designed in the following manner: 200 mM NaCl (final concentration) was added to the *cis* compartment after initial recordings obtained in (300/100 mM) KCl solutions, with equal volume of 100 mM KCl being added to the *trans* compartment to minimize any volume difference across the bilayer. If the cation channels observed in KCl solutions (see above)

were equally permeable to K^+ and Na^+, we would expect to see a hyperpolarizing shift in the reversal potential towards the new equilibrium potential produced by the total cation gradient (500/100 mM), in this case, −41 mV. If the channels were highly selective for K^+, no shift in the reversal potential would be expected upon addition of NaCl. Another possibility would be a much greater hyperpolarizing shift in reversal potential upon addition of NaCl, $\gg -41$ mV, if the channels were more selective for

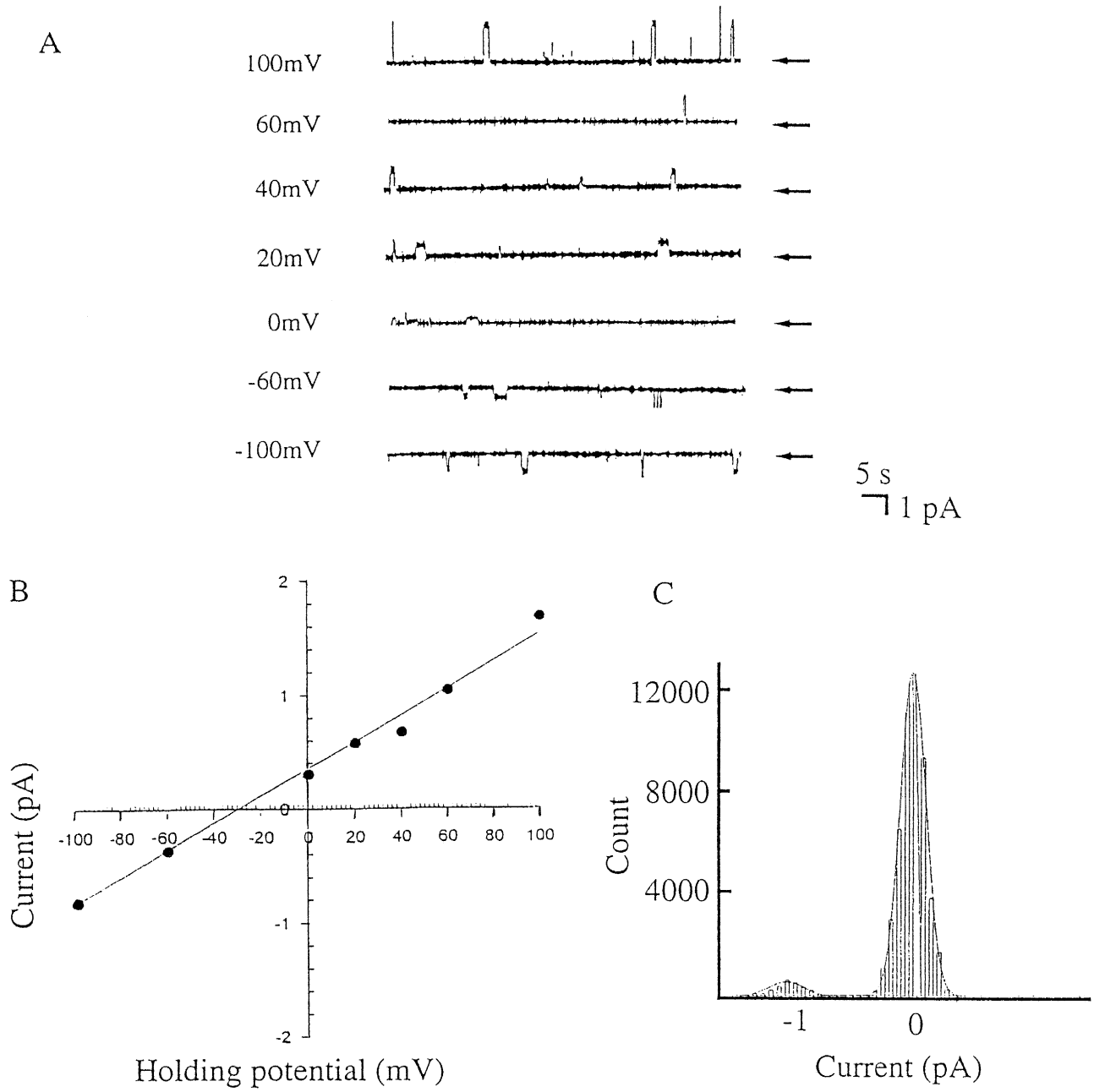

Fig. 1. Single channel currents observed from rat sperm in KCl solutions. (A) Channel events observed at seven different holding voltages with arrows indicating the closed state of the channel. (B) Corresponding current–voltage relationship. (C) Current amplitude histograms obtained at −100 mV. The conductance was 12 pS and reversal potential was −30 mV. The concentration gradient was 300 (*cis*) to 100 (*trans*) mM KCl.

Na^+ over K^+. In five such experiments, three cases initially exhibited cation-selective channel events in KCl solutions similar to those described in last section, with an averaged reversal potential of −14 mV. Of the three cases, the reversal potentials observed in two bilayers were not greatly altered (±6 mV) upon addition of 200 NaCl to the *cis* compartment. In addition, the currents recorded after addition of NaCl remained unaltered as shown in Fig. 4, suggesting that the channels were highly selective for K^+ over Na^+. In one of the three cases, a hyperpolarizing shift of −11 mV was observed indicating certain degree of Na^+ permeability of the channel. However, the channel was still more selective for K^+ over Na^+

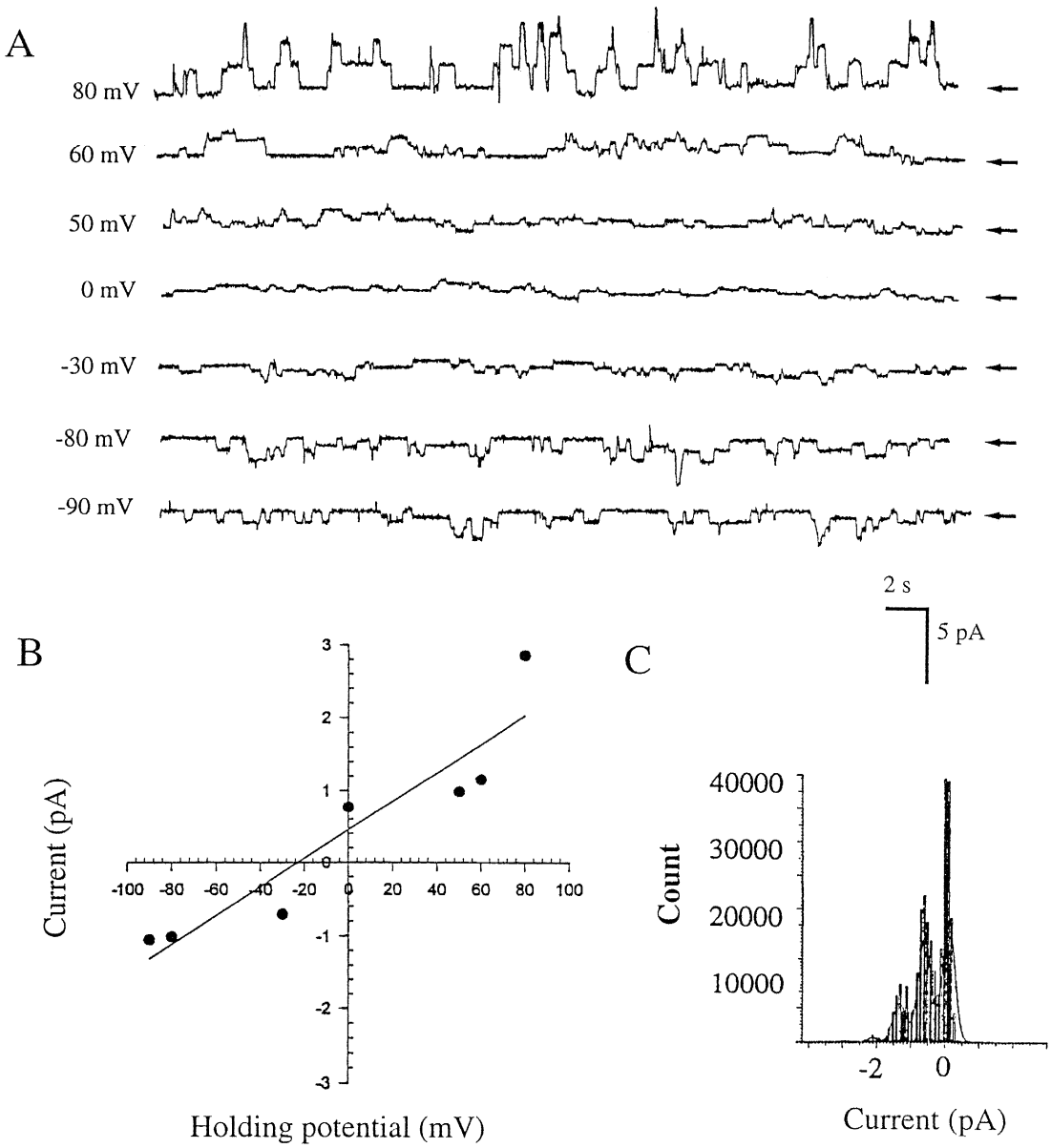

Fig. 2. Another type of single channel currents observed from rat sperm in KCl solutions. (A) Channel events observed at seven different holding voltages with arrows indicating the closed state. (B) Corresponding current–voltage relationship. (C) Current amplitude histograms obtained at −80 mV. The conductance was 19.6 pS and reversal potential was −14 mV. The concentration gradient was similar to that in Fig. 1.

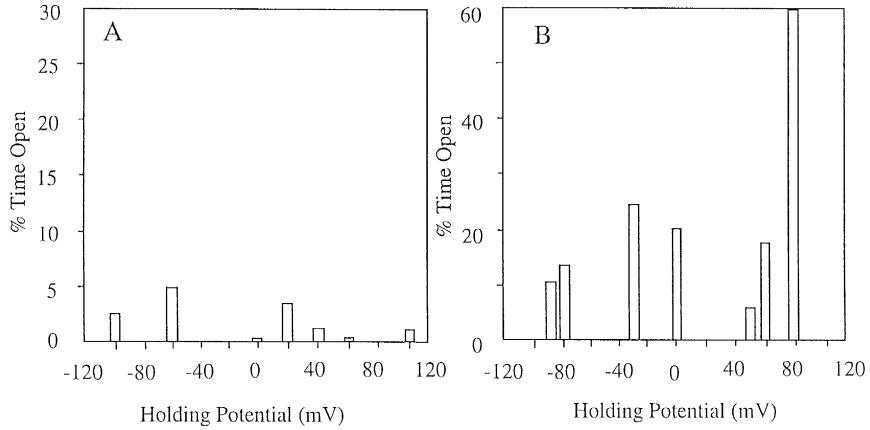

Fig. 3. Percentage of channel open time at indicated holding potentials for channels observed in bilayers shown in Fig. 1 (A) and Fig. 2 (B). There was no apparent voltage dependence.

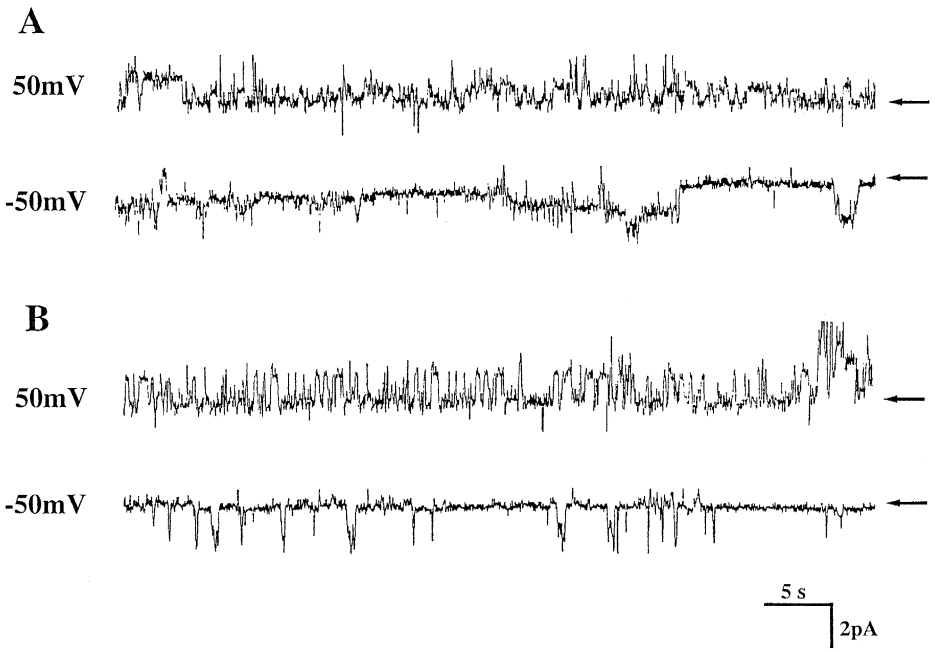

Fig. 4. Channel events observed from rat sperm in KCl (A) and in mixture of KCl and NaCl (B). Single channel currents at 50 and −50 mV were first recorded in KCl(300/100 mM). After addition of 200 mM (final concentration) of NaCl to the *cis* compartment, similar channel activity was observed.

Fig. 5. Sensitivity to K^+ channel blocker, TEA. Currents observed from rat sperm at 40 mV in KCl (300/100 mM) was greatly reduced upon addition of 0.1 mM of TEA to the *cis* compartment. Arrows indicate the closed state.

since the final reversal potential was close to K^+ equilibrium rather than to the new cation equilibrium ($K^+ + Na^+$). The other two cases in the five experiments exhibited a slight anion selectivity judged from the depolarizing reversal potentials observed in KCl solutions (+5 mV).

The K^+ channel blocker, TEA (0.1 mM), was also used to identify the above mentioned channel. In two

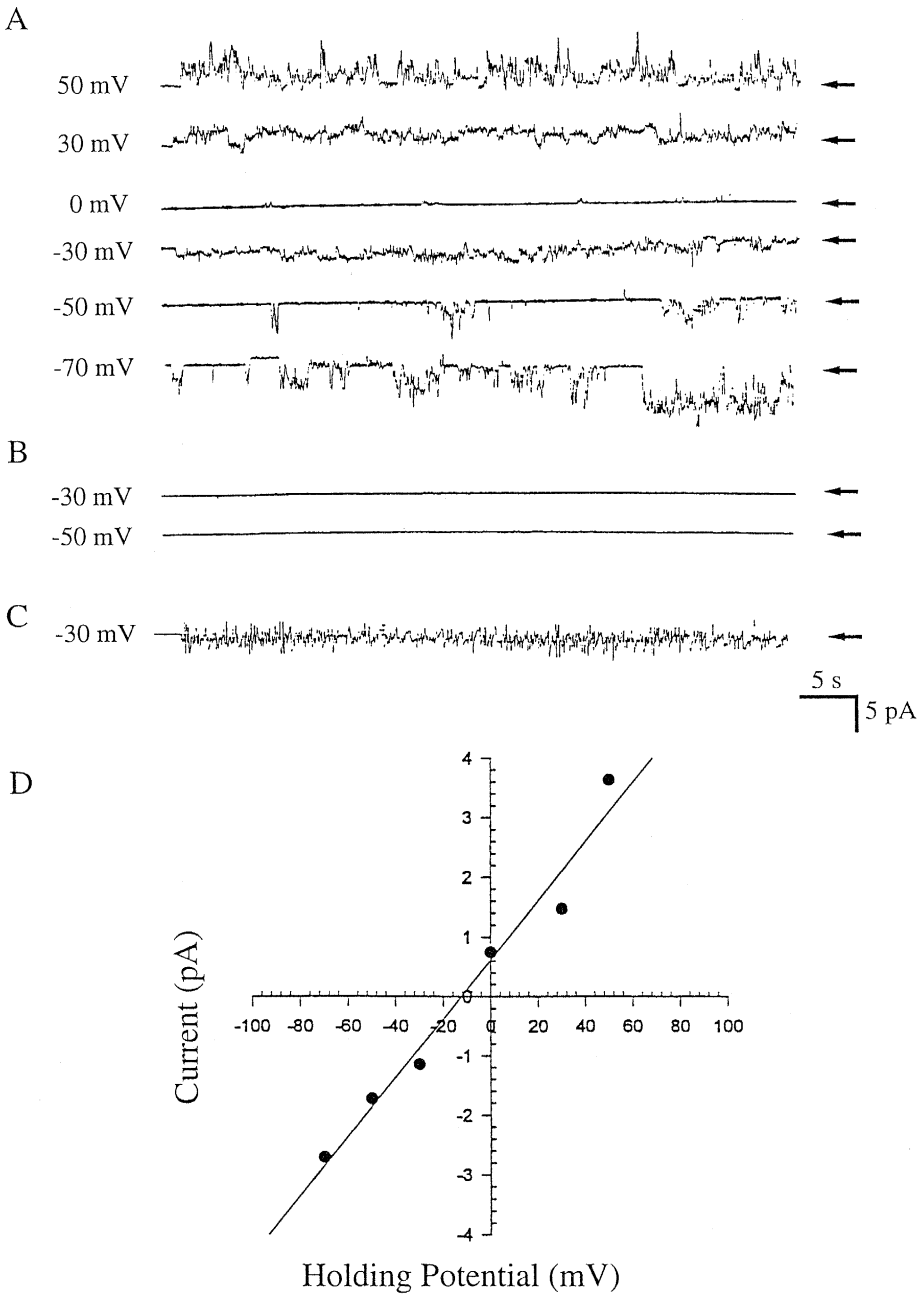

Fig. 6. Demonstration of Na^+-selective channels in rat sperm. (A) Channel events observed at six different holding voltages in NaCl (200/100 mM). (B) No current was observed when bathing solutions were changed to KCl via perfusion. (C) Reappearance of channel activity upon reperfusion of NaCl. (D) Current–voltage relationship for channel events observed in (A). The conductance was 49 pS and reversal potential was −16 mV.

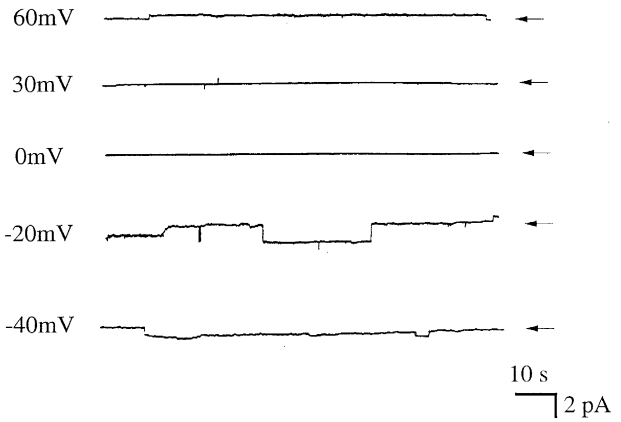

Fig. 7. Anion selective channel observed from rat sperm in NMDG-Cl solutions (200/100 mM). Channel events observed at five different holding voltages exhibited slow open and closed kinetics. Note the time scale is 10 s.

successful experiments, TEA suppressed channel activity observed in either KCl solutions or in mixed KCl and NaCl solutions. As shown in Fig. 5, channel activity detected at 40 mV was greatly, but not entirely, reduced by the treatment with TEA indicating the presence of TEA-sensitive K^+ channels in rat sperm.

3.2. Na^+ channels

Experiments were also performed where NaCl instead of KCl solutions were used. A gradient of 200/100 mM NaCl was used because it was difficult to obtain stable bilayer membranes in 300/100 mM NaCl. The observed reversal potentials in two successful experiments were -18 and -19 mV, giving rise to a permeability ratio of Na^+ to Cl^- greater than 13. In a total of three experiments, single unit conductance was measured ranging from 52 to 184 pS (109 ± 47 pS, $n = 3$), significantly larger than that found in KCl solutions ($P < 0.05$). We were able, with very limited success, to change bath solutions, from NaCl to KCl then back to NaCl again. As shown in Fig. 6, channel activity was only observed in NaCl but not KCl solutions, indicating a Na-selective nature of this channel.

3.3. Anion channels

The residual channel activity observed after addition of TEA and the occasionally observed slightly anion-selective channel events in KCl solutions prompted us to investigate further the anion-selective channel in rat sperm. Impermeant NMDG was used to replace permeant cations leaving Cl^- as the major permeant species in the bathing solutions so that any anion channels which might be masked by high conductance cation channels observed in KCl or NaCl solutions could be revealed. When bathed in NMDG-Cl solutions, bilayers formed were highly unstable and the successful rate of observing channels was

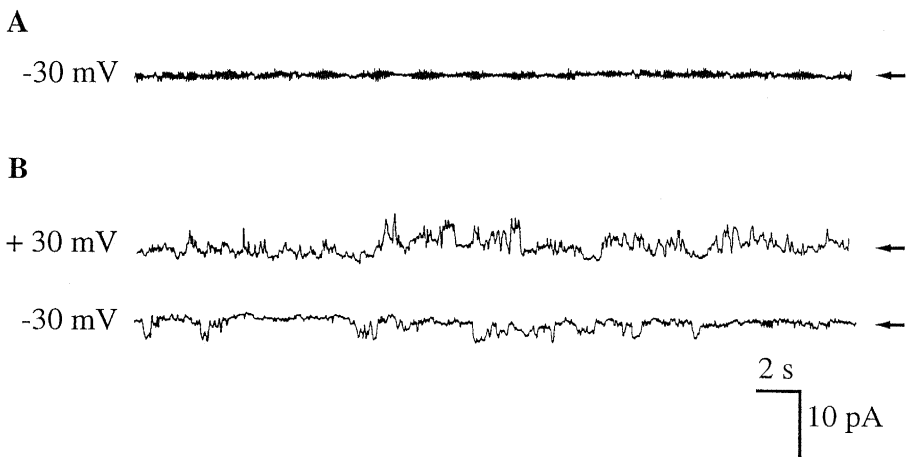

Fig. 8. Demonstration of Ca^{2+} permeability in rat sperm plasma membrane. (A) No channel events were observed when the bilayer was held at -30 mV, the reversal potential for K^+ (small current fluctuation was due to noise). (B) After addition of 100 mM Ca^{2+} to the *cis* compartment, channel events were observed at both 30 mV and -30 mV, reversal potential for Cl^- and K^+, respectively.

much lower probably due to the difficulty of inserting channels to the bilayers in these solutions. In a total of 10 attempts, only two experiments were successful. As shown in Fig. 7, the channel observed in NMDG-Cl solutions had much longer open and closed times as compared to that observed in KCl and NaCl solutions. The conductance was also smaller, about 15 pS.

3.4. Ca^{2+} permeability

Since changes in Ca^{2+} flux had been reported to be associated with acrosome reaction, it was of interest to identify the Ca^{2+} channel or channels that were permeable to Ca^{2+}. We tried to examine channel activity in $CaCl_2$ solutions. The channel events thus found exhibited depolarizing reversal potentials, +5 to +19 mV, indicating slightly higher selectivity for anion over cation. This could be due to the activation of anion channel by high Ca^{2+} present in the solutions, which could mask the cation channel interested. We went back to do experiments in KCl solutions, holding the bilayers at −30 mV, theoretical K^+ equilibrium potential, at which no channel activity was observed initially as shown in Fig. 8. After addition of Ca^{2+} (100 mM final concentration) to the *trans* compartment, channel activity started to appear at the equilibrium potential for K^+ ($n = 6$). To ensure that the channel events observed were not due to activation of anion channel upon addition of Ca^{2+}, channel activity at +30 mV (Cl^- equilibrium) after addition of Ca^{2+} was also examined. The current observed at both −30 and +30 mV could not have been due to either K^+ or Cl^- current, but rather, must have been due to Ca^{2+} current.

3.5. Channels observed in plasma membrane of human spermatozoa

No ion channel study on human spermatozoa had been reported previously. In the present study we tried to investigate channel populations in human sperm. The successful rate in detecting channel activity from human sperm plasma membrane appeared to be greater, 60% as compared to that obtained from rat sperm, 30%. With limited supply of human samples, data obtained from human sperm, which were from subjects of proven fertility, were far less than that obtained from rat sperm. The channel populations observed might not be representative, but nevertheless, valuable. In four experiments performed in 200/100 KCl solutions, two cases clearly showed cation-selective characteristics with an averaged P_K/P_{Cl} ratio of greater than 10 (Fig. 9). The single channel conductances thus observed were 29 and 65 pS, close to the values found in 20% of the samples from rat sperm under the same condition. On the other hand, two experiments demonstrated strong anion selectivity (P_K/P_{Cl}, 0.38). The anionic conductances observed in two successful preparations of

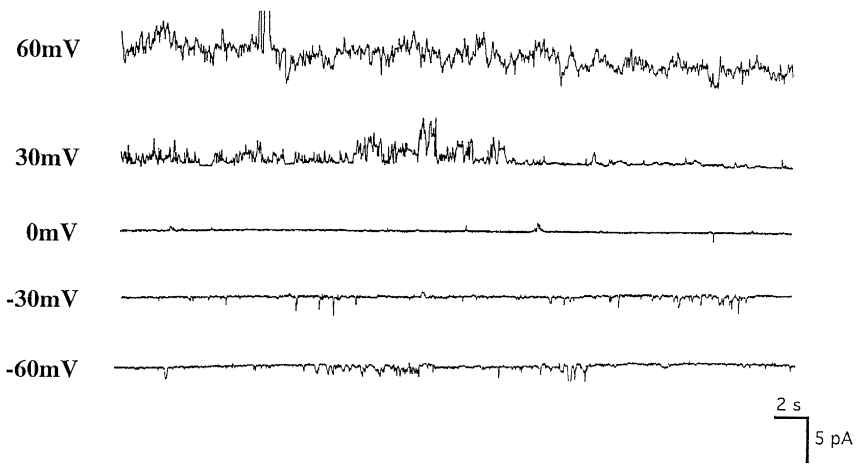

Fig. 9. Single channel currents observed from human sperm in KCl solutions at five different holding voltages. The concentration gradient was 200 (*cis*) to 100 (*trans*) mM KCl.

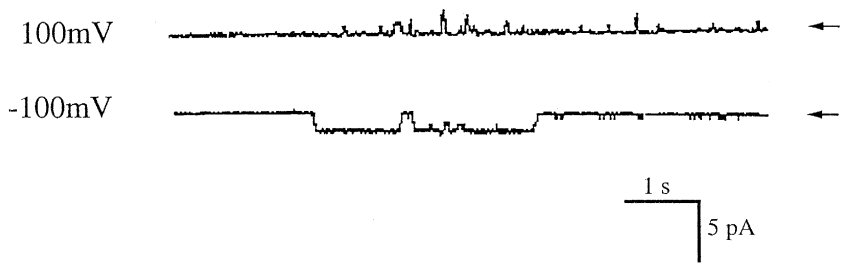

Fig. 10. Anion selective channel observed from human sperm in NMDG-Cl solutions (300/100 mM) at −100 and 100 mV exhibiting slow open and closed kinetics.

human sperm were 8.2 and 19.8 pS, which were similar to that observed in rat sperm, 15 pS. Similar to that observed in rat sperm, experiments using NMDG-Cl solutions ($n = 6$) revealed that the anion channel in human sperm exhibited a slower open and closed kinetics (Fig. 10) as compared to that observed for cation channels (Fig. 9).

4. Discussion

Characterization of sperm ion channels, which are important for sperm functions, has been limited by their small size. Reconstitution of ion channels in planar bilayer from sperm plasma membrane has provided a useful method to investigate the electrophysiological properties of spermatozoa. A number of studies using the planar bilayer technique to study ion channels in sea urchin, mouse and boar spermatozoa have been reported [7–11]. However, very few channel types have been documented. The present study has shown the presence of different types of cation ion channels as well as anion channels in both rat and human spermatozoa.

Similar to the findings from a previous study on plasma membranes of boar spermatozoa [9], we have found in the present study that the channels reconstituted from rat sperm membranes were mostly cation-selective when KCl solutions were used. The present study has found no apparent dependence of channel open time on voltage as reported in boar sperm [9]. However, single unit conductances of the channels found in rat sperm were much smaller than that found in boar sperm, 24 pS as compare to >100 pS. Among the rat sperm channels observed in KCl solutions, some were subject to further characterization by adding NaCl solution to the *cis* compartment. The channel activity and reversal potentials observed in KCl were not greatly affected by addition of NaCl suggesting the presence of K^+-selective channels in the bilayers examined. The K^+-selective nature of the channels was also indicated by its sensitivity to K^+ channel blocker, TEA. Although reconstituted sperm K^+ channels have not been reported in any bilayer study, activation of K^+ channels by the egg peptide speract has been shown in patch-clamped hypoosmotically swollen intact sea urchin sperm [15]. Similar to the present finding in rat sperm, the speract-activated K^+ channels in intact sea urchin sperm also exhibit TEA sensitivity. Changes in membrane potential and intracellular Ca^{2+}, as well as increases in flagellar responses, have also been observed in sea urchin sperm upon activation by speract. A mechanism of sperm chemotaxis by which egg peptides control intracellular Ca^{2+}, via activation of K^+ channels and changes in membrane potential, to regulate flagellar responses has been put forward [16]. These coordinated responses may enable sperm to sense the alterations in egg peptide concentration presumably encountered as it approaches the egg, and to change its swimming pattern around the egg, thereby, enhancing fertilization. It remains to be elucidated whether a similar mechanism would be used by mammalian sperm. Based on the present data, a similar sperm chemotaxis mechanism in mammalian sperm seems possible since the key component, K^+ channels, which exhibits similar sensitivity to TEA as that observed in sea urchin sperm, are found to be present in rat sperm.

It has been established that an elevation of intracellular Ca^{2+}, which is dependent on external Ca^{2+}, is required for sperm acrosome reaction to occur [5].

Efforts have been made in the present study to identify the ion channel responsible for Ca^{2+} entry in rat sperm. Two approaches were used. We first examined channel activity in the presence of a Ca^{2+} gradient across the bilayer. We encountered the same problem as that observed in the study of boar sperm [9]. Experiments were much less successful in the presence of divalent ions than with monovalent ions. The limited data that we did obtain under this condition revealed a selectivity for anion over Ca^{2+}, perhaps due to the activation of a Ca^{2+}-dependent anion channel. We then used another approach to assess divalent permeability. Experiments were performed in KCl solutions, voltage was held at a value close to the reversal potential for K^+ or Cl^-, at which no channel activity was first observed. Upon addition of Ca^{2+}, channel events started to appear indicating permeability to Ca^{2+}. The same approach has been used in the study for boar sperm [9]. The authors of that study concluded that the channel observed in their study was a non-selective cation channel which conducted K^+, Na^+, Cs^+, Ca^{2+} and Ba^{2+}. Since we could only observed divalent permeability at two reversal potentials and no detail characterization of the responsible channel was performed, we could not distinguish whether the same channel shown to be permeable to K^+ was able to conduct Ca^{2+} as well, or a Ca^{2+}-selective channel was indeed responsible for conducting Ca^{2+} current. Further experiments such as examining the sensitivity of channel activity to specific Ca^{2+} channel blockers are needed to characterize the channel involved. This approach has been used recently to characterize Ca^{2+}-selective channels in boar sperm plasma membrane [11]. It is also interesting to note that a high-conductance voltage-dependent Ca^{2+} channel has been found in sea urchin and mouse spermatozoa [10].

Although the cation channel previously observed in boar sperm plasma membrane has been shown to be permeable to Na^+, the channel was said to be non-selective based on the observation that channel activity could be detected in various cation-containing solutions [9]. In the present study, cation-selective channel activity was indeed observed in different bilayers either bathed in KCl or NaCl solutions. However, detecting channel activity from different bilayers in KCl or NaCl solutions does not necessarily indicate a channel with non-selective nature since the ion channel actually being incorporated into a bilayer from one preparation may not be the same as that incorporated into another. Therefore, it is possible that different channels with different selectivities could be observed in different preparations. In the present study, the presence of a Na^+-selective channel in rat sperm plasma membrane was indicated by ion substitution experiment in which channel activity initially observed in NaCl solution disappeared upon perfusion with KCl and re-appeared when NaCl solutions were again introduced. The role of this highly Na^+-selective channel in sperm function is unclear. It has been observed that activation of K^+ channels by egg peptide speract caused a hyperpolarization of membrane potential in sea urchin sperm which was followed by repolarization of the membrane [15]. It was suggested that repolarization had a different ionic basis other than K^+ since change in extracellular Na^+ was shown to promote such repolarization. Therefore, in addition to K^+ channels, the Na^+ channel observed in the present study could also be involved in the mechanism of sperm chemotaxis described earlier as part of the coordinated responses in the initiation of sperm and egg interaction. Further study investigating the voltage dependence of the gating properties of the Na^+ channel would be of interest since it would be desirable if the Na^+ channel could be activated by membrane hyperpolarization as a subsequent event to K^+ channel activation by speract.

The present study has also provided strong evidence for the presence of anion channel in rat sperm plasma membrane. It is well documented that cAMP is involved in acrosome reaction [17] and that bicarbonate activates sperm adenylate cyclase in a number of species [18–20]. The involvement of a bicarbonate transport system in sperm acrosome reaction has been demonstrated indirectly by inhibition of acrosome reaction with anion channel blockers [13,21]. As far as we are aware no sperm anion channels have been described in any species. In the present study we did observe anion-selective channels. First, a small percentage (13%) of the channels observed in KCl solutions exhibited a P_K/P_{Cl} ratio of less than 1, indicating these channels are more selective for anions over cations. Secondly, channel activity was also observed in bathing solutions containing impermeant cation, $NMDG^+$. The current observed under this condition

should be largely due to Cl^- permeability through anion channels. These channels exhibited much slower open and closed kinetics as compared to that of the other cation channels observed in the present study. However, further study is needed to establish the sensitivity of the anion channel to various anion channel blockers. It would be of interest to compare its permeability to bicarbonate with that to Cl^-.

This study is the first to report observation of channel activity in human sperm plasma membrane. Although we have not been able to study human sperm channels to the same extent as that for the rat sperm due to limited supply of human sperm, our study did indicate the presence of both cation and anion channels in human sperm. Similar to the findings from rat sperm, both cation and anion selective channel events were observed in KCl solutions, with an apparently higher percentage of anion channel being observed in human sperm (50%) than that observed in rat sperm (13%). Since the total number of experiments in human sperm is far less than that in rat sperm, a comparison of distribution of channel population between the two based on the present data could not be firmly established. The single unit conductance for the cation channel observed in human sperm, >30 pS, is similar to the values found in 20% of rat sperm cation channels observed under the same condition. The single unit conductance for human sperm anion channel observed in KCl solutions is similar to that found in rat sperm. Detail study of the anion channel using NMDG-Cl solutions indicates that both anion channels found in rat and human sperm exhibited very slow channel open and closed kinetics. It is uncertain whether the slow kinetics is an intrinsic channel property or it is induced by the NMDG-containing solutions. It is interesting to note that when purified cystic fibrosis transmembrane conductance regulator, shown to be a Cl^- channel, was reconstituted into lipid bilayer, it also exhibited a very slow open and closed kinetics even in KCl solutions [22].

Due to enormous technical difficulties, such as bilayer stability and noise level encountered in the present study, the channels presented here could not be characterized to the extent as one would like to. Especially, since high filter was used in order to lower the noise level, some channel events were undoubtedly altered or missed, resulting in underestimation of total channel activities. It should also be addressed that the present results were obtained from total sperm membrane preparations, which include channel populations in the sperm head and tail. Therefore, we are not able to correlate the channel properties with specialized functions of the sperm head and tail. In addition, since we have no control over the process of a particular channel protein being incorporated into a lipid bilayer, this also makes it difficult to systematically characterize individual channels. It would be desirable to prepare plasma membrane from either the sperm head or tail so that channel populations in each preparation may be reduced to facilitate further characterization of these channels.

The present study has revealed similarities between the channels observed in rat and human sperm suggesting that both species may possess similar cation and anion channels. The presence of different types of cation and anion channels in both rat and human sperm suggests that these channels may play different roles in mammalian sperm functions. Further study to compare ion channels in normal and pathologic human sperm may reveal the ionic basis underlying some cases of male infertility.

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