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K_{ATP} channels in mouse spermatogenic cells and sperm, and their role in capacitation

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Abstract

Mammalian sperm must undergo a series of physiological changes after leaving the testis to become competent for fertilization. These changes, collectively known as capacitation, occur in the female reproductive tract where the sperm plasma membrane is modified in terms of its components and ionic permeability. Among other events, mouse sperm capacitation leads to an increase in the intracellular Ca^{2+} and pH as well as to a hyperpolarization of the membrane potential. It is well known that ion channels play a crucial role in these events, though the molecular identity of the particular channels involved in capacitation is poorly defined. In the present work, we report the identification and potential functional role of K_{ATP} channels in mouse spermatogenic cells and sperm. By using whole-cell patch clamp recordings in mouse spermatogenic cells, we found K^+ inwardly rectifying (K_{ir}) currents that are sensitive to Ba^{2+}, glucose and the sulfonylureas (tolbutamide and glibenclamide) that block K_{ATP} channels. The presence of these channels was confirmed using inhibitors of the ATP synthesis and K_{ATP} channel activators. Furthermore, RT-PCR assays allowed us to detect transcripts for the K_{ATP} subunits SUR1, SUR2, $K_{ir}6.1$ and $K_{ir}6.2$ in total RNA from elongated spermatids. In addition, immunoconfocal microscopy revealed the presence of these K_{ATP} subunits in mouse spermatogenic cells and sperm. Notably, incubation of sperm with tolbutamide during capacitation abolished hyperpolarization and significantly decreased the percentage of AR in a dose-dependent fashion. Together, our results provide evidence for the presence of K_{ATP} channels in mouse spermatogenic cells and sperm and disclose the contribution of these channels to the capacitation-associated hyperpolarization.

Keywords: Sperm; Mammalian sperm; Spermatogenic cells; Ion channels; Sperm capacitation; Sperm acrosome reaction; K^+ channels; KATP channels; Inward-rectifying channels; SUR subunits

Introduction

Mammalian sperm are unable to fertilize eggs immediately after ejaculation. They acquire fertilization capacity after residing in the female tract for a finite period of time. The physiological changes occurring in the female reproductive tract rendering sperm able to fertilize constitute the process of "capacitation". Capacitation is associated with changes in tyrosine (tyr) phosphorylation of a subset of sperm proteins (Visconti et al., 1995a, 2002; Baker et al., 2004). Both capacitation and tyr-phosphorylation have been shown to be regulated by a cAMP-dependent pathway involving protein kinase A (PKA) (Visconti et al., 1995b; Galantino-Homer et al., 1997; Nolan et al., 2004). In addition, capacitation in mammalian sperm is accompanied by a hyperpolarization of the membrane potential (Em) (Zeng et al., 1995; Arnoult et al., 1999) and increases in intracellular pH (pHi) (Parrish et al., 1989; Zeng et al., 1996; Galantino-Homer et al., 2004) and Ca^{2+} ($[Ca^{2+}]i$) (Baldi et al., 1991; DasGupta et al., 1993). Mouse spermatogenic cells and mature sperm have voltage dependent Ca^{2+} (Ca_V) channels of the T-type (Ca_V3) that participate in the acrosome reaction (AR) (Arnoult et al.,

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1996; Lievano et al., 1996). A sperm hyperpolarization associated to capacitation could be important to remove inactivation from T-channels, driving them from an inactive state to a closed state from which they could be activated by zona pellucida (ZP) to trigger AR (Darszon et al., 2001; Florman et al., 1998).

The hyperpolarization that accompanies mouse sperm capacitation is influenced by external K^+ and K^+ channel blockers. It is thus thought that a K^+ permeability contributes to this process (Zeng et al., 1995; Arnoult et al., 1999; Muñoz-Garay et al., 2001). Molecular and functional studies of K^+ channels in mammalian male germ cells and mature sperm have indicated the presence of voltage-gated (Hagiwara and Kawa, 1984; Schreiber et al., 1998; Wu et al., 1998; Jacob et al., 2000; Felix et al., 2002) and inward rectifier (K_{ir}) channels (Salvatore et al., 1999; Muñoz-Garay et al., 2001; Felix et al., 2002). However, little is known about the regulation of the capacitation-associated hyperpolarization.

It was suggested that a pH-regulated K^+ channel with strong inward rectification contributes to the capacitation-associated hyperpolarization. The addition of Ba^{2+}, a K_{ir} channel blocker, eliminated inwardly rectifying K^+ currents in spermatogenic cells and prevented both the development of membrane hyperpolarization and partially the sperm AR (Muñoz-Garay et al., 2001). Therefore, an elevation in pHi, as it occurs during capacitation, could increase the open probability of these channels (Muñoz-Garay et al., 2001) driving Em towards the K^+ equilibrium potential and hyperpolarizing sperm. Considering that the K_{ir} family has 7 subgroups (1–7) at the present time (Coetzee et al., 1999; Bichet et al., 2003), it seemed important to identify the molecular identity of those present in spermatogenic cells and their possible influence on sperm physiology, particularly during capacitation.

Here, we report what is to our knowledge the first functional evidence for the presence of a weak inwardly rectifying K_{ATP} channel in spermatogenic cells. These channels are ubiquitously expressed in a variety of cell types, including pancreatic β cells, cardiac myocytes, skeletal muscle cells, neurons and pituitary cells (Ashcroft and Gribble, 1998). Molecular studies indicate that K_{ATP} channels are double tetramers formed from four K_{ir} channel (6.1 and/or 6.2) and four sulfonylurea receptor (SUR1, SUR2 A and B) subunits. When the ATP/ADP ratio rises in the cytoplasm, K_{ATP} channels close and the cell depolarizes. It is well known that in pancreatic β-cells this mechanism regulates insulin release (Nichols and Koster, 2002). In the current work we report K_{ir} currents from mouse spermatogenic cells that are sensitive to Ba^{2+}, glucose and sulfonylureas (tolbutamide and glibenclamide). Notably, these currents were augmented by K_{ATP} openers (pinacidil and diazoxide) as well as by agents that inhibit the production of ATP (2-deoxyglucose and 2,4-dinitrophenol). Furthermore, RNA messengers for SUR1, SUR2, K_{ir}6.1 and 6.2 were found in mouse spermatogenic cells, and specific antibodies to these K_{ATP} subunits detected all the proteins in this cells and mature sperm. The possible role of these channels during sperm capacitation is discussed.

Materials and methods

Chemicals

Tolbutamide, glibenclamide, 2-deoxyglucose, 2,4-dinitrophenol, diazoxide and pinacidil were purchased from Sigma-Aldrich (St. Louis, MO). All other reagents were analytical grade.

Cell preparation

Spermatogenic cells for electrophysiological recording were obtained as previously described (Muñoz-Garay et al., 2001). Briefly, testes from adult CD1 mice were excised and suspended in ice-cold dissociation solution containing (in mM) 130 NaCl; 3 KCl; 10 $CaCl_2$, 2 $MgCl_2$; 1 $NaHCO_3$; 0.5 NaH_2PO_4; 5 HEPES; 10 glucose (pH 7.4/NaOH). The tunica albuginea was removed and the seminiferous tubules separated. Tubules were dispersed into individual cells or symplasts using Pasteur pipettes. The cells were stored at 8°C until assayed. Subsequently, 100 μl aliquots of cell suspension were dispensed into a recording chamber (500-μl total volume) and subjected to electrophysiological recording.

Electrophysiology

K^+ currents were recorded according to the whole-cell patch-clamp technique (Marty and Neher, 1995). All recordings were performed at room temperature using an Axopatch 200A patch-clamp amplifier (Axon Instruments, Foster City, CA) and 2- to 4-MΩ micropipettes. Cells were clamped at a holding potential of 0 mV and currents were evoked by 200 ms voltage steps (0.5 Hz) to test potentials ranging from −100 to +40 mV. Pulse protocols, data capture, and analysis of recordings were performed using pCLAMP software (Axon). Current records were captured on-line and digitized at a sampling rate of 5–10 kHz following filtering of the currents (2 kHz; internal 4-pole Bessel filter) using a personal computer attached to a DigiData 1200 interface (Axon). To record inwardly rectifying K^+ currents, cells were bathed in a solution containing (in mM): 150 K-methanesulfonate ($MeSO_3$); 6.5 $CaCl_2$; 1 $MgCl_2$; 10 HEPES; 10 Glucose (pH 7.4/KOH). The internal solution consisted of (mM): 122 K-$MeSO_3$; 20 KF; 8 KCl; 2.5 $CaCl_2$; 1 $MgCl_2$; 5 EGTA; 10 HEPES; (pH 7.3/KOH). Tolbutamide, glibenclamide and pinacidil were prepared as 100 mM stock solutions in DMSO. Ba^{2+}, 2-deoxyglucose and 2,4-dinitrophenol were made at 100 mM in external solution and diazoxide 50 mM in 0.1 N NaOH. These compounds were diluted in external solution to the indicated concentration and perfused by gravity at ~1 ml/min. Controls with the highest DMSO volume used in the experiments with inhibitors or activators had no effect on the recorded currents.

Assay for capacitation and acrosome reaction

Caudal epididymal mouse sperm were collected from CD1 mice and placed in capped 1.5-ml microcentrifuge tubes containing medium 199 supplemented with BSA (0.4% wt/vol), Na^+ pyruvate (30 mg/l) and $NaHCO_3$ (2.2 g/l) at 37°C ($4–5 \times 10^6$ cells/ml). The swim-up method (Henkel and Schill, 2003) was used to separate sperm with >90% motility. The sperm suspension was incubated for 10 min and the top ~1 ml separated and capacitated incubating it 30 min at 37°C (Visconti et al., 1999). AR was induced after capacitation in a 30 μl aliquot by adding 5 eq/μl *zona pellucida*. The percentage of AR, which also measures capacitation indirectly, was determined 30 min later, after adding an equal volume of fixative (10% formaldehyde in phosphate-buffered saline). Following fixation, 10 μl aliquots of the sperm suspension were spread onto glass slides and air-dried. The slides were stained with 0.22% Coomassie Blue G-250 in 50% methanol and 10% glacial acetic acid for ~5 min, rinsed and mounted with 50% (v/v) glycerol in phosphate-buffered saline (Muñoz-Garay et al., 2001). At least 100 sperm were assayed per experimental condition to calculate the percentage of AR.

Measurement of membrane potential

Mature sperm were capacitated in vitro as mentioned above. After a 30-min incubation, the potential sensitive dye 3,3′-dipropylthiocarbocyanine iodide