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Pharmacology of hSlo3 channels and their contribution in the capacitation-associated hyperpolarization of human sperm

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ABSTRACT

Slo3 channels (mSlo3) primarily mediate mouse sperm K^+ currents and are essential for the capacitation-associated hyperpolarization (CAH). Whether Slo3 and/or Slo1, two Slo family K^+ channels are functionally expressed in human sperm is controversial. Our recent pharmacological studies of the human sperm CAH suggested the participation of both. Lack of a detailed pharmacology of heterologously expressed human Slo3 (hSlo3) prevented precisely identifying the K^+ channel(s) involved. In the present report, we compare the pharmacological profile of expressed hSlo3 in CHO cells with that of the CAH to advance this matter. Whole-cell patch-clamp recordings showed that hSlo3 currents are inhibited: significantly by progesterone, Ba^{2+} and quinidine; partially by Penitrem A and Charybdotoxin; and poorly by Iberiotoxin and Slotoxin. Surprisingly, hSlo3 currents were resistant to Clofilium and 60 mM TEA^+ which inhibit mSlo3. Pharmacological comparison of the CAH and hSlo3 profiles indicates in addition to hSlo3, other K^+ channels, possibly Slo1, may participate in CAH. The pharmacological profile of heterologously expressed hSlo3 channels differs from that of mSlo3 K^+ channels, consistent with species-specific differences observed among other sperm ion channels. While the pharmacological correlation analysis of the hSlo3 currents and the CAH confirmed the participation of hSlo3 channels, it suggests that additional K^+ channels may be involved, in particular Slo1 channels.

1. Introduction

In mouse sperm, membrane hyperpolarization during capacitation is necessary and sufficient for the acrosome reaction to take place [1]. Sperm from Slo3 K^+ channel null mice do not hyperpolarize during capacitation, suggesting that this channel is fundamental in the process [2,3]. Recently, using flow cytometry, we reported that human spermatozoa also undergo capacitation-associated hyperpolarization (CAH), and that it is sensitive to different K^+ channel antagonists [4]. Based on the known pharmacological profiles for K^+ currents determined either directly on mouse sperm or on heterologously expressed mSlo3, mSlo1 and hSlo1 channels, we suggested the participation of both Slo1 and Slo3 in human CAH. The molecular identity of the K^+ channels present in human sperm remains controversial [5–7], given that the K^+ currents measured in human sperm exhibit biophysical and pharmacological properties that lie in between those reported for mSlo1 and mSlo3 channels. It is known that homologous channels from mouse and human sperm can have considerably different properties; therefore, knowledge of the specific pharmacological profile of hSlo3 is of utmost importance.

In the present report, we showed that the pharmacological profile of heterologously expressed Slo3 channels differs in a species-specific manner. In addition, the pharmacological correlation analysis of the hSlo3 currents and the CAH confirmed the

List of abbreviations: CAH, capacitated-associated hyperpolarization; hSlo3, human Slo3 K^+ channel; $DiSC_3(5)$, 3,3′-Dipropylthiadicarbocyanine Iodide; PI, propidium iodide; CbTX, Charybdotoxin; IbTX, Iberiotoxin; SloTX, Slotoxin; TEA^+, Tetraethylamonia; LRRC52, leucine rich repeated containing protein 52; Pg, progesterone.

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participation of hSlo3 channels, it suggests that additional K^+ channels may be involved, probably Slo1 channels.

2. Materials and methods

2.1. Constructs preparation

hLRRC52 (γ2 subunit) was subcloned from the pCR4TOPO-hLRRC52 construct (Clone Id 40147005, purchased from Open Biosystems (Thermo Fisher Scientific; Pittsburg, PA)) into the mammalian expression vector pIRES-EYFP (BD Biosciences Clontech; Mountain View, USA). Subcloning was performed by adding NotI (5′ TAC GGC GGC CGC ATG TCC CTT 3′) and EcoRI (5′ GCC AAA GAA TTC AAA CTA AAT AAG CTG AGG GAA 3′) sites through PCR. The pcDNA3.1(+)-hSlo3 and pcDNA3.1(−)-GFP constructs was the generous gift of Benjamin Kaupp (Center of Advanced European Studies and Research; Bonn, Germany) and Ricardo Félix (CINVESTAV-IPN, México), respectively.

2.2. Cell culture and transient expression of hSlo3 channels

CHO cells were seeded in plastic Petri dishes using Advanced Dulbecco's modified Eagle's medium (Gibco, Life Technologies; Grand Island, USA) supplemented with 1% antibiotics and 10% bovine fetal serum (Gibco, Life Technologies; Grand Island, USA); they were grown in a humidity-controlled incubator at 37 °C and 5% CO_2 (VWR Scientific 2100) to 50% of confluence. At this stage, CHO cells were co-transfected using Lipofectamine (Invitrogen, Life Technologies; Carlsbad, USA) with the following constructs: hSlo3, hLRRC52 and GFP as reporter in a 1:1:0.3 mass ratio. Transfected CHO cells were used 48 h *post*-transfection.

2.3. Electrophysiology

Disaggregated CHO cells were placed on the stage of an inverted microscope (Diaphot 300, Nikon). Membrane currents were recorded with an Axopatch 200B amplifier (Molecular Devices, Sunnyvale, USA) at a sampling frequency of 20 kHz (50 μs) and filtered at 5 kHz (four-pole Bessel filter). Ion currents were digitized using a Digidata 1550 interface (Molecular Devices, Sunnyvale, USA) and analyzed with the pCLAMP 10.5 and SigmaPlot software suites, respectively. All experiments were carried out at room temperature (~22 °C) and the holding potential (HP) was −100 mV. The recording extracellular solution contained (in mM): 140 NaCl, 5.4 KCl, 1 $MgCl_2$, 1.8 $CaCl_2$, 10 glucose, and 5 N-2-hydroxyethylpiperazine-W-2-ethanesulfonic acid (HEPES). Intracellular solution contained (in mM): 130 K-Aspartate, 10 NaCl, 0.5 $CaCl_2$ and 10 HEPES. The osmolality of the external and internal solutions was adjusted to 310 and 290 mosmol/kg with dextrose, respectively. In both cases pH was adjusted to 7.4 and 7.3 with NaOH or KOH for external and internal solutions, respectively. Patch pipettes were made from borosilicate glass and were pulled with a laser micropipette puller P-2000 (Sutter Instruments Co; Novato, USA). The typical micropipette electrical resistance was 3–8 MΩ when filled with internal solutions. Cells had an average capacitance of 16 ± 1 pF (n = 25) and input resistances in the range of 8–16 MΩ.

2.4. Human sperm swim-up and capacitation

Briefly, ejaculated samples (obtained by masturbation) fulfilling the parameters established by the World Health Organization laboratory manual were used for the experiments. Motile sperm were obtained as described in Mata-Martinez et al., 2013 [8]. When required, sperm samples were incubated at 37 °C under 5% CO_2 for 13 h to promote capacitation in the presence of 1% Antibiotic-Antimycotic (Gibco, Life technologies; Grand Island, NY). Non-capacitated sperm were incubated the same time but in a media lacking bovine serum albumin. For the pharmacological assays, K^+ channel blockers were incubated during the whole capacitation time.

2.5. Flow cytometry

Sperm plasma membrane potential was monitored using $DiSC_3(5)$, according to Lopez-González et al., 2014 [4]. Briefly, after 13 h incubation under either non-capacitating or capacitating conditions, samples were centrifuged at 750 g for 5 min. Sperm were resuspended in Human Sperm Medium (HSM; 120 mM NaCl, 4 mM KCl, 2 mM $CaCl_2$, 1 mM $MgCl_2$, 30 mM HEPES, 10 mM Na lactate, 5 mM D-glucose, 1 mM Na pyruvate; pH 7.4) and their concentration adjusted to 3×10^6 cells/mL. For each experimental condition to be tested, 500 μL of cell suspension was loaded with fluorescent dyes by incubation with 50 nM $DiSC_3(5)$, during 3 min; and 100 nM propidium iodide (PI) (to monitor viability) for 30 s at room temperature. Data were recorded as individual cellular events using a FACSCanto II™ cytometer (Becton Dickinson; San Jose, USA). Forward scatter (FSC) and side scatter (SSC) data were collected from 20,000 cells per sample. Threshold levels for FSC and SSC were set to exclude signals from cellular debris. Appropriate cytometer settings were selected for $DiSC_3(5)$ and PI using allophycocyanine and PI filters, respectively. The following controls were used to set up compensation parameters: unstained sperm, sperm stained with $DiSC_3(5)$ or PI-stained dead sperm (sperm suspended in 0.1% Triton X-100 in HSM and incubated 10 min at room temperature). Data were analyzed using FACS Diva and FlowJo version 9.3.3 software (Tree Star Inc., Ashland, USA).

2.6. Statistical analysis of data

Statistical analysis was performed using the Sigmaplot 10 and GraphPad Prism 5 programs. Analysis of variance (ANOVA) and Tukey's test for multiple comparisons were used to compare either the percentages of inhibition of the K^+ current amplitude of CHO cells or the CAH inhibition in the presence of different K^+ channel blockers. Data were expressed as the mean ± standard error of the mean (SEM). A $p < 0.05$ was considered significant. Pearson's correlation analysis was used to compare the hSlo3 K^+ currents reduction versus the percentage of CAH inhibition in the presence of different K^+ channel blockers. A $\rho = 0.95$ was considered significant.

3. Results and discussion

3.1. hSlo3 K^+ currents showed a species-specific pharmacological profile

As previously reported, heterologous expression of hSlo3 requires cotransfection with the LRRC52 regulatory subunit (γ2) [7]. Our whole-cell electrophysiological recordings of hSlo3 K^+ currents + LRRC52 (I_{hSlo3}) obtained on acute disaggregated CHO cells showed the classical family of non-inactivating outward currents previously described for hSlo3 channels [7] (Fig. 1A); with a mean current density of 94 ± 10 pA/pF (n = 14 cells). These currents were clearly different from CHO endogenous K^+ currents [9] and from those of CHO cells transfected only with the hLRRC52 subunit (Fig. 1A); which presented a mean current density of 6.5 ± 2 and 24.5 ± 5 pA/pF, respectively. The I–V curve of I_{hSlo3} presented an activation threshold at around −50 mV (Fig. 1K–M, control).

In this report we further explored the pharmacological