Mechanism of inhibition of mouse Slo3 (K\textsubscript{Ca}5.1) potassium channels by quinine, quinidine and barium

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BACKGROUND AND PURPOSE
The Slo3 (K\textsubscript{Ca}5.1) channel is a major component of mammalian KSper (sperm potassium conductance) channels and inhibition of these channels by quinine and barium alters sperm motility. The aim of this investigation was to determine the mechanism by which these drugs inhibit Slo3 channels.

EXPERIMENTAL APPROACH
Mouse (m) Slo3 (K\textsubscript{Ca}5.1) channels or mutant forms were expressed in Xenopus oocytes and currents recorded with 2-electrode voltage-clamp. Gain-of-function mSlo3 mutations were used to explore the state-dependence of the inhibition. The interaction between quinidine and mSlo3 channels was modelled by in silico docking.

KEY RESULTS
Several drugs known to block KSper also affected mSlo3 channels with similar levels of inhibition. The inhibition induced by extracellular barium was prevented by increasing the extracellular potassium concentration. R196Q and F304Y mutations in the mSlo3 voltage sensor and pore, respectively, both increased channel activity. The F304Y mutation did not alter the effects of barium, but increased the potency of inhibition by both quinine and quinidine approximately 10-fold; this effect was not observed with the R196Q mutation.

CONCLUSIONS AND IMPLICATIONS
Block of mSlo3 channels by quinine, quinidine and barium is not state-dependent. Barium inhibits mSlo3 outside the cell by interacting with the selectivity filter, whereas quinine and quinidine act from the inside, by binding in a hydrophobic pocket formed by the S6 segment of each subunit. Furthermore, we propose that the Slo3 channel activation gate lies deep within the pore between F304 in the S6 segment and the selectivity filter.

Abbreviations
KSper, sperm potassium conductance; mSlo3, mouse Slo3 (K\textsubscript{Ca}5.1) channel; WT, wild type
The large-conductance Ca2+-activated K+ channel has recently been shown to have proton channel activity (Lishko et al., 2007) as a result of proton transport by human voltage-gated maxi-K, KCa1.1, and is strongly expressed in testes (Schreiber et al., 1998). The mouse (m) Slo3 (KCa5.1) channel is activated by increased cytosolic pH and not by intracellular Ca2+ (Schreiber et al., 1998; Zhang et al., 2006). In mouse spermatozoa, a Na+-dependent Cl-/HCO3-exchanger is responsible for intracellular alkalinization during capacitation (Zeng et al., 1996), which is thought to activate KSPer and hyperpolarize the cell membrane to more negative potentials. This could increase the driving force for Ca2+ influx through CatSper channels, thereby increasing sperm motility (Navarro et al., 2007), although an indirect link between Slo3 and CatSper function involving H+ transporters has also been proposed (Chavez et al., 2014). Male mice deficient in Slo3 K+ channel subunits (KCNU1 knockout) are infertile and have reduced alkalinization-induced K+ conductance (Santi et al., 2010; Zeng et al., 2011, 2013). In contrast, human spermatozoa undergo cytoplasmic alkalinization during capacitation as a result of proton transport by human voltage-gated proton channel activity (Lishko et al., 2010). Interestingly, the human Slo3 (KCa5.1) channel has recently been shown to be activated by Ca2+ and less reliant on alkalinization, but still a critical component of KSPer channels (Brenker et al., 2014). Given its importance in sperm physiology, human mutations in the gene encoding Slo3, KCNU1, are likely to be linked to male fertility. Hence, sperm ion channels are promising targets for drugs that either suppress or enhance male fertility (Lishko et al., 2012).

Despite the technical difficulties in recording KSPer currents from spermatozoa and the poor heterologous expression of mSlo3 subunits, some of their functional and pharmacological properties have been correlated. Both KSPer and mSlo3 are blocked by Ba2+, exhibit weak sensitivity to TEA− relative to other K+ channels (Schreiber et al., 1998; Navarro et al., 2007; Martinez-Lopez et al., 2009), are enhanced by PKA (Martinez-Lopez et al., 2009), and inhibited by depletion of phosphatidylinositol 4,5-bisphosphate (Tang et al., 2010a). Native KSPer channels are also inhibited by quinine, clofibrate, mibebradil and S-(N-ethyl-N-isopropyl)-amiloride (Navarro et al., 2007). Detailed aspects of the pharmacology of Slo3 channels, including their block by 4-aminopyridine and quinidine, have been investigated by generating and expressing an mSlo3 chimera, MC13, which incorporates transmembrane regions from Slo1 (KCa1.1) channels to improve expression in Xenopus oocytes (Tang et al., 2010b).

The aim of this study was to investigate the effects of inhibitors on full-length mSlo3 channels expressed in Xenopus oocytes. We were particularly interested in the state dependence of block by quinine, quinidine and Ba2+, and in comparing their effects on those reported with native KSPer and the MC13 Slo1/Slo3 chimera (Navarro et al., 2007; Tang et al., 2010b). To explore state dependence, we generated gain-of-function mutants based on mutations previously shown to increase the open probability of Slo1 channels: R196Q is equivalent to an S4 mutation that enhances voltage activation (Diaz et al., 1998), whereas F304Y is analogous to a pore mutation that directly increases open probability (Lippiat et al., 2000). We found that channel inhibition by these substances was not dependent on channel activity; however, the binding site for quinine and quinidine was affected by the F304Y mutation in the mSlo3 pore.

### Methods

#### Molecular biology and oocyte injection

A plasmid containing the full-length mSlo3 sequence was a gift from Dr L. Salkoff (Washington University School of Medicine) and the open-reading frame was subcloned into pBF (Baukrowitz et al., 1999), a vector optimized for in vitro mRNA synthesis and Xenopus oocyte expression. This ion channel subunit also has the designation KCa5.1 (Alexander et al., 2013), but is termed mSlo3 in this paper. The R196Q and F304Y mutations were introduced by Quikchange site-directed mutagenesis (Agilent Technologies, Stockport, UK) and confirmed by automated sequencing. In vitro transcription was carried out using the mMessage mMachine SP6 kit (Ambion, Huntingdon, UK) to produce capped mRNA. Individual oocytes were obtained from humanely killed female Xenopus laevis toads (housed either at the University of Leeds, UK or from the European Xenopus Resource Centre, University of Portsmouth, UK) and were injected with 10–50 ng RNA in a 40 nL volume. Oocytes were incubated for at least 2 days at 18°C in a modified Barth’s solution comprising, in mM, 84 NaCl, 1 KCl, 2.4 NaHCO3, 0.82 MgSO4, 0.41 CaCl2,

### Introduction

A number of substances, including Ba2+ and quinine, affect spermatozoa motility and cell volume regulation by inhibiting K+ channels (Yeung and Cooper, 2001; Yeung et al., 2003; Barfield et al., 2005a,b). Slo3 (KCa5.1) is a strong candidate for the predominant K+ channel in spermatozoa membranes, KSPer (sperm potassium conductance) (Navarro et al., 2007; Santi et al., 2010a; Zeng et al., 2011; 2013; Brenker et al., 2014; Mansell et al., 2014). It is also the closest molecular relative of the large-conductance Ca2+ -activated K+ channel Slo1 (Bkca, maxi-K, Kc5.1), and is strongly expressed in testes (Schreiber et al., 1998). The mouse (m) Slo3 (KCa5.1) channel is activated by increased cytosolic pH and not by intracellular Ca2+ (Schreiber et al., 1998; Zhang et al., 2006). In mouse spermatozoa, a Na+-dependent Cl-/HCO3-exchanger is responsible for intracellular alkalinization during capacitation (Zeng et al., 1996), which is thought to activate KSPer and hyperpolarize the cell membrane to more negative potentials. This could increase the driving force for Ca2+ influx through CatSper channels, thereby increasing sperm motility (Navarro et al., 2007), although an indirect link between Slo3 and CatSper function involving H+ transporters has also been proposed (Chavez et al., 2014). Male mice deficient in Slo3 K+ channel subunits (KCNU1 knockout) are infertile and have reduced alkalinization-induced K+ conductance (Santi et al., 2010; Zeng et al., 2011, 2013). In contrast, human spermatozoa undergo cytoplasmic alkalinization during capacitation as a result of proton transport by human voltage-gated proton channel activity (Lishko et al., 2010). Interestingly, the human Slo3 (KCa5.1) channel has recently been shown to be activated by Ca2+ and less reliant on alkalinization, but still a critical component of KSPer channels (Brenker et al., 2014). Given its importance in sperm physiology, human mutations in the gene encoding Slo3, KCNU1, are likely to be linked to male fertility. Hence, sperm ion channels are promising targets for drugs that either suppress or enhance male fertility (Lishko et al., 2012).

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### Targets

| Targets  |  |
|----------|---|
| KCa1.1 (Slo1; BKca) |  |
| KCa5.1 (Slo3) |  |

### Ligands

| Ligands  |  |
|----------|---|
| Ba2+ (BaCl) |  |
| Mibebradil |  |
| Quinidine |  |

These Tables list key protein targets and ligands in this article which are hyperlinked to corresponding entries in http://www.guidetopharmacology.org, the common portal for data from the IUPHAR/BPS Guide to PHARMACOLOGY (Pawson et al., 2014) and are permanently archived in the Concise Guide to PHARMACOLOGY 2013/14 (Alexander et al., 2013).
0.33 Ca(NO₃)₂, 5 HEPES, adjusted to pH 7.4 with NaOH and supplemented with 100 IU·mL⁻¹ penicillin/streptomycin, 2 mM pyruvate and 50 mg·mL⁻¹ gentamycin. For oocytes injected with mutant Slo3 mRNA, survival was improved by raising the KCl concentration of the Barth’s solution to 23 mM. This brought the resting membrane potential, estimated from the Goldman–Hodgkin–Katz equation, reported oocyte intracellular ion concentrations (Costa et al., 1989), and an increased K⁺ conductance, closer to -30 mV and within the range of that of a non-injected oocyte.

**Electrophysiology**

Microelectrodes were pulled from GC100F borosilicate glass capillaries (Harvard Apparatus, Cambridge, UK) and had resistances of between 0.3 and 5 MΩ when filled with 3 M KCl. Oocytes were voltage-clamped by an Axon GeneClamp 500 amplifier (Molecular Devices, Sunnydale, CA, USA) and currents were digitized with a NI USB-6211 interface (National Instruments, Newbury, UK) and recorded using WinWCP v4.0.5 (Strathclyde Electrophysiology Software, University of Strathclyde, UK). To generate current–voltage relationships, oocytes were held at ~80 mV and pulses from -100 to +140 mV were applied at 0.2 Hz. For studies of drug block, pulses were applied to +100 mV followed by a 1.5 s voltage ramp from -100 mV to +100 mV. Oocytes were perfused at a rate of approximately 1 mL·min⁻¹. 100 mM K⁺ solution was made by an equimolar replacement of NaCl with KCl.

**Homology modelling and drug docking**

Two homology models for the mSlo3 transmembrane domains were generated using PHYRE2 (Kelley and Sternberg, 2009) and SWISS-MODEL (Arnold et al., 2006), using the coordinates of a prokaryotic cyclic nucleotide-gated K⁺ channel (PDB : 3BEH) as a template, and the tetrameric structural models were created by fitting the individual subunits into the tetrameric crystal structure (Clayton et al., 2008) using DeepView (Swiss Institute of Bioinformatics, Lausanne, Switzerland). The binding of quinidine to mSlo3 homology models was studied in silico with SwissDock (Grosdidier et al., 2011). Electrostatic calculations were performed by pdb2pqr (Dolinsky et al., 2004). Molecular models were visualized and presented using PyMol (Schrödinger LLC, New York, NY, USA).

**Materials**

All chemicals were reagent grade and obtained from Sigma-Aldrich (Poole, UK), unless stated otherwise. Quinine (Acrors Organics, Loughborough, UK), quinidine, mibebradil, clofilium, barium chloride and tetraethylammonium chloride were dissolved in either DMSO or Ringers solution to generate stock solutions, and then diluted to the required concentrations in Ringers solution.

**Data analysis and statistical procedures**

Data were analysed with WinWCP, OriginPro 7.5 (OriginLab, Northampton, MA, USA), and GraphPad Prism 6 software (GraphPad Software, San Diego, CA, USA). For drug inhibition analysis, drug-containing solutions were perfused until there was no further change in current amplitudes (typically 3–5 min), which were measured towards the end of depolarizing steps to +100 mV. Current (I)-inhibition plots were fitted with the equation: I = (I₀ - C)/(1 + ((B)/IC₅₀)ⁿH + C) where I₀ is the current in the absence of inhibitor, B the inhibitor concentration, IC₅₀ the concentration that achieves half-maximal inhibition, nH the Hill coefficient, and C the drug-insensitive component of the current. To measure the voltage dependence of current inhibition by quinine, quinidine and Ba²⁺, current amplitudes evoked by a voltage ramp were measured at sections corresponding to different voltages. The IC₅₀–voltage relationships were fitted with the Woodhull equation in the form ln(IC₅₀) = ln(IC₅₀₀)e⁻δvRT, where IC₅₀₀ is the apparent IC₅₀ at 0 mV, δ the fraction of the voltage field sensed by the blocking ion, z is the valence of the blocking ion (+1 for quinine and quinidine, and +2 for Ba²⁺), and R, T have their standard thermodynamic meanings. Data are presented as the mean ± SEM of n individual oocytes and statistical significance tested using Student’s t-test, ANOVA (with Bonferroni post hoc comparison of means), or by Kruskal–Wallis test (with Dunn’s post hoc comparison) as indicated in the Results.

**Results**

**Expression of wild-type (WT) and mutant mSlo3 channels**

Full-length WT, R196Q and F304Y mSlo3 K⁺ channel subunits were expressed in Xenopus oocytes and currents recorded by two-electrode voltage clamp. Oocytes injected with WT mSlo3 gave rise to outwardly rectifying currents, which were absent in non-injected oocytes in the 0–100 mV range (Figure 1A and B). Oocytes injected with mRNA encoding the mSlo3 mutants R196Q and F304Y also yielded large outwardly rectifying currents, but exhibited channel activity at voltages below the threshold for WT mSlo3 activation (Figure 1A and B). Expressing R196Q and F304Y mSlo3 resulted in a significantly more negative oocyte resting membrane potential (Figure 1C) compared with control oocytes or those expressing WT mSlo3. We observed a reduction in the survival of oocytes expressing the gain-of-function mutants in normal Barth’s medium, which was rectified by raising the K⁺ concentration (see Methods).

**Effects of KSper inhibitors on WT mSlo3 channels**

We next studied the effects of drugs that have previously been shown to inhibit the sperm KSper channel (Navarro et al., 2007) on WT mSlo3 currents. Like KSper (Navarro et al., 2007), Slo3 was weakly inhibited by 5 μM mibebradil and 20 mM TEA⁺, but more strongly inhibited by 500 μM quinine and 50 μM clofilium (Figure 2). WT mSlo3 was also strongly inhibited by 2 mM Ba²⁺, an effect that was prevented by raising the extracellular K⁺ concentration to 100 mM (Figure 2). The inhibition by each drug was reversible, although we observed that the amplitude of the current after washing out mibebradil was often larger than the control currents.
We studied the effects of quinine and quinidine in more detail. Quinine inhibited WT mSlo3 currents evoked by voltage pulses to $+100 \text{ mV}$ with an IC$_{50}$ of $169 \pm 40 \mu\text{M}$ and Hill slope of $1.0 \pm 0.21$ ($n=12$; Figure 3A and C). F304Y mSlo3 was also blocked by quinine, but with a significantly lower IC$_{50}$ of $15.9 \pm 3.31 \mu\text{M}$ ($n=16$, $P<0.0005$; Hill slope of 0.69 $\pm 0.02$). Potency was not significantly altered with the R196Q mSlo3 mutant, which was blocked by quinine with an IC$_{50}$ of $166 \pm 27.5 \mu\text{M}$ and Hill slope of $1.3 \pm 0.04$ ($n=4$). The effects of quinidine, a stereoisomer of quinine, have previously been studied on the MC13 Slo1/Slo3 chimera (Tang et al., 2010b). Quinidine blocked WT mSlo3 channels with an IC$_{50}$ of $19.9 \pm 1.41 \mu\text{M}$ and Hill slope of 1.15 $\pm 0.15$ ($n=7$; Figure 3B and D). Again, the potency of inhibition by quinidine was higher for F304Y mSlo3 (IC$_{50}$ of $2.42 \pm 0.60 \mu\text{M}$, $n=9$, $P<0.005$, ANOVA; Hill slope of 0.98 $\pm 0.12$), but lower with R196Q mSlo3 (IC$_{50}$ of $38.4 \pm 6.77 \mu\text{M}$, $n=5$, $P<0.001$, ANOVA; Hill slope of 1.05 $\pm 0.16$). The inhibition of F304Y mSlo by quinidine was observed to have some time dependence (Figure 3B). To gain an insight into the mechanisms by which quinine and quinidine block WT and F304Y mSlo3 channels with altered potencies, we estimated the voltage dependence of the IC$_{50}$ at different voltages. Exemplar currents evoked by voltage ramps and inhibition by quinidine are shown in Supporting Figure 1.
Information Figure S2. We found that the F304Y mutation significantly increased the electrical distance, $\delta$, sensed by the blockers from $-0.12 \pm 0.07$ ($n = 12$, $P < 0.01$) for block by quinine (Figure 3E), and from $-0.12 \pm 0.40$ ($n = 7$) to $-0.46 \pm 0.15$ ($n = 9$, $P < 0.05$) for block by quinidine (Figure 3F; two-way ANOVA). The negative values for the electrical distance, $\delta$, represent a positively charged molecule moving into the pore from the intracellular face of the channel.

**Inhibition by $\text{Ba}^{2+}$ ions**

To determine if the F304Y mutation had more wide-ranging effects on the structure of the pore we studied inhibition by $\text{Ba}^{2+}$ ions. Concentration–inhibition curves were generated for both WT and F304Y mSlo3 currents evoked by depolarizing steps to $+100$ mV. Despite the differences in channel activity, the properties of inhibition by $\text{Ba}^{2+}$ were similar. The IC$_{50}$ was 646 $\pm$ 100 $\mu$M ($n = 6$) and 525 $\pm$ 97 $\mu$M ($n = 5$), with Hill slopes of 0.88 $\pm$ 0.11 and 1.05 $\pm$ 0.12 for WT and F304Y mSlo3 respectively (Figure 4A and B). Inhibiting concentrations of $\text{Ba}^{2+}$ also appeared to slow the activation of the currents upon depolarization (Figure 4A).

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**Figure 3**

Concentration-dependent inhibition of WT, F304Y and R196Q mSlo3 currents by quinine and quinidine. Representative traces recorded before (0) and in the presence of quinine (A) and quinidine (B) (concentrations in $\mu$M as indicated). The dashed line represents the zero-current levels and scale bars represent equivalent current amplitudes and timescales. Mean (± SEM) concentration–inhibition plots for quinine (C) and quinidine (D) inhibition of WT mSlo3, R196Q mSlo3 and F304Y mSlo3 at $+100$ mV, fitted by the Hill equation provided in the Methods. Apparent voltage-dependence of the IC$_{50}$ for quinine (E) and quinidine (F) of WT and F304Y mSlo3 currents. The data are described and analysed further in the main text. For key to symbols used see Figure 1.
Structural model of mSlo3 and inhibition by quinidine

Both Phyre2 and SWISS-MODEL produced homology models of the mSlo3 transmembrane domain, which were highly similar to each other showing a root mean square deviation of 0.43 Å, giving confidence to the resulting model. The models based on the bacterial cyclic nucleotide-regulated ion channel (PDB: 3BEH) or other K⁺ channel structures showed a common architecture. Docking of quinidine by SwissDock produced a number of predicted mSlo3 binding sites. Most sites were located in the periphery of the K⁺ channel protein at the protein-lipid interface, likely due to the hydrophobic nature of quinidine and, due to the distance from the pore, therefore false-positive results. However, SwissDock also predicted a quinidine-binding site, which involved pore-lining residues F304, I308 and V312. Analysis of the model shows how quinidine is accommodated within a hydrophobic pocket, complementing the hydrophobic character of the inhibitor. Moreover, the replacement of F304 by tyrosine results in the phenolic group being sufficiently close to potentially form a hydrogen bond to the nitrogen on the methoxyquinoline group of quinidine (Figure 5C), which might improve the efficacy of quinidine binding to F304Y mSlo3. Interestingly, SwissDock did not identify any binding sites within the pore for quinidine when F304 was changed to Y304 in the homology model. This may be caused by the bulkier nature of the tyrosine side chain not accommodating the bound inhibitor, since binding site plasticity and side-chain movement is not accounted for in SwissDock.

Discussion

We investigated the characteristics of mSlo3 channel inhibition by known blockers by generating mutations that alter open probability. The observation that the R196Q and F304Y mutations both resulted in increased channel activity, particularly at more negative voltages, demonstrates a close structure-function relationship with Slo1 (BKCa, Kc.1.1) subu-
nits. The hyperpolarized membrane potentials with R196Q and F304Y mSlo3 expression demonstrate that the K⁺ selectivity of the channel, previously thought to be weak (Schreiber et al., 1998), is sufficient to hyperpolarize the membrane to strongly negative potentials when channels are activated. Increased activity at negative potentials in vivo is thought to be brought about by co-expression with accessory proteins; candidate accessory proteins are Sloβ4 (Yang et al., 2009) and LRRC52 (Yang et al., 2011; Zeng et al., 2015). We found that the mSlo3 channel has a pharmacological profile similar to that of native mouse KSper channels (Navarro et al., 2007) providing further support that mSlo3 underlies, at least in part, this current.

The results with quinine and quinidine indicate that they both block the channel by the same mechanism and site, although with slightly different potency. Quinidine inhibited WT mSlo3 at +100 mV with similar concentrations as those described with the Slo1/Slo3 chimera containing the mSlo3 pore (Tang et al., 2010b). The voltage-dependence of inhibition by quinidine was also found to be weak, although no inhibitory effect was observed with voltages more positive than those used in the present study (Tang et al., 2010b). The negative slopes that we obtained from the Woodhull plots with block by quinine and quinidine indicate that the drugs enter the mSlo3 channel pore from the intracellular side, presumably by traversing the membrane in the unprotonated state. The efficiency of protonation of the drugs in the cytoplasm, which may be affected by the pH buffering of the oocyte, may contribute to the variability in IC50 values. None-

The alleviation of the Ba²⁺-induced block of WT mSlo3 observed on raising the extracellular K⁺ concentration is consistent with increased occupancy by K⁺ at a site in the pore analogous to the Slo1 (BKCa) 'external lock-in', which prevents Ba²⁺ from entering the pore and binding at a deeper site. The relative locations of K⁺ and Ba²⁺ ions in a K⁺-selective pore have also been demonstrated crystallographically with the KCa channel (Jiang and MacKinnon, 2000), and we propose that these corresponding cation binding sites exist in mSlo3. Extracellular K⁺ concentration is therefore a major factor affecting potency of block by extracellular Ba²⁺. Furthermore, the similar results obtained from studies of Ba²⁺ blockade of WT and F304Y mSlo3 demonstrate that this mutation did not affect the selectivity filter. Ba²⁺ blocked both WT and F304Y mSlo3 with similar potency and voltage-dependence and the positive slope of the Woodhull plot reinforces the idea that externally applied Ba²⁺ enters the pore from outside of the cell. The slower current activation in the presence of Ba²⁺ is consistent with the block being alleviated upon depolarization and further supports the idea that this ion can block the channel whilst it is closed. The negative resting membrane potentials that were obtained by expressing either R196Q or F304Y mSlo3 indicate that the latter mutation had no appreciable effect on the ion selectivity of the mSlo3 pore.

Finally, our results suggest that the location of the activation gate in Slo3 channels may be similar to that of the closely related Slo1 (BKCa) channel. We conclude that inhibition of mSlo3 by barium and quinine/quinidine are not dependent on the channel being in the open or closed state. This indicates that the activation gate lies between the Ba²⁺ and quinine/quinidine-binding sites in WT mSlo3. Structurally, this points to a region deep within the pore between the selectivity filter and F304, and not at an S6 helix bundle at the intracellular face. This idea corresponds well with the conclusions of previous studies on BKCa channels in that an S6 helix bundle is unlikely to form a gate to permeating ions (Wilkens and Aldrich, 2006; Zhou et al., 2011), and that gating may involve side-chain reorientation of S6 residues deep within the pore and close to the selectivity filter (Chen et al., 2014).

To conclude, the extracellular K⁺ concentration negatively influences the potency of Ba²⁺ in blocking Slo3 K⁺ channels, and probably, therefore, KSper. We propose that quinine and quinidine inhibit Slo3 channels by binding to hydrophobic side chains found at the intracellular region of the channel pore. We also described two mutations in mSlo3 that increased channel activity and demonstrate a close structure-function relationship to Slo1 (BKCa, KCa1.1) channels. It is expected that pharmacological modulation of Slo3 K⁺ channels will affect the function of spermatozoa and this ion channel presents a novel therapeutic target in the treatment or control of male fertility.

Acknowledgements

Supported by the BBRC. We thank Mr Tim Munsey for helping with oocyte preparation and injection. S. P. M. is supported by an MRC career development grant (G100567).
Author contribution

D. C. W. and J. D. L. designed the research study, generated new reagents and conducted the electrophysiological experiments. S. P. M. and J. D. L. constructed the homology models and conducted the docking experiments. All of the authors analysed data, prepared and edited the manuscript.

Conflicts of interests

The authors declare that there is no conflict of interest regarding this research.

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## Supporting information

Additional Supporting Information may be found in the online version of this article at the publisher’s web-site:

http://dx.doi.org/10.1111/bph.13214

**Figure S1** A–C. Currents recorded from oocytes expressing WT and mutant mSlo3 as indicated following perfusion of bath solution containing 98 mM KCl and no sodium. The holding potential was −80 mV and pulses were applied in 10 mV steps to up to +140 mV. The scale bars apply to each panel and the zero-current levels are indicated by the dashed lines. Mean ± SEM. (n = 5 or 6) current- (D) and conductance-voltage (E) relationships from oocytes expressing WT (●), F304Y (▲) and R196Q (▼) mSlo3. F Relationship between reversal potentials measured and bathing [K+], where [Na+] = 98 mM − [K+].

**Figure S2** Block by quinidine of currents evoked by a voltage ramp protocol from oocytes expressing WT (A), F304Y (B), and R196Q (C) mSlo3. (D) The time-course of the voltage ramp. The concentration of quinidine, in μM, is shown to the right of the current trace, with 0 the control current prior to perfusing quinidine.