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Functional characterization of malaria parasites deficient in the K⁺ channel Kch2

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Abstract

K⁺ channels are integral membrane proteins, which contribute to maintain vital parameters such as the cellular membrane potential and cell volume. Malaria parasites encode two K⁺ channel homologues, Kch1 and Kch2, which are well-conserved among members of the *Plasmodium* genus. In the rodent malaria parasite *P. berghei*, the functional significance of K⁺ channel homologue PbKch2 was studied using targeted gene knock-out. The knockout parasites were characterized in a mouse model in terms of growth-kinetics and infectivity in the mosquito vector. Furthermore, using a tracer-uptake technique with ⁸⁶Rb⁺ as a K⁺ congener, the K⁺ transporting properties of the knockout parasites were assessed.

Results—Genetic disruption of Kch2 did not grossly affect the phenotype in terms of asexual replication and pathogenicity in a mouse model. In contrast to Kch1-null parasites, Kch2-null parasites were fully capable of forming oocysts in female *Anopheles stephensi* mosquitoes. ⁸⁶Rb⁺ uptake in Kch2-deficient blood-stage *P. berghei* parasites (Kch2-null) did not differ from that of wild-type (WT) parasites. About two-third of the ⁸⁶Rb⁺ uptake in WT and in Kch2-null parasites could be inhibited by K⁺ channel blockers and could be inferred to the presence of functional Kch1 in Kch2 knockout parasites. Kch2 is therefore not required for transport of K⁺ in *P. berghei* and is not essential to mosquito-stage sporogonic development of the parasite.

Keywords

Malaria; K⁺ channels; *P. berghei*; gene knock out

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1. Introduction

The membrane potential of higher eukaryotic cells is a diffusion potential dominated by K^+ . The magnitude of the potential is determined *i*) by the K^+ permeability of the plasma membrane and *ii*) by the transmembrane K^+ gradient, which is maintained by the action of the Na^+/K^+ ATPase. The membrane potential of the human malaria parasite *Plasmodium falciparum*, like that of plant cells, appears to be generated through the action of a membrane-bound proton pump, a V-type ATPase [1,2,3]. The parasite's membrane potential has been assessed to be more negative than the equilibrium potential for K^+ and therefore drives an inward K^+ transport across the parasite's plasma membrane, which consequently is depolarized [1].

Only two putative K^+ channels (PfK(ch)1 and PfK(ch)2) are encoded in the genome of *P. falciparum* parasites [4,5,6], and highly conserved homologues are found in other *Plasmodium* species, where they are expected to perform homologous functions. In the rodent malaria parasite *P. berghei*, targeted gene disruption showed that Kch1 contributes to K^+ transport across the parasite's plasma membrane and appears to critically influence parasite development in the mosquito vector [7]. The function of the second K^+ channel, Kch2, however, is unknown. In this study we sought to further characterize mechanisms of K^+ transport, survival and transmission competence of *Plasmodium* parasites using targeted gene knock-out of Kch2.

2. Materials and methods

2.1. Identification and cloning of PbKch2-null parasites

The orthologue to Kch2 in *P. berghei* (PB001143.01.0; gene ID 3425110) was identified by a BLAST search of PlasmoDB (version 8.0; <http://plasmodb.org/plasmo/>) using the PfK(ch)2 amino acid sequence as a query sequence. Nucleotide and amino-acid-sequence alignments were made with the MultAlin algorithm (<http://bioinfo.genopole-toulouse.prd.fr/multalin/multalin.html>).

2.2. Targeted disruption of the PbKch2 gene

Targeting plasmid (pB3DPbKch2) was constructed by cloning fragments from the 5' and 3' flanking regions of the deduced six transmembrane domains-encoding part of the PbKch2 gene. The 5' fragment was PCR amplified from genomic DNA of *P. berghei* by using the forward primer 5'-GGTACCATAACCCTAATGAGC-3' and the reverse primer 5'-AAGCTTAAACGCACTTGGAGAAC-3'. The 3' fragment was PCR amplified from genomic DNA of *P. berghei* by using the forward primer 5'-GGATCCTCTATTATATAGTGTCAATGC-3' and the reverse primer 5'-GCGGCCGCTGGTTATTATTAATGTGAGAAG-3'. The fragments contained sites for *KpnI* and *HindIII* at the 5' and 3' ends of the 5' flanking fragment, and *BamHI* and *NoI* at the 5' and 3' ends of the 3' flanking fragment. These sites were used for cloning of the fragments into the pB3D vector (kindly provided by Andrew Waters) on either side of the *Toxoplasma gondii* dihydrofolate reductase (*Tg-DHFR*) cassette. Before transfection, pB3DPbKch2 was linearized with three restriction enzymes (*KpnI*, *NoI*, and *ScaI*).

Schizont-stage parasites of *P. berghei* 2.34 ANKA strain were transfected with ~100 µg of linearized targeting plasmid using Amaxa Nucleofactor® device, program T-001 [7]. Transfected parasites were immediately injected (intra venous) into female Swiss-Webster mice (four-weeks old) and mice were treated with pyrimethamine in the drinking water (70 µg/ml), starting 24 hours after transfection. Pyrimethamine-resistant parasite populations were detected seven days after infection in mice. Integration of the plasmid into the PbKch2 locus was confirmed by PCR analysis. Parasite clones were obtained by the method of limiting dilution and verified by diagnostic PCR for disruption of genomic locus.

All animal experiments were carried out in accordance with NIH guidelines for the care and use of laboratory animals.

2.3. $^{86}\text{Rb}^+$ transport in *P. berghei* parasites

The alkali ion $^{86}\text{Rb}^+$ was used as a tracer for K^+ in an uptake protocol [7]. To detect differences in $^{86}\text{Rb}^+$ transport between WT, PbKch1-null [7], and PbKch2-null parasites, infected mouse red blood cells were harvested and treated with saponin to allow functional access to the parasites plasma membrane [7]. Saponin permeabilizes the cholesterol-containing red blood cell membrane and parasitophorous vacuole membrane, but leaves the parasite's plasma membrane intact [3]. Saponin-treated infected red blood cells, referred hereafter as isolated parasites, were used for transport studies.

Blood was harvested from isoflurane-anesthetized, infected mice (parasitaemia 20–25 %) by axillary venesection. The blood was diluted to 10 % hematocrit in HEPES-buffered saline (NaCl 125 mM, HEPES 25 mM, glucose 10 mM, KCl 5.4 mM, MgCl_2 0.4 mM, pH 7.4), treated for 30 sec at room temperature (RT) with 0.05 % saponin and immediately centrifuged at $2000\times g$ for 5 min. The parasite pellet was washed twice at RT in HEPES-buffered saline to remove free hemoglobin and most of the red cell ghosts. Parasites were resuspended in HEPES-buffered saline to a final density of $6\text{--}50 \times 10^7$ cells/ml, and placed in a water bath at 37 °C. Microscopy revealed free parasites of all stages, and, only occasionally, residual erythrocyte ghosts. Trace amounts of $^{86}\text{RbCl}$ was added ($1.4\text{--}1.9 \times 10^5$ Bq/ml; specific activity $3.9\text{--}4.2 \times 10^{13}$ Bq/mol), and at various time points (0–30 min), 200 µl samples were centrifuged ($16,000\times g$ for 20 sec) through 0.25 ml oil cushions, consisting of a 5:4 mixture of dibutyl:diethyl phthalate, overlaid with 0.8 ml ice-cold, isotonic saline. In studies employing K^+ channel-blocking compounds, samples were analyzed at a fixed time point of 10 min. The cell pellets were lysed with 0.1 % Triton X-100 and the protein precipitated with 5 % trichloroacetic acid. After centrifugation ($10,000\times g$ for 10 min), supernatants were transferred to plastic vials and counted in a β -scintillation counter. K^+ -uptake rates were calculated from $^{86}\text{Rb}^+$ uptake using the specific activity of the isotope, the $[\text{Rb}^+]/[\text{K}^+]$ ratio in the uptake buffer ($\sim 7.9 \times 10^{-4}$), and the counting efficiency of the β -scintillation counter (30 %).

2.4. In vivo asexual growth kinetics and virulence of PbKch2-null parasites

To detect differences in parasite growth during blood-stage development between Kch2-null and WT parasites, four-week old female Swiss-Webster mice were infected intra-

peritoneally with either 10^5 Kch2-null parasites or WT parasites. Parasite growth was monitored by Giemsa-stained blood smears, and survival of the mice was assessed daily.

2.5. Mosquito transmission capability

Starved adult (5–6 days old) *A. stephensi* mosquitoes were allowed to feed on mice infected for four days with equal numbers of WT or Kch2-null parasites (three mice in each group). Blood-fed mosquitoes were maintained at 20 °C in insectary chambers at 70–80 % relative humidity. Ten to twelve days after blood feeding, mosquito midguts were dissected and oocyst numbers enumerated after staining with 0.1 % mercurochrome as described [7].

2.6. Statistical analysis

Unpaired t test was used for comparing two sets of data. The log rank test was used to compare survival curves.

3. Results

3.1. Identification of PbKch2, disruption of the gene in *P. berghei* and validation of the genotype

The PfKch2 amino acid sequence [5] was used as a query in a standard protein BLAST search to identify the orthologous sequence in *P. berghei*. According to the annotation, *P. berghei* Kch2 (PbKch2) is comprised of 1468 amino acids, compared to the 1461 amino acids of *P. falciparum* Kch2 (PfKch2). Overall amino acid sequence identity between PbKch2 and PfKch2 was 53.4 % (67 % identity at nucleotide level), while the putative six transmembrane domains of PbKch2 shared 77 % amino acid sequence identity and 82 % similarity with homologous domains of PfKch2. The amino acid sequence of the pore loop, which determines ion selectivity and inhibitor profile, was identical in PfKch2 and PbKch2 (SISTVGYGDI(I/F)P), but differed from the pore loop in PfKch1 and PbKch1 (TMSTVGYGDYTP) (Fig. 1).

Gene knockout plasmids was constructed as described in Materials and Methods and used for transfection of *P. berghei* parasites. Pyrimethamine resistant parasites were cloned by limiting dilution, and to verify the correct integration of the plasmid in the parasite's genome, genomic DNA from wild type (WT) parasites and from the cloned transfectant parasites were analyzed by PCR using integration-specific primers (Supplementary Fig. S1).

3.2. In vivo parasite growth kinetics, survival of mice and mosquito transmission studies

To evaluate biological differences between WT and Kch2-null parasites, mice were infected by intra-peritoneal route using equal number of parasites (10^5), and blood stage infection was monitored by microscopic examination of Giemsa-stained blood smears. Mice infected with WT and Kch2-null parasites became parasitaemic on day 3 and parasitaemias increased steadily afterwards. The growth patterns were not significantly different between the two strains (Supplementary Fig. S2), and median survival for Kch2-null infected mice was 6.5 days compared with 7 days for WT infected mice (no significant difference).

To test the transmission potential of Kch2-null parasites, mosquitoes were allowed to feed on WT and Kch2-null-infected mice and the numbers of oocysts in the mosquito midguts counted, 10–12 days after infection. The median oocyst numbers obtained with WT and KCh2-null parasites were comparable (Supplementary Fig. S3), indicating unperturbed transmission capability of both Kch2-null and WT parasites.

3.3. K⁺ permeability of the parasite's plasma membrane

To compare the plasma membrane K⁺ permeability of WT, Kch1-null and Kch2-null parasites, infected erythrocytes were treated with saponin to permeabilize the red blood cell membrane and gain access to the parasite's plasma membrane. Within one hour of isolation, ⁸⁶Rb⁺ uptake was measured as described in Materials and Methods.

Total ⁸⁶Rb⁺ uptake at 30 min time point in isolated Kch2-null *P. berghei* parasites did not differ from basal uptake in WT parasites. Uptake in isolated Kch1-null parasites measured in parallel showed, as reported earlier [7], a 60 % decrease in ⁸⁶Rb⁺ uptake compared to WT and Kch2-null parasites (Fig. 2). ⁸⁶Rb⁺ uptake data during the initial 1½ min was used to calculate the initial rate of uptake, and similar values were obtained for WT and Kch2-null parasites: $7.1 \pm 1.7 \times 10^{-9}$ mol K⁺/(10⁹ cells × min) for WT and $8.2 \pm 1.4 \times 10^{-9}$ mol K⁺/(10⁹ cells × min) for Kch2-null parasites. In contrast, the rate for Kch1-null parasites was nearly half of that for WT or Kch2-null parasites ($3.4 \pm 0.8 \times 10^{-9}$ mol K⁺/(10⁹ cells × min)). A series of K⁺ channel blockers inhibited ⁸⁶Rb⁺ uptake in WT and in Kch2-null parasites in a dose-dependent manner, while uptake in Kch1-null parasites was unaffected by the K⁺ channel blockers (Fig. 3). The most potent blocker was clofilium with an IC₅₀ of about 18 µM in WT and Kch2-null parasites.

4. Discussion

In the present study, ⁸⁶Rb⁺ uptake was exploited as a surrogate marker for K⁺ transport in isolated, asexual *P. berghei* parasites. Uptake in Kch2-null parasites was comparable to that in WT parasites, and uptake was inhibited to the same extent in Kch2-null and in WT parasites by various channel blockers. It is therefore unlikely that Kch2 contributes to K⁺ transport across the parasite's plasma membrane in asexual stage parasites. In contrast, Kch1-null parasites had previously shown markedly reduced uptake of ⁸⁶Rb⁺ as compared to WT parasites. The remaining transport of K⁺ could not be inhibited by K⁺ channel blockers, and therefore most likely is mediated by so far uncharacterized K⁺ transporters.

Selected K⁺ channel blockers were shown to have anti-plasmodial effect against *P. falciparum* in vitro [8]. The identified blockers, however, were rather nonspecific, and the growth-inhibition assay was not paralleled by assessment of K⁺ permeability in the parasites. Thus, it remains unclear, if the antiplasmodial effect of the employed blockers was mediated through blockage of K⁺ channels. Genetic disruption of Kch1 [7] and Kch2 (current study) did not have any significant impact on the kinetics of in vivo asexual parasite growth and lethal outcome in mice. Whether simultaneous disruption of both Kch1 and Kch2 may be deleterious to the parasites could not be investigated due to lack of two different selectable markers required for selection of double knockouts. Identification of specific pharmacological inhibitors of either K⁺ channels may help in future to identify the

role of individual Kch in *Plasmodium*. Many chemical blockers of mammalian K⁺ channels interact with the pore region [9]. The predicted pore regions of Kch1 and Kch2 differ in several aspects from the pore regions of mammalian K⁺ channels, and it should be possible to identify specific inhibitors of *Plasmodium* K⁺ channels without affecting human K⁺ channels [10]. However, identification of such agents requires a robust assay for the *Plasmodium* K⁺ channels in a suitable heterologous expression system, a task which has so far not been accomplished.

In *P. falciparum*, Kch2 is expressed in all stages of blood-stage parasites [11,5]. The expression level, assessed from the amount of mRNA encoding Kch2, gradually increases as the parasites mature, which is a general feature of most other proteins in the parasite. Using immunofluorescence technique, previous studies have shown Kch2 to be expressed preferentially in schizonts and merozoites of *P. falciparum* blood-stages, however the exact sub-cellular localization has been more difficult to discern from those studies [6]. We did not detect any significant contribution of Kch2 to the K⁺ permeability of the parasite's plasma membrane, implying that if a proportion of Kch2 is located in the parasite's plasma membrane, it is either closed or non-functional. Amino acid sequence analysis supports the notion that Kch2 may be present in closed form under the experimental conditions due to inadequate potential difference [5]. Voltage-dependency of K⁺ channels is based upon several charged amino acids, usually arginine residues, in the S4 segment of the channel. Kch2 from both *P. falciparum* and *P. berghei* has in its putative S4 segment only two arginine residues, while the required minimal number is usually four for conferring voltage-dependency.

The parasite's K⁺ channels are also expressed in exo-erythrocytic stages [11] and channel-mediated K⁺ transport has been shown to be important for sporozoite invasion of host cells [12]. Unfortunately, when we knocked out Kch1 in *Plasmodium*, we were unable to obtain Kch1-null sporozoites due to transmission defect [7] and it is not possible to conclude which of the two K⁺ channels is important for sporozoite invasion. Kch2-null parasites produced in the described study, on the other hand, were able to complete a normal blood stage as well as sporogonic development life cycle.

In conclusion, the function of Kch2 was neither critical for the survival of the parasites in the intraerythrocytic stages nor in the mosquito stages. The K⁺ permeability of the parasite's plasma membrane was unaffected by the disruption of Kch2. Kch2 may, however, be involved in a function at the subcellular level not affecting bulk K⁺ transport, and a phenotype of Kch2-null should be further pursued. Our studies, however, clearly establish that Kch1 is the major, if not the sole, plasma membrane K⁺ channel of blood-stage *Plasmodium* parasites.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

PbKch(1 & 2)	<i>Plasmodium berghei</i> K ⁺ channel 1 & 2
PfKch(1 & 2)	<i>Plasmodium falciparum</i> K ⁺ channel 1 & 2
TEA	tetraethylammonium
Tg-DHFR	<i>Toxoplasma gondii</i> dihydrofolate reductase
WT	wild-type

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-----S1-----
PFL1315w FRGFYIYTKH RFVHYLSYEP IWIVAILIRI VLWCIVWIWA ASYMERKPKN
PVX_123990 FRGVSLYVKH RFMHYLSYEP VWIAILIRI VLWCIVWLWA ASYMERKPKN
PKH_144400 FRGVSLYIKH RFMHYLSYEP VWIAILIRI VLWCIVWLWA ASYMERKPKN
PBANKA_144200 FRGLTLYIKH QFIQHLSYEP VWIAILIRI ILWCIVWLWA AGYMRPPKN
PY05131 FRGLTLYIKH QFIQHLSYEP VWIAILIRI ILWCIVWLWA AGYMRPPKN
PCHAS_144400 LRGLTLYIKH QFIQHLSYEP VWIAILIRI ILWCIVWLWA AGYMRPPKN

PF14_0622 YM.LKIYNSN FNYYFCN... . . . . .IRD MCYTIIWYIS LYWRRDFTYN
PVX_117190 YM.LKIYNSI FNYYFCN... . . . . .VRD VLYAFIYWS LYWRRDFTYD
PKH_124010 YM.LKIYNST FNYYFCN... . . . . .MRD VLYAFIYWS LYWRRDFTYD
PBANKA_132890 YM.LKIYNSD FNCYFCN... . . . . .TRD ILYAIIWYIS LYWRRDEYN
PY02006 YM.LKIYNSD FNCYFCN... . . . . .IRD ILYAIIWYIS LYWRRDEYN
PCHAS_133350 YM.LKIYNSD FNCYFCT... . . . . .IRD ILYAIIWYIS LYWRRDEYN

-----S2-----
PFL1315w FEITEWNMKN VPSFYGYIEC TFQWCGVFDY FFLYFSNNK LKYIFSFFSL
PVX_123990 FQITEWNMKN VPSLYGYIEC TFQWCGVFDY FFLYFTNNK LKYIFSFFSL
PKH_144400 FKITEWNMKN VPSLYGYIEC TFQWCGVFDY FFLYFTNNK LKYIFSFFSV
PBANKA_144200 YKINAWNMKS IPPLYGYIEC TFQWCGVLDY LFLYFSNNK LKYIFSFFSL
PY05131 YKINAWNMKS IPPLYGYIEC TFQWCGVLDY LFLYFSNNK LKYIFSFFSL
PCHAS_144400 YKINAWNMKS IPPLYGYIEC TFQWCGVLDY LFLYFSNNK LKYIFSFFSL

PF14_0622 M...IWGFNK IPTYIYNMLL ILLSTSYIDL VMVSIYNKS KYLMSKSKLL
PVX_117190 T...IWNFDN IPTYIHNILI VLLSSSYIDL VMVLSYSKS KHYVMKSKLL
PKH_124010 K...MWNFDN IPTYIHNILI VLLSSSYIDL VMVLSYSKS KYVVMKSKFL
PBANKA_132890 I...LWSFNK IPIYIYNILL ILLSSSYIDL VMIIISYNKS KYHMMKSKLL
PY02006 I...LWSFNK IPIYIYNILL ILLSSSYIDL VMIIISYNKS KYHMMKSKLL
PCHAS_133350 I...LWSFNK IPIYLYNILL ILLSSSYLDL VMIIISYNKS KYHIMKSKLL

S3-----S4
PFL1315w IDFITTPVSS LIMNFFVQDN INHNYWFLIL GPLRFLRL... .VRAESTISS
PVX_123990 IDFITTPVSS LIMNAMCENK YSQNYWFLIL GPLRFLRL... .VRAESTISS
PKH_144400 IDFITTPVSS LIMNTLCENK YSQNYWFLIL GPLRFLRL... .VRAESTISS
PBANKA_144200 IDFITTPVSS FIMNFWQND YHQTWFLIL GPLRFLRL... .VRAESTMSS
PY05131 IDFITTPVSS FIMNFWKND YHQTWFLIL GPLRFLRL... .VRAESTMSS
PCHAS_144400 IDFITTPVSS LIMNFCQTD YHQTWFLIL GPLRFLRL... .VRAESTMSS

PF14_0622 IDIFFSAPCT YLFSKFIFVF EHQIDIYFMM GFLRNIKIFL NVSYVRIEHN
PVX_117190 IDAFFSAPSV FFFSKYLLVL DNKIDIYFIM GFLRNIKIFL NVSYVRVEQN
PKH_124010 IDAFFSAPSV FFFSKYLFVL DNKIDIYFIM GFLRNIKIFL NVSYVRVEQN
PBANKA_132890 IDVFFSSPSA FFFSRHFFVL ENGIDIYFLM GFLRIKVFL NVSYAKTEQS
PY02006 IDVFFSSPSA FFFSRHFFVL ENGIDIYFLM GFLRIKVFL NVSYAKTEQS
PCHAS_133350 IDVFFSSPSA FFFSRHFFGL ENGIDIYFLM GFLRVIKVFL NVSYAKTEHN

S4-----S5-----
PFL1315w CFFWLNDVKI ..IIIGIIL ALAI...LFT FSGIM.YILE APDIERDFVK
PVX_123990 CFFWLSDVKI ..IIIGIIL ALAI...LFT FSGIM.YILE APDVERAFVT
PKH_144400 CFFWLSDVKI ..IIIGIIL ALAI...LFT FSGIM.YILE APDIERDFVS
PBANKA_144200 CFFWLSDVKI ..IIIGIIL SLAI...LFT FSGIM.YILE APDIERQFIS
PY05131 CFFWLSDVKI ..IIIGIIL SLAI...LFT FSGIM.YILE APDIERQFIS
PCHAS_144400 CFFWLSDVKI ..IIIGIVIL SLAI...LFT FSGIM.YILE APDIERQFIS

PF14_0622 SILTNTIEMI IRIVLGVLLL CNAFASTIYT IQAIHPYNLD NENFNFLNS
PVX_117190 SILTNTIEMI VRIVLGVLLL CNAFASTVYT IQAIHPYNLE NGNFSYFLNS
PKH_124010 SILTNTIEMI VRIVLGVLLL CNAFASTVYT IQAIHPYNLE NGNFSYFLNT
PBANKA_132890 YILTNTIEMI IRIVLGVLLL CNAFASTLYT IQGIHPYNID NHDLYYILNN
PY02006 YILTNTIEMI IRIVLGVLLL CNAFASTLYT IQGIHPYNID NHDLYYILNN

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PCHAS_133350 YILTNTTEIKV IRIILGVLLL CNAFASTLYT IQGIHPYNID NHDLYYILSN

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          -----Pore-----*   **-----S6-----
PFL1315w  PLDFVYFGVI TMSTVGYGDI TPVTKAGKFL TMFIIITCIS FVAAQFKRLK
PVX_123990 PLDFVYFGVI TMSTVGYGDI TPVTPAGKYL TMFIIITCIS FVGAQFKRLK
PKH_144400 PLDFVYFGVI TMSTVGYGDI TPVTPAGKCL TMFIIIACIS FVGAQFKRLK
PBANKA_144200 PLDFVYFGVI TMSTVGYGDI TPVTPAGKCL TMFIIIVTCFT FVGAQLKRLK
PY05131  PLDFVYFGVI TMSTVGYGDI TPVTPAGKCL TMFIIIVTCFT FVGAQLKRLK
PCHAS_144400 PLDFVYFGVI TMSTVGYGDI TPVTPAGKCL TMFIIIVTCFT FVGAQLKRLK

PF14_0622 YLDYFYFSII SISTVGYGDI FPINKLSKVV CIIFIFWTFI WVPIQFNDLI
PVX_117190 YLDYFYFSII SISTVGYGDI FPINKLSKVV CIIFIFWTFI WVPIQFNDLI
PKH_124010 YLDYFYFSII SISTVGYGDI FPINKLSKVV CIIFIFWTFI WVPIQFNDLI
PBANKA_132890 YLDYFYFSII SISTVGYGDI IPTNKLSRVI CIIFFIFWTFI WVPIQFNDLI
PY02006  YLDYFYFSII SISTVGYGDI IPTNKLSRVV CIIFFIFWTFI WVPIQFNDLI
PCHAS_133350 YLDYFYFSII SISTVGYGDI IPTNKLSRVV CIIFFIFWTFI WVPIQFNDLI

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Figure 1. Amino acid sequence alignment of Kch1 and Kch2 in different *Plasmodium* species

Only the parts of the different proteins, which comprise the six transmembrane segments (S1–S6, light-grey colour) are included. Several features of voltage-gated K⁺ channels are recognizable (dark-grey colour): The charged amino acids in the S4 segment; the K⁺ channel signature sequence ((T/S)XXTXGYGD) in the pore loop; a proline residue, which delimits the pore region of voltage-gated K⁺ channels (*); a charged amino acid (lysine or arginine), which delimits the sixth transmembrane segment (**). Orthologues to Kch1 shown are: PFL1315w (PfKch1) from *P. falciparum* (1966 aa), PVX_123990 from *P. vivax* (2027 aa), PKH_144400 from *P. knowlesi* (2003 aa), PBANKA_144200 (PbKch1) from *P. berghei* (1936 aa), PY05131 from *P. yoelii* (1265 aa), and PCHAS_144400 from *P. chabaudi* (1924 aa). Orthologues to Kch2 shown are: PF14_0622 (PfKch2) from *P. falciparum* (1461 aa), PVX_117190 from *P. vivax* (1449 aa), PKH_124010 from *P. knowlesi* (1437 aa), PBANKA_132890 (PbKch2) from *P. berghei* (1468 aa), PY02006 from *P. yoeli* (1317 aa), and PCHAS_133350 from *P. chabaudi* (6430 aa).

Figure 2. Characterization of $^{86}\text{Rb}^+$ transport in isolated parasites

Erythrocytes from mice infected with WT parasites (squares), PbKch1-null parasites (triangles), or PbKch2-null parasites (circles) were treated with saponin, thereby allowing functional access to the parasite's plasma membrane. The saponin-treated parasites were incubated with trace amounts of $^{86}\text{Rb}^+$ and samples were taken at the indicated time points. Each point represents mean value $\pm$ SEM ($n = 3$).

Figure 3. A–D. Pharmacological characterization of $^{86}\text{Rb}^+$ uptake in isolated parasites
Uptake was assessed at a fixed time point ($t = 10$ min) in the presence of serial dilutions of different K^+ channel blockers. Uptake in WT parasites (squares) and in PbKch2-null parasites (circles) was inhibited by the K^+ channel blockers in a dose-dependent manner. Uptake in PbKch1-null parasites [7] (triangles) was unaffected by K^+ channel blockers (small inserts). Each point represents mean value $\pm$ SEM ($n = 3$).