Kv1.3 is the exclusive voltage-gated $K^+$ channel of platelets and megakaryocytes: roles in membrane potential, $Ca^{2+}$ signalling and platelet count

Conor McCloskey¹, Sarah Jones¹, Stefan Amisten⁶, Roger T. Snowden³, Leonard K. Kaczmarek⁴, David Erlinge⁵, Alison H. Goodall², Ian D. Forsythe³ and Martyn P. Mahaut-Smith¹

¹Department of Cell Physiology & Pharmacology, ²Department of Cardiovascular Sciences and ³MRC Toxicology Unit, University of Leicester, Leicester, UK
⁴Department of Cellular and Molecular Physiology, Yale University School of Medicine, New Haven, CT, USA
⁵Department of Cardiology, Lund University Hospital, Lund University, Lund, Sweden
⁶Oxford Centre for Diabetes, Endocrinology and Metabolism, Churchill Hospital, University of Oxford, Oxford, OX3 7LJ, UK

A delayed rectifier voltage-gated $K^+$ channel (Kv) represents the largest ionic conductance of platelets and megakaryocytes, but is undefined at the molecular level. Quantitative RT-PCR of all known Kvα and ancillary subunits showed that only Kv1.3 ($KCNA3$) is substantially expressed in human platelets. Furthermore, megakaryocytes from Kv1.3−/− mice or from wild-type mice exposed to the Kv1.3 blocker margatoxin completely lacked Kv currents and displayed substantially depolarised resting membrane potentials. In human platelets, margatoxin reduced the P2X₁ and thromboxaneA₂ receptor-evoked $[Ca^{2+}]_i$ increases and delayed the onset of store-operated $Ca^{2+}$ influx. Megakaryocyte development was normal in Kv1.3−/− mice, but the platelet count was increased, consistent with a role of Kv1.3 in apoptosis or decreased platelet activation. We conclude that Kv1.3 forms the Kv channel of the platelet and megakaryocyte, which sets the resting membrane potential, regulates agonist-evoked $Ca^{2+}$ increases and influences circulating platelet numbers.

(Received 2 February 2010; accepted after revision 22 February 2010; first published online 22 March 2010)

Corresponding author M. Mahaut-Smith: Department of Cell Physiology & Pharmacology, University of Leicester, Medical Sciences Building, University Road, LE1 9HN, UK. Email: mpms1@le.ac.uk

Abbreviations ACD, acid citrate dextrose; PRP, platelet-rich plasma; Kv channel, voltage-gated $K^+$-selective channel.

Introduction

Ion channels are a large and diverse family of transmembrane proteins that play important roles in all cell types. Their functions in the platelet are poorly understood (reviewed in Mahaut-Smith, 2004), although it is clear from patch clamp studies of platelets and megakaryocytes that the largest amplitude ionic currents are conducted through voltage-gated $K^+$-selective (Kv) channels (Maruyama, 1987; Kawa, 1990; Kapural et al. 1995; Romero & Sullivan, 1997). These channels activate on depolarisation to potentials positive to $-60$ mV, are steeply voltage dependent over the range $-40$ to $-10$ mV and are maximally activated at potentials above $0$ mV (Maruyama, 1987). They open and close with a relatively slow timecourse and are therefore comparable to the ‘delayed rectifier’ $K^+$ channels of excitable tissues that contribute to action potential repolarisation. In the platelet, this $K^+$ conductance could serve to stabilise the membrane potential at rest or following influx of $Ca^{2+}$ or $Na^+$ through agonist-evoked channels such as Orai1, P2X₁ and TRPC6 (Varga-Szabo et al. 2009). Voltage-dependent $K^+$ channels also play crucial roles in volume regulation and cell proliferation of lymphocytes (Lewis & Cahalan, 1995; Chandy et al. 2004). However, the molecular composition of the voltage-gated $K^+$ channel(s) in platelets, and their precursor cell the megakaryocyte, is unknown. Pharmacological studies (Maruyama, 1987; Kawa, 1990; Romero & Sullivan, 1997) indicate that one or more members of the Kv1 or Kv3 families could contribute, as reported for lymphocytes (Grissmer et al. 1992; Lewis & Cahalan, 1995). Here we show for the first time that the voltage-gated $K^+$ channel of the platelet and megakaryocyte is formed by Kv1.3 subunits, with no
evidence for a significant contribution from K\(^+\) channel subunits of other Kv families. We also show that the channel is not essential for megakaryocyte development, but that it influences the number of circulating platelets and promotes agonist-evoked increases in intracellular Ca\(^{2+}\), a key second messenger during platelet-dependent thrombosis.

**Methods**

**Materials and salines**

Standard external saline contained (in mM): 145 NaCl, 5 KCl, 1 CaCl\(_2\), 1 MgCl\(_2\), 10 Heps, 10 D-glucose, pH 7.35 with NaOH. CaCl\(_2\) was omitted for nominally Ca\(^{2+}\)-free saline. The patch pipette saline contained (in mM): 150 KCl, 2 MgCl\(_2\), 10 Heps, 10 D-glucose, pH 7.2 with KOH. Acid citrate dextrose (ACD) contained (in mM): 85 trisodium citrate, 78 citric acid, 111 glucose. Fura-2 was from Molecular Probes-Invitrogen (the Netherlands). Margatoxin and apyrase (type VII) were from Sigma (Poole, Dorset, UK).

**Cell source and preparation**

Marrow was removed from the femoral and tibial marrow of C57/bl6 or Kv1.3\(^{-/-}\) mice by flushing with standard saline containing 0.32 U ml\(^{-1}\) apyrase. For immunohistochemical studies, clumps of marrow were immediately frozen in Tissue-Tek\(^{\text{®}}\) O.C.T.\(^{\text{TM}}\) compound (Sakura, the Netherlands). For electrophysiological recordings, marrow was gently triturated to disperse the cells and maintained on a rotor at room temperature for use within 12 h. The generation of Kv1.3\(^{-/-}\) mice (C57/bl6 background) has been described previously (Koni et al. 2003); control mice (bred in-house at the University of Leicester or from Charles River, UK) were matched for age and sex. For studies of human platelets, standard phlebotomy techniques were used to draw blood from informed, consenting donors according to a protocol approved by the local ethics committees of the University of Leicester and the University of Lund. For intracellular Ca\(^{2+}\) measurements, blood was anti-coagulated with ACD, platelet-rich plasma (PRP) prepared by centrifugation at 700 g for 5 min and washed platelet suspensions prepared by centrifugation at 350 g for 20 min. Platelets were treated with aspirin (100 \(\mu M\)) and apyrase (0.32 U ml\(^{-1}\)). For cDNA preparation, whole human blood was collected into ACD supplemented with 2 mM EDTA, 0.1 \(\mu M\) PGE\(_1\), and 300 \(\mu M\) aspirin as described elsewhere (Amisten et al. 2008). Mouse blood was withdrawn into ACD by cardiac puncture under terminal gaseous anaesthesia according to UK Home Office guidelines. All procedures within this study were performed in accordance with ethical standards as outlined in Drummond (2009).

**Intracellular Ca\(^{2+}\) measurements**

Ratiometric Ca\(^{2+}\) measurements from washed suspensions of fura-2-loaded platelets were conducted as described in detail previously (Rolf et al. 2001) using a Cairn cuvette spectrophotometer (Cairn Research Ltd, Faversham, UK). Platelets were loaded with fura-2 by incubation of PRP with 2 \(\mu M\) fura-2 AM for 45 min at 37\(^{\circ}\)C and initially resuspended in nominally Ca\(^{2+}\)-free saline in the presence of apyrase (0.32 U ml\(^{-1}\)). CaCl\(_2\) was added to obtain external Ca\(^{2+}\) as required by the specific protocol. Fura-2 was calibrated extracellularly using a \(K_d\) of 224 nM following release by digitonin (50 \(\mu M\)) and determination of \(R_{\text{min}}\) and \(R_{\text{max}}\) values in Ca\(^{2+}\)-free (10 mM EGTA) or Ca\(^{2+}\)-containing (2 mM CaCl\(_2\)) salines at neutral pH. All fura-2 recordings were corrected for background fluorescence, determined by quenching with MnCl\(_2\).

**Patch clamp recordings**

Conventional whole-cell patch clamp recordings were conducted using an Axopatch 200B, as described in detail elsewhere (Mahaut-Smith, 2004). Megakaryocytes were identified by their large size and multilobed nucleus (Mahaut-Smith, 2004). For voltage clamp recordings, series resistance (\(R_s\)) compensation of \(\geq 70\%\) was applied. Whole-cell capacitance, which is a quantitative measurement of the megakaryocyte platelet-generating demarcation membrane system (Mahaut-Smith et al. 2003), was read directly from the patch clamp amplifier following compensation of the current transients evoked by a 5 mV voltage step from −80 mV, a potential range that does not activate voltage-gated channels in these cells (Mahaut-Smith et al. 2003; Mahaut-Smith, 2004).

**Platelet counting**

Murine platelets in ACD-anticoagulated whole blood were counted by flow cytometry according to the method developed by Alugupalli et al. (2001). Briefly, platelets were labelled with a FITC-conjugated anti-CD41 antibody (BD Pharmingen; 1 in 300 dilution) for 1 h and counted at a final dilution of \(>1000\) in Ca\(^{2+}\)-free (1 mM EGTA) standard platelet saline in the presence of 5.5 \(\mu M\) diameter fluorescent beads of known density (SPHERO ACFP-50-5, Spherotech, Lake Forest, IL, USA). Fluorescence was measured at low rate in a single channel (488 nm excitation) of a FACSCalibur flow cytometer (BD Biosciences), which provided clear separation of platelets, beads and unlabelled cells (see online Supplementary figure). The platelet count was calculated from the ratio of beads to platelets, the dilution factor, and the density of beads.
cDNA generation and quantitative PCR

Platelets were purified and cDNA was generated as described elsewhere (Amisten et al. 2008). qPCR was performed using Quantifast SYBR Green PCR kit and QuantiTect Primer Assays (Qiagen, Venlo, the Netherlands) in a Roto-Gene 2000 thermal cycler (Corbett Life Science, NSW, Australia) according to the manufacturers’ instructions. Gene expression was calculated according to the \( \Delta \Delta Ct \) method with GAPDH as a reference (Pfaffl, 2009).

Megakaryocyte size distribution

Sections 12 μm thick were cut from clumps of marrow frozen in Tissue-Tek\textsuperscript{®} O.C.T.\textsuperscript{TM} compound and thaw-mounted on slides. Sections were incubated in FITC-conjugated rat antimouse CD41 monoclonal antibody (BD Pharmingen) for 1 h and washed prior to analysis on an Olympus IX81 FV1000 confocal microscope. Fluorescence images (488 nm excitation, >500 nm emission) were acquired of randomly selected 0.1 mm\(^2\) fields of view (six from each of 7 WT and 7 Kv1.3\(^{-/-}\) mice). Olympus FV1000 analysis software was used to draw around the periphery of stained cells and thus to compute megakaryocyte area.

Statistics

Statistical significance was assessed using either Student’s \( t \) test (paired for intracellular Ca\(^{2+}\) recordings and unpaired for membrane capacitance, megakaryocyte size distribution and platelet count) or one-way analysis of variance (membrane potential). Significance is indicated at levels of 0.05 (\(*\)), 0.01 (\(*\)*), 0.005 (\(*\)**) or 0.001 (\(*\)***).

Figure 1. Kv1.3 forms the voltage-gated K\(^+\) channel of human platelets and murine megakaryocytes

A, expression of Kv subunits relative to GAPDH in human platelets. Of all 51 known Kv subunits (see Supplementary information) only three (KCNA3, KCNAB2 and KCNE3) were detected by quantitative PCR at levels above background. B and C, typical whole-cell currents (B) and average peak current densities (C) in response to 3 s duration voltage steps from \(-80\) mV to potentials in the range \(-120\) to 60 mV (see voltage protocol in B) for wild-type (WT) megakaryocytes, Kv1.3-deficient megakaryocytes (Kv1.3\(^{-/-}\)) and WT megakaryocytes after 10 min exposure to 10 nM margatoxin (mgtx).
Results and Discussion

To identify components of the voltage-dependent K\(^+\) conductance of the human platelet, we used real-time PCR to screen purified platelet cDNA for transcripts of all known \(\alpha\) (pore-forming) and \(\beta\) or other ancillary subunits of the Kv family of ion channels (51 targets, see Supplementary Table 1). A single \(\alpha\) subunit transcript was detected, for the gene KCNA3, which encodes Kv1.3. The only other subunits detected were for KCNAB2 and KCNE3 (which encode Kv\(\beta\)2 and Mirp2, respectively), but both were expressed at extremely low levels (<1% of Kv1.3; Fig. 1A). The primary megakaryocyte is an authentic surrogate for electrophysiological studies of the small and fragile platelet (Tolhurst et al. 2005), therefore we used whole-cell patch clamp to investigate Kv currents of megakaryocytes from wild-type and Kv1.3-deficient mice. Using pseudophysiological internal and external salines, voltage steps from a holding potential of \(-80\) mV activated a large transient outward current at potentials positive to about \(-40\) mV in wild-type megakaryocytes (Fig. 1B top panel and Fig. 1C), with a mean magnitude of \(11.8 \pm 1.0\) nA at 0 mV \((n = 14)\). This voltage-gated outward current was totally absent in Kv1.3-deficient megakaryocytes (Fig. 1B centre panel and Fig. 1C), consistent with the quantitative PCR screen and implies that this channel is a homomultimer of Kv1.3. In contrast to murine lymphocytes (Koni et al. 2003), we failed to detect any compensatory anion currents in more than 30 recordings from Kv1.3\(^{-/-}\) megakaryocytes. Margatoxin (10 nM) is a relatively selective inhibitor of Kv1.3 (Chandy et al. 2004; Gutman et al. 2005) and this blocked the outward currents (Fig. 1B lower panel and Fig. 1C), further supporting the conclusion that Kv1.3 channels mediate the voltage-gated K\(^+\) conductance of the megakaryocyte and platelet.

In whole-cell current clamp recordings, the average membrane potential \((V_m)\) of unstimulated megakaryocytes from wild-type mice was \(-46.6 \pm 2\) mV, \(n = 7\) (Fig. 2B), around which regular spontaneous fluctuations of \(2–3\) mV were observed (see sample recording in Fig. 2A). Margatoxin (10 nM) slowly depolarised this resting potential over a timecourse that varied between cells (see, for example, Fig. 2A), taking between 4 and 8 min to reach a stable \(V_m\) of \(-14.7 \pm 3\) mV \((n = 7; P < 0.001;\) Fig. 2B). A similar range of timecourses was observed for the block of voltage-dependent outward currents by 10 nM margatoxin in voltage clamp experiments (Fig. 1C). The margatoxin-induced depolarisation was slowly, and only partially, reversible (not shown), consistent with the slow wash-off (>20 min) reported previously for this toxin (Garcia-Calvo et al. 1993). However, the depolarisation caused by margatoxin was specifically related to block of Kv1.3 as there was no significant loss of membrane potential during the first 8 min of whole-cell recording in untreated wild-type megakaryocytes \((-43.9 \pm 0.9\) mV, \(P > 0.05;\) Fig. 2B). The \(V_m\) of Kv1.3-deficient megakaryocytes was also substantially depolarised, on average to \(-10 \pm 2\) mV \((n = 5; P < 0.001,\) Fig. 2B), further

![Figure 2. Kv1.3 is the major determinant of the membrane potential in mouse megakaryocytes](image)

**Figure 2.** Kv1.3 is the major determinant of the membrane potential in mouse megakaryocytes

A, current clamp recording of membrane potential in a wild type megakaryocyte during exposure to 10 nM margatoxin (mgtx). B, average membrane potential in wild-type megakaryocytes in the presence and absence of margatoxin at the times indicated after transition to whole-cell recording, and in Kv1.3\(^{-/-}\) megakaryocytes. C, time-course of block of voltage-gated K\(^+\) currents by 10 nM margatoxin in 4 different wild-type megakaryocytes.
supporting a major role for this channel in the resting potential.

Kv1.3 may influence platelet activation by increased agonist-evoked Ca\(^{2+}\) influx, through maintenance of the initial driving force for Ca\(^{2+}\) entry; furthermore, influx of cations will substantially depolarise the cell if counter-ion movement is not provided. In fura-2-loaded human platelets, margatoxin significantly reduced the peak Ca\(^{2+}\) increase following stimulation of P2X\(_1\) (1 \(\mu\)M \(\alpha\beta\)meATP) and thromboxaneA\(_2\) (500 nM U46619) receptors to 62 ± 10% and 76 ± 8% (\(P < 0.05\)) of control, respectively (Fig. 3A). In addition, margatoxin delayed the initial phase of store-operated Ca\(^{2+}\) influx, tested by addition of external Ca\(^{2+}\) after 10 min exposure to thapsigargin. The \([\text{Ca}^{2+}]_i\) increase stimulated after 10 s was reduced to 69 ± 8% (\(P < 0.05\)) of control; however, there was no significant effect on the peak Ca\(^{2+}\) increase (Fig. 3B).

Together, these results suggest that Kv1.3 promotes early Ca\(^{2+}\) influx through multiple pathways stimulated during platelet activation, including P2X\(_1\) ionotropic receptors and store-operated Ca\(^{2+}\) channels. An increase in \([\text{Ca}^{2+}]_i\) is a key signal during platelet activation (Varga-Szabo et al. 2009) and in vivo studies have demonstrated important roles for P2X\(_1\) and Orai1 store-operated Ca\(^{2+}\) channel subunits in arterial thrombosis. Thus, Kv1.3 may also facilitate haemostasis and thrombosis; however, further work is required to address this issue. Direct interactions between Kv channels and integrins have been described in several cell types, including lymphocytes (Levite et al. 2000); thus, Kv1.3 may influence platelet aggregation and adhesion independently of effects on intracellular Ca\(^{2+}\). Additional studies are also required to determine which other channels act as counter-ion pathways for Ca\(^{2+}\) influx in the absence of Kv1.3; however, intermediate conductance Ca\(^{2+}\)-activated K\(^+\) channels previously reported in human platelets are a likely candidate (Mahaut-Smith, 1995).

A role for Kv channels in thymocyte development has been described (Freedman et al. 1995), but no differences could be detected between Kv1.3\(^{-/-}\) and age/sex-matched wild-type mice in the size distribution (Fig. 4A; \(P > 0.05\)) of bone marrow megakaryocytes, suggesting that megakaryocyte development is normal. In addition, the high specific membrane capacitance of the

![Graph](https://example.com/graph.png)

**Figure 3. Role for Kv1.3 in human platelet Ca\(^{2+}\) signalling**

A and B, typical (a) and average (b) \([\text{Ca}^{2+}]_i\) responses in fura-2-loaded washed human platelet suspensions in the presence and absence of 10 nM margatoxin (mgtx). A, Responses following activation of P2X\(_1\) and thromboxane A\(_2\) receptors by \(\alpha\beta\)meATP (1 \(\mu\)M) and U46619 (500 nM), respectively. B, store-operated \([\text{Ca}^{2+}]_i\) increases induced by addition of CaCl\(_2\) (0.25 mM) after 10 min in 1 \(\mu\)M thapsigargin; \([\text{Ca}^{2+}]_i\) increases were measured after 10 s and at the peak of the response.
megakaryocyte, which reflects the amount of demarcation membrane system (Mahaut-Smith et al. 2003), was unaffected by loss of Kv1.3 (8.19 ± 0.24 μF cm⁻² for WT and 7.98 ± 0.42 μF cm⁻² for Kv1.3⁻/⁻, P > 0.05; Fig. 4B). This plasma membrane invagination system provides the additional membrane required for the process of thrombopoiesis (Schulze et al. 2006); therefore these data indicate that the platelet-generating capacity of the megakaryocyte is normal in Kv1.3⁻/⁻ mice. In contrast, platelet counts were significantly increased in Kv1.3⁻/⁻ mice compared to control mice (1.47 × 10⁶ vs. 1.12 × 10⁶ μl⁻¹, n = 7, P < 0.005; Fig. 4C). The underlying basis of this increased platelet count is unknown and currently under investigation. One possibility is the role of mitochondrial Kv1.3 channels in apoptosis, as recently described in lymphocytes (Szabo et al. 2008), since platelet lifespan is controlled by an intrinsic programme of apoptosis (Mason et al. 2007). Alternatively, the reduced Ca²⁺ responses of Kv1.3-deficient platelets may decrease activation and thereby increase survival of circulating platelets. This is opposite to the situation in mice that express a constitutively active Stim1 mutant, whose platelets have elevated cytosolic Ca²⁺ levels and shorter circulation life-time leading to thrombocytopenia (Grosse et al. 2007).

In conclusion, this study demonstrates for the first time that Kv1.3 channels are responsible for the major K⁺ conductance and the resting potential of the platelet. Consequently, blockade of Kv1.3 reduces Ca²⁺ entry through a number of different agonist-stimulated pathways. Kv1.3 is known to play roles in immune responses, olfaction and glucose homeostasis (Fadool et al. 2004; Xu et al. 2004; Cahalan & Chandy, 2009) and proposed as a target for treatment of multiple sclerosis (Rangaraju et al. 2009). The present study extends the roles of Kv1.3 to the platelet where it influences the membrane potential, Ca²⁺ responses and circulating platelet numbers.
References

Alugupalli KR, Michelson AD, Barnard MR & Leong JM (2001). Serial determinations of platelet counts in mice by flow cytometry. *Thromb Haemost* **86**, 668–671.

Amisten S, Braun OO, Bengtsson A & Erlinge D (2008). Gene expression profiling for the identification of G-protein coupled receptors in human platelets. *Thromb Res* **122**, 47–57.

Cahalan MD & Chandy KG (2009). The functional network of ion channels in T lymphocytes. *Immunol Rev* **231**, 59–87.

Chandy KG, Wulff H, Beeton C, Pennington M, Gutman GA & Cahalan MD (2004). K+ channels as targets for specific immunomodulation. *Trends Pharmacol Sci* **25**, 280–289.

Drummond GB (2009). Reporting ethical matters in *The Journal of Physiology*: standards and advice. *J Physiol* **587**, 713–719.

Fadool DA, Tucker K, Perkins R, Fasciani G, Thompson RN, Parsons AD et al. (2004). Kv1.3 channel gene-targeted deletion produces ‘Super-Smeller Mice’ with altered glomeruli, interacting scaffolding proteins, and biophysics. *Neuron* **41**, 389–404.

Freedman BD, Fleischmann BK, Punt JA, Gaulton G, Hashimoto Y & Kotlikoff MI (1995). Identification of Kv1.1 expression by murine CD4+CD8+ thymocytes. A role for voltage-dependent K+ channels in murine thymocyte development. *J Biol Chem* **270**, 22406–22411.

Garcia-Calvo M, Leonard RJ, Novick J, Stevens SP, Schmalhofer W, Kaczorowski GJ et al. (1993). Purification, characterization, and biosynthesis of margatoxin, a component of *Centruroides margaritatus* venom that selectively inhibits voltage-dependent potassium channels. *J Biol Chem* **268**, 18866–18874.

Grissmer S, Ghanshani S, Dethlefs B, McPherson JD, Wasmuth JJ, Gutman GA et al. (1992). The *Shaw*-related potassium channel gene, Kv3.1, on human chromosome 11, encodes the type 1 K+ channel in T cells. *J Biol Chem* **267**, 20971–20979.

Grosse J, Braun A, Varga-Szabo D, Beyersdorf N, Schneider B, Zeitlmann L et al. (2007). An EF hand mutation in Stim1 causes premature platelet activation and bleeding in mice. *J Clini Invest* **117**, 3540–3550.

Gutman GA, Chandy KG, Grissmer S, Lazdunsi M, McKinnon D, Pardo LA et al. (2005). International Union of Pharmacology. LIll. Nomenclature and molecular relationships of voltage-gated potassium channels. *Pharmacol Rev* **57**, 473–508.

Kapural L, Feinstein MB, O’Rourke F & Fein A (1995). Suppression of the delayed rectifier type of voltage gated K+ outward current in megakaryocytes from patients with myelogenous leukemias. *Blood* **86**, 1043–1055.

Kawa K (1990). Voltage-gated calcium and potassium currents in megakaryocytes dissociated from guinea-pig bone marrow. *J Physiol* **431**, 187–206.

Koni PA, Khanna R, Chang MC, Tang MD, Kaczmarek LK, Schlichter LC et al. (2003). Compensatory anion currents in Kv1.3 channel-deficient thymocytes. *J Biol Chem* **278**, 39443–39451.

Levite M, Cahalon L, Peretz A, Hershkoviz R, Sobko A, Ariel A et al. 2000. Extracellular K+ and opening of voltage-gated potassium channels activate T cell integrin function: physical and functional association between Kv1.3 channels and β1 integrins. *J Exp Med* **191**, 1167–1176.

Lewis RS & Cahalan MD (1995). Potassium and calcium channels in lymphocytes. *Annu Rev Immunol* **13**, 623–653.

Mahaut-Smith MP (1995). Calcium-activated potassium channels in human platelets. *J Physiol* **484**, 15–24.

Mahaut-Smith MP (2004). Patch-clamp recordings of electrophysiological events in the platelet and megakaryocyte. *Methods Mol Biol* **273**, 277–300.

Mahaut-Smith MP, Thomas D, Higham AB, Usher-Smith JA, Hussain JF, Martinez-Pinna J et al. (2003). Properties of the demarcation membrane system in living rat megakaryocytes. *Biophys J* **84**, 2646–2654.

Maruyama Y (1987). A patch-clamp study of mammalian platelets and their voltage-gated potassium current. *J Physiol* **391**, 467–485.

Mason KD, Carpinelli MR, Fletcher JJ, Collinge JE, Hilton AA, Ellis S et al. (2007). Programmed anuclear cell death delimits platelet life span. *Cell* **128**, 1173–1186.

Pfaffl M (2009). A new mathematical model for quantification in real-time RT-PCR. *Nucleic Acids Res* **29**, e45.

Rangaraju S, Chi V, Pennington MW & Chandy KG (2009). Kv1.3 potassium channels as a therapeutic target in multiple sclerosis. *Expert Opin Ther Targets* **13**, 909–924.

Rolf MG, Brearley CA & Mahaut-Smith MP (2001). Platelet shape change evoked by selective activation of P2X1 purinoceptors with α,β-methylene ATP. *Thromb Haemost* **85**, 303–308.

Romero E & Sullivan R (1997). Complexity of the outward K+ current of the rat megakaryocyte. *Am J Physiol Cell Physiol* **272**, C1525–C1531.

Schulze H, Korpal M, Hurov J, Kim SW, Zhang J, Cantley LC et al. (2006). Characterization of the megakaryocyte demarcation membrane system and its role in thrombopoiesis. *Blood* **107**, 3868–3875.

Szafo I, Bock J, Grassme H, Soddemann M, Wilker B, Lang F et al. (2008). Mitochondrial potassium channel Kv1.3 mediates Bax-induced apoptosis in lymphocytes. *Proc Natl Acad Sci U S A* **105**, 14861–14866.

Tollhurst G, Vial C, Leon C, Gachet C, Evans RJ & Mahaut-Smith MP (2005). Interplay between P2Y1, P2Y12, and P2X1 receptors in the activation of megakaryocyte cation influx currents by ADP: evidence that the primary megakaryocyte represents a fully functional model of platelet P2 receptor signalling. *Blood* **106**, 1644–1651.

Varga-Szabo D, Braun A & Nieszwandt B (2009). Calcium signalling in platelets. *J Thromb Haemost* **7**, 1057–1066.

Xu J, Wang P, Li Y, Li G, Kaczmarek LK, Wu Y et al. (2004). The voltage-gated potassium channel Kv1.3 regulates peripheral insulin sensitivity. *Proc Natl Acad Sci U S A* **101**, 3112–3117.

Author contributions

Experiments were carried out in the Department of Cell Physiology and Pharmacology and the MRC Toxicology

© 2010 The Authors. Journal compilation © 2010 The Physiological Society
Unit, University of Leicester. C.M. conducted and analysed the patch clamp, intracellular \(\text{Ca}^{2+}\) and megakaryocyte size distribution measurements; C.M., S.J., M.P.M.-S., A.H.G. and R.T.S. conducted and analysed the murine platelet counts; S.J. prepared all platelet samples except those used in qPCR studies; S.A. and D.E. conducted and analysed the platelet qPCR studies; I.D.F., S.J. and A.H.G. contributed essential discussion; I.D.F. and L.K. contributed essential materials; M.P.M.-S. designed the research and wrote the paper, which was approved by all authors.

Acknowledgments

This work was funded by the British Heart Foundation (PG 06/028 and 05/014) and the Medical Research Council. We thank Gwen Tolhurst and Richard Carter for their participation in preliminary studies.