

Cation Channels in Erythrocytes - Historical and Future Perspective

Lars Kaestner*

Institute for Molecular Cell Biology, Medical Faculty, Saarland University, 66421 Homburg/Saar, Germany

Abstract: Compared to ion pumps and carriers, ion channels have been quite late recognised as individual transport identities in red blood cells. Here the transition from cell population based flux experiments to single cell based patch-clamp investigations are described in detail. The present knowledge of cation channels in red blood cells from their molecular identity to their electrophysiological properties are summarised. Tendencies and novel concepts for future research concerning ion-transport across the red blood cell membrane are discussed.

Keywords: Red blood cells, calcium homeostasis, cation flux, electrophysiology, patch-clamp.

INTRODUCTION

Cation channels are versatile transporters, responsible for the adjustment of the resting membrane potential in most cells, for the induction of the action potential in excitable cells and the transmembrane transduction of many signalling cascades and ion-exchange in general. Historically, methods of tracer flux experiments using red blood cells have been extremely popular for investigating transmembrane transport processes (e.g. [1, 2]). The major reasons for this popularity are summarised in Box 1.

(i)	availability (also in large numbers) without seriously affecting the donor - such they are the easiest available living cells from humans
(ii)	individual cells (not connected as in tissue), so if the surrounding medium is changed they are immediately and completely covered by the new medium
(iii)	especially mammalian red blood cells do not contain cell organelles or subcellular compartments (cp. Fig. 1A) that could be used as ion stores
(iv)	the cellular population seems very homogeneous, cell to cell differences are minor since growth and development are almost stagnating in red blood cells.

Box 1. Reasons for popularity of red blood cells in investigating transmembrane transport processes.

Even nowadays the availability (reason (i) in Box 1), especially the aspect of using human cells, holds a strong argument. Nevertheless, a decent number of studies have been performed on animal red blood cells. In the following the reasons for using animal cells are discussed:

- the size of the cells - it is not by chance that the first microscopically observed cells at all (probably around 1660) have been frog red blood cells [3]. For an illustration of differing cell sizes refer to Fig. (1A);
- to avoid the special condition of lacking cell organelles and sub-cellular structures (argument (iii) in

Box 1) by investigating red blood cells of e.g. birds or amphibians, that contain a few organelles like a nucleus or mitochondria and are therefore more representative for cells in general;

- disease models might be more advantageous on certain animals, for instance the malaria *Plasmodium gallinaceum* in chicken red blood cells (cp. Fig. (1B) and Thomas *et al.* 2001 [4]) is not infectious to humans and therefore eases cell handling;
- animal red blood cells may display certain properties that are absent or not inducible in human red blood cells - examples are transgenic animals or lamprey erythrocytes having an inactive band 3 protein [5,6].

Within this review the focus is set to human red blood cells, however, animal red blood cells are covered if their investigation made a significant contribution to the cation channel identification or investigation.

With the advent of methods addressing membrane properties more directly than flux experiments, namely the patch-clamp technique, but also single molecule imaging techniques or cloning and over-expressions of channels e.g. in oocytes, on the one hand a lot of open questions in the field of red blood cell transmembrane transport could be resolved (see below). On the other hand other cell types, not meeting the conditions of being individual cells per se and not containing cell organelles (reasons (ii) and (iii) in Box 1, respectively), became easier to investigate and ousted red blood cells somewhat from their central role of transport investigations. However, in the recent years it became evident, that the homogeneous cell population (argument (iv) in Box 1) is of limited validity. There are subpopulations behaving different from the rest of the cells indicating that red blood cells are much more versatile than one would expect from membrane-bags just holding the haemoglobin.

THE DISCOVERY OF CATION CHANNELS IN ERYTHROCYTES: FROM FLUX-MEASUREMENTS TO ELECTROPHYSIOLOGY

The transport mediated by the cation channels in the red blood cell membrane was in the major fraction observed

*Address correspondence to this author at the Institute for Molecular Cell Biology, Medical Faculty, Saarland University, 66421 Homburg/Saar, Germany; Tel: +49 6841 1626 103; Fax: +49 6841 1626104; E-mail: dr.lars.kaestner@uniklinikum-saarland.de

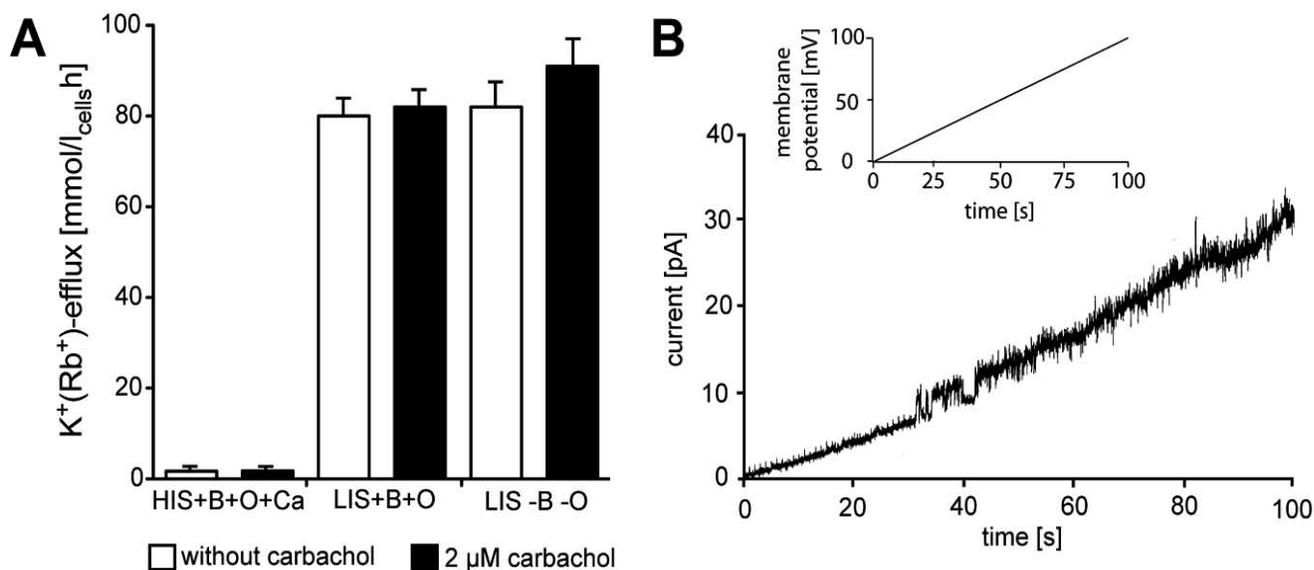


Fig. (2). Pannel A displays a radioactive tracer flux measurement. Each bar is the mean (\pm SEM) of measurements from 3 independent blood samples from healthy donors. To address channel activity both major K^+ -transport entities, namely the $Na^+/K^+/2Cl^-$ -cotransporter and the Na^+/K^+ -pump were inhibited with 100 μ M bumetanide (B) and 100 μ M ouabain (O), respectively. To be able to monitor an indirect channel activity (Gardos-channel mediated K^+ -efflux induced by Ca^{2+} -influx), 2 mM Ca^{2+} was added to the external solution in the experiments performed in solutions of high ionic strength (HIS: 145 mM NaCl, 10 mM MesTRIS, 10 mM glucose). To depolarise the cells, measurements were performed in solution of low ionic strength (LIS: 10 mM MesTRIS, 10 mM glucose, 250 mM sucrose) leading to a measured membrane potential of approximately 45 mV [72]. Since carbachol activates the non-selective voltage activated cation channel [34], a significant difference should be observed in LIS-solutions in the presence and in the absence of carbachol. This change could not be measured. To ensure that bumetanide and ouabain do not interfere with the non-selective voltage activated cation channel, the experiments in LIS-solution were repeated without the presence of the mentioned inhibitors. Again, a significant difference between the presence and absence of carbachol was not detectable. Pannel B is a plot of a current trace recorded from an excised patch showing a number of biophysical properties of the non-selective voltage activated cation channel. The pipette and bath solution contained 20 mM Na-tartrate and 70 mM Na-tartrate, respectively. Additionally, both solutions contained 2.5 mM $BaCl_2$, 10 mM glucose, 100 μ M bumetanide, 100 μ M ouabain and 10 mM MOPS. The tonicity of the solutions was adjusted to 300 mOsm with sucrose and the pH-value was adjusted to 7.4 with NaOH. The inset shows the applied voltage ramp in the voltage-clamp mode, while the current trace nicely reflects the open probability of the channel being zero up to a membrane potential of approximately 25 mV and being about 1 from 75 mV upwards. The conductance is approximately 21 pS and the current-voltage relationship is within the range of the open probability roughly linear.

measured by Donlon and Rothstein, namely the second phase of the three-phasic increase is channel mediated [20, 21].

CLASSIFICATION AND PROPERTIES OF CHANNELS ACHIEVED BY THE PATCH-CLAMP TECHNIQUE

The patch-clamp technique as it was introduced by Neher, Sakmann and colleagues provided a tool for direct ion transport measurements through single channels and such provided a direct functional assay for a biophysical investigation of ion channels even allowing for versatile manipulation options. During the initial 20 years of patch-clamp measurements on human red blood cells, investigations were limited to the cell-attached configuration and excised patches, where channels in the piece of membrane touched by the micropipette could be recorded, either with the rest of the cell between the two electrodes or under direct voltage control of the membrane, respectively. This is caused by the difficulty to achieve whole cell recordings from human red blood cells, due to their small size and the special shape, which is determined by the cytoskeleton [7]. In the early days of patch-clamping red blood cells, the possibility

to achieve whole cell recordings on human red blood cells was thought to be impossible [22]. However, with the establishment of automatised pipette pullers, whole cell recordings on red blood cells became a routine method.

The first person investigating ion channels in red blood cells was Hamill, who published current traces in an abstract [23] clearly depicting single channel openings of the Gardos-channel giving a single channel conductance of 18 pS. This initial report was followed by a comprehensive chapter about „Potassium and Chloride Channels in Red Blood Cells“ in the 1st edition of the classic „Single Channel Recording“ book edited by Sakmann and Neher [24]. This chapter was focusing on frog red blood cells, but contained all classical patch-clamp approaches including whole cell recordings. The first comprehensive analysis of the Gardos channel in human red blood cells was performed by Grygorczyk and Schwarz [25] revealing basic properties such as the K^+ to Na^+ selectivity of 15:1, inward rectification and an estimation of (in average) 10 channels per cell. After this initial report a number of follow-up paper appeared correlating the patch-clamp data to the flux measurements [26] and further characterising the Gardos-channel (cp. Table 1). The following years more detailed reports were published, e.g. [27, 28]

culminating in two papers by Leinders *et al.* [29, 30] summarising and complementing the knowledge of the opening

behaviour of the Gardos channel.

Table 1. Overview of all Cation Channels and their Properties Described in Red Blood Cells

I. The Gardos Channel		
<i>Property</i>	<i>Reference</i>	<i>Remark</i>
flux measurement based evidence	Gardos 1958 [14]	short, but precise report published as "preliminary notes"
microelectrode measurements of Ca ²⁺ induced hyperpolarisation	Lassen <i>et al.</i> 1974, 1976 [15,73], Gardos <i>et al.</i> 1976 [74]	performed on giant red blood cells from <i>Amphiuma means</i>
electrophysiological identification as a 18 pS channel by patch-clamp	Hamill 1981, 1983 [23,24]	major part of characterisation performed on frog red blood cells
electrophysiological characterisation such as modulation of single channel conductance, selectivity of K ⁺ over Na ⁺ being 17:1, temperature dependence	Grygorczyk and Schwarz 1983 [25], Grygorczyk <i>et al.</i> 1984 [26], Grygorczyk and Schwarz 1985 [69], Grygorczyk 1987 [70]	all measurements performed on human red blood cells - inside out patches
Ca ²⁺ dependence of opening behaviour and the concentration dependent action of various metal ions	Leinders <i>et al.</i> 1992a, b [29,30]	2 side by side articles, all measurements performed on human red blood cells - inside out patches
molecular identity (hSK4)	Hoffman <i>et al.</i> 2003 [43]	Western blots from ghost membranes
whole cell conductance was noninactivating and inward rectifying for K ⁺ ; selectivity of K ⁺ :Rb ⁺ :Cs ⁺ :Na ⁺ :Li ⁺ , NMDG:NH ₄ ⁺ was 51:5:>1:0; inhibition (EC50) by charybdotoxin (28 nM), clotrimazole (153 nM), nitrendipine (27 nM), <i>Stychodactyla</i> toxin (291 nM), margatoxin (459 nM), miconazole (785 nM) econazole (2.4 μM), cetiedil (79 μM); activation (EC50) by 1-ethyl-2-benzimidazolinone (74 μM)	Jensen <i>et al.</i> 1998 [42]	channel properties not investigated in red blood cells, although the characterisation was before the molecular identification of hSK4 in red blood cells it was already proposed to be the Gardos-channel
II. Low Conductance Cation Channel		
<i>Property</i>	<i>Reference</i>	<i>Remark</i>
15 pS caused by inward current	Grygorczyk and Schwarz 1983 [25]	cell attached configuration with the pipette solution in mM: 140 KCl, 1 EGTA, 1 MgCl ₂ , 10 MOPS
17 pS, K ⁺ selectivity	Bennekou and Christophersen 1988 [31]	abstract
8.4 pS in physiological ionic strength, 17 pS at approximately bisected ionic strength	Kaestner and Bernhardt 2002 [32]	when in inside out patches (symmetrical 150 mM KCl solution) the bath solution was exchanged for 75 mM KCl no shift in the reversal potential could be observed
III. The Voltage-activated Non-selective Cation Channel		
<i>Property</i>	<i>Reference</i>	<i>Remark</i>
flux measurement based evidence for voltage activation < 40 mV, permeability for Na ⁺ , K ⁺ and Ca ²⁺ inhibition by ruthenium red	Halperin <i>et al.</i> 1989 [17]	potential was adjusted by either varying the K ⁺ concentration gradient in the presence of valinomycin or by varying the concentration gradient of anion nitrate in the presence of DIDS
electrophysiological identification, zero current conductance of 35 pS, open probability increases between -30 mV and +30 mV from 0 to 100%	Christophersen and Bennekou 1991 [33]	all measurements were performed in symmetrical 500 mM salt solutions
acetylcholine sensitivity	Bennekou 1993 [34]	measurements were performed in symmetrical 500 mM salt solutions
electrophysiological characterisation under physiological conditions, permeability for divalent cations	Kaestner <i>et al.</i> 1999, 2000 [20,44]	the 2000 paper reconciles the discrepancy between the report of Christophersen and Bennekou and the 1999 paper
hysteretic behaviour of the open probability	Kaestner <i>et al.</i> 2000 [44], Bennekou <i>et al.</i> 2004 [57]	the property was initially found in patch-clamp experiments, but confirmed in cell suspension

(Table 1) Contd.....

III. The Voltage-activated Non-selective Cation Channel		
Property	Reference	Remark
whole-cell investigations	Rodighiero <i>et al.</i> 2004 [58]	confirmation of previous single channel recordings
activation by clotrimazole and analogues; inhibition: 30% by 100 μM La^{3+} , max. 70% by ruthenium red (IC_{50} of 3.7 μM); inactivation by N-ethyl-maleimide (IC_{50} of 660 μM), iodoacetamide (IC_{50} of 480 μM), 2,4'-dibromoacetophenone and declined pH values down to 6.0	Barksmann <i>et al.</i> 2004 [68], Bennekou <i>et al.</i> 2004, 2006 [59,60]	paper trilogy based on flux experiments; CCCP-estimated membrane potentials
indications for identity $\text{Ca}_v2.1$	Andrews <i>et al.</i> 2002 [48]	Western blots from ghost membranes and ω -agatoxin pharmacology in flow cytometry
for $\text{Ca}_v2.1$ no activators are known, but is blocked by ω -conotoxin MVIIC, piperidines, substituted diphenylpiperidines, piperazines, volatile anesthetics, gabapentin, mibefradil, peptide toxins DW13.3 and ω -conotoxin SVIB	Catterall <i>et al.</i> 2005 [49]	review, $\text{Ca}_v2.1$ channel properties not investigated in red blood cells
IV. The Receptor-activated Non-selective Cation Channel		
Property	Reference	Remark
flux measurement based evidence	Li <i>et al.</i> 1996 [67], Yang <i>et al.</i> 2000 [61]	indirect measurement by Gardos-channel activity in the 1996 paper; $^{45}\text{Ca}^{2+}$ flux and Fluo-3 based FACS in the 2000 paper
activation by osmotic shock and oxidative stress	Huber <i>et al.</i> 2001 [37] Duranton <i>et al.</i> 2002 [38]	performed in whole cell configuration on red blood cell ghosts
activation by prostaglandine E_2 and lysophosphatidic acid	Kaestner and Bernhardt 2002 [32], Kaestner <i>et al.</i> 2004 [35]	at the time of publication believed to be a property of the voltage-activated non-selective cation channel; still not completely resolved
inhibition by ethylisopropylamiloride and erythropoietin	Lang <i>et al.</i> 2003 [62], Myssina <i>et al.</i> 2003 [63]	channel inhibition protects against eryptosis
V. TRP C6 Channel		
Property	Reference	Remark
identification (Western blot)	Föller <i>et al.</i> 2008 [46]	the particular Western blot was provided by V. Flockerzi, M. Meissner and M. Freichel (Saarland University, Germany)
Ca^{2+} entry changes into human ghosts upon antibody incubation	Föller <i>et al.</i> 2008 [46]	performed by FACS analysis
no selective or high affinity ligands for activation or inhibition; activation by phospholipase C signalling or directly by diacylglycerol	Abramowitz and Birnbaumer 2009 [64]	review, channel properties not exclusively investigated in red blood cells
VI. NMDA-Channel		
Property	Reference	Remark
identification (Western blot, immunohistochemistry)	Makhro <i>et al.</i> 2010 [47]	performed on rat red cells, predominantly present in reticulocytes and young erythrocytes
identification in human red blood cells	Bogdanova <i>et al.</i> 2009 [65]	increased occurrence in sickle-cell patients
agonists: L-glutamate, glycine, D-2-amino-5-phosphonovalerate, 7-chlorokynurenate; sensitivity and affinity depends on heteromeric subunit composition	Yamakura and Shimoji 1999 [66]	channel properties not investigated in red blood cells

Along with the recordings of the Gardos channel, a small conductance of about 17 pS was observed by Grygorczyk and Schwarz [25] without further characterisation. Bennekou and Christophersen [31] confirmed the finding. Kaestner and Bernhardt [32] reported a channel of a similar conductance, showing no shift in the reversal potential when the KCl concentration in the bath was bisected. Therefore they concluded to face a proton channel. It is unknown if this

channel is identical with the previous reports and an independent confirmation of these results is lacking.

Single channel currents of the voltage-activated non-selective cation channel in human red blood cells were firstly measured by Christophersen and Bennekou [33]. Measurements have been performed in 500 mM salt solutions and as turned-out later on, showed a frequent appearance because it is coupled to an acetylcholine receptor of nicotinic type and

a contamination of utensils with nicotine due to tobacco smoking in the lab took place [34]. However, the channel could be verified in physiological solutions under agonist free conditions, with an extremely low open probability [20]. The biophysical properties of the channel are nicely displayed by a single current trace depicted in Fig. (2B): The ramp reflects the I-V relationship and the channel follows the typical open probability - for a detailed description refer to the figure legend of Fig. (2B).

In 2002 the first electrophysiological report of a receptor-like channel not showing voltage dependent activation behaviour appeared [32]. At the time of publication it was in similarity to the follow-up reports [35, 36] believed that this channel is identical with the non-selective voltage-activated cation channel. Whether this is the case or not could still not be resolved completely. As a working hypothesis these reports were grouped in table 1 with other observations by the group of Florian Lang depicting channel activation by osmotic shock and oxidative stress [37, 38].

Once the channels are identified on a molecular level (see section 4, below), a huge number of reports about the channels from other cell types become available. A full coverage of these sources would blast the frame of the present review. Therefore it is just pinpointed to comprehensive reviews (see Table 1, last line for each channel, if applicable).

TOWARDS THE MOLECULAR IDENTITY OF THE CHANNELS

Identifying the amino-acid blueprint of ion-channels in red blood cells needs a different strategy compared to other cell types, where cloning the protein is the method of choice. Looking at the recent work of proteomics in red blood cells approximately 150 membrane proteins were identified [39, 40]. However, there are basically no obvious candidates for ion channels, although their existence is clearly proved by the patch-clamp technique (compare section 3, above). All identification concepts so far mainly on antibody recognition, either in Western blots or in immunohistochemistry.

As for the Gardos channel at the end of the 1990s a Ca^{2+} -activated K^{+} -channel (hSK4) was cloned from, among other tissues, human T-lymphocytes [41] and was assigned to the KCNN4-gene. Shortly afterwards this cloned channel was characterised [42] and from that time the hSK4-channel was the hottest candidate for the Gardos channel, because electrophysiological characterisation as well as the pharmacology of the channel was virtually identical. In 2003 Joe Hoffman and co-workers presented a paper [43] providing further evidence: The Western blots of erythrocyte membranes displayed a clear band for SK4. Additionally, the developmental route was followed from Northern and Western blots of human erythroid progenitor cells to RT-PCR of RNA from reticulocytes also discriminating all other isoforms of the SK-family. These results whipped away all doubts for the hSK4 being identical with the Gardos channel.

For the non-selective cation conductance the situation is slightly more complex. It seems to be clear that the TRPC6 and the NMDA channels are present in red blood cells. For the TRPC6 and the NMDA-channel, the groups of Flockerzi/Freichel and Bogdanowa provided the Western

blots, respectively (for references refer to Table 1). However, both channels can not completely account for Ca^{2+} -entry measured by patch-clamp, flow cytometry and fluorescence imaging before [37, 44, 45]. The TRPC6-channel covers just a fraction of the non-selective cation-influx ([46] and own unpublished results). The NMDA-channel is just found in a fraction (subpopulation) of the cells [47]. In conjunction with the Ca^{2+} -permeability a report described Western blots against the α_{1A} -subunit of the voltage activated $\text{Ca}_v2.1$ -channel in human red blood cells [48]. From the electrophysiological characterisation it is very hard to compare the data of the voltage-activated non-selective cation channel in red blood cells to $\text{Ca}_v2.1$ -channel recordings in other cells, since these properties are differentially affected by the coexpression level of particular β -subunits as well as by alternative splicing of the α_{1A} -subunit [49]. To clarify this, further investigations are compulsory.

For the low-conductance cation channel it is not even clear whether these are substates of channels covered above or if they have their own identity. No molecular data are available.

RESEARCH PERSPECTIVE

The future basic research of red blood cells relies - as for most other cell types - on genetic manipulation. This manipulation might be more difficult in red blood cells, since the lack of protein transcription and translation machinery in mature mammalian red blood cells provokes more sophisticated methods. Presently there are two major strategies: (i) manipulation of stem cells and consecutive differentiation into red blood cells as described by Douay and coworkers [50]. A proof of principle that the expression of fluorescent fusion proteins works out in red blood cells was shown by Dickinson and coworkers when using the GFP expression to follow blood flow properties [51], (ii) the use of transgenic animals. Animals having a protein knocked-out or protein over expression, can be investigated independent of their initial purpose of breeding if the method to breed the transgene animal leads to a global and tissue-unspecific gene manipulation. Since the generation of a transgenic mouse is very labour intensive such synergic effects with other research fields can be used. However, within the recent years it became more popular to breed tissue specific and inducible transgenic animals. This may prevent the re-use of transgenic animals in red cell research that have been originally dedicated to other research areas.

Furthermore, the future mode of investigation will be single cell based such as patch-clamp, flow cytometry or live cell imaging, because it enables to identify also smaller subpopulations of cells that can hardly be addressed by measurements of huge populations of cells such as tracer flux analysis.

In that respect it has to be mentioned that these detection methods need to be further exploited. Presently it is e.g. still not possible to measure intracellular calcium concentrations in red blood cells in a quantitative manner. It has been proposed but never performed to solve that problem by fluorescence lifetime imaging [45].

Further concepts imply the expression of genetically encoded biosensors (GEBs) that have been proved to be useful tools in many different cell types, either as biomarker [52, 53] or as a functional sensor [54, 55]. As for other protein manipulation, the challenges described above lead to the concept of GEB expression in erythroid progenitor cells and consecutive differentiation into red blood cells still containing the sensor.

Independent of the direction of future technological developments, investigations of red blood cell cation channels in health and disease will foster the understanding of blood related pathologies and may provide deeper insight in general cellular physiology [56].

ACKNOWLEDGEMENTS

I like to thank the Saarland Ministry for Economy and Science for financial support and Prof. Ingolf Bernhardt for proofreading the manuscript.

REFERENCES

- [1] Schatzmann HJ. Why red cells? In: Raess BU, Tunnicliff G, Eds. *The Red Cell Membrane A Model for solute Transport*. Clifton: Humana Press 1989; pp. 3-17.
- [2] Lew VL, Glynn IM, Ellory JC. Net synthesis of ATP by reversal of the sodium pump. *Nature* 1970; 225: 865-6.
- [3] Swammerdam J. *Bybel der Natuur*. In: Hill J, Ed. *The book of nature*. London: Bookseller C. G. Seyffert 1737.
- [4] Thomas SL, Egee S, Lapaix F, Kaestner L, Staines HM, Ellory JC. Malaria parasite Plasmodium gallinaceum up-regulates host red blood cell channels. *FEBS Lett* 2001; 500: 45-51.
- [5] Lapaix F, Egee S, Gibert L, Decherf G, Thomas SL. ATP-sensitive K^+ and Ca^{2+} -activated K^+ channels in lamprey (*Petromyzon marinus*) red blood cell membrane. *Pflugers Arch* 2002; 445: 152-60.
- [6] Virkki LV, Nikinmaa M. Two distinct K^+ channels in lamprey (*Lampetra fluviatilis*) erythrocyte membrane characterized by single channel patch clamp. *J Membr Biol* 1998; 163: 47-53.
- [7] Bennekou P, Christophersen P. Ion Channels. In: Bernhardt I, Ellory JC, Eds. *Red cell membrane transport in health and disease*. Berlin: Springer Verlag 2003; pp. 139-52.
- [8] Abderhalden E. Über den blut kalium gehalt verschiedener säugethiere. *Hoppe-Seyler's Zeitschrift für Physiologische Chemie* 1889; 25: 65-115.
- [9] Bang I. *Physiko-chemische verhältnisse der blutkörperchen*. *Biochemische Zeitschrift* 1909; 16: 255-76.
- [10] Cohn WE, Cohn ET. Permeability of red corpuscles of the dog to sodium ion. *Proc Soc Exp Biol Med* 1939; 41: 445-9.
- [11] Lew VL, Beaugé L. Passive Cation Fluxes in Red Cell Membranes. In: Giebisch G, Tosteson DC, Ussing HH, Eds. *Transport across biological membranes*. Vol. II. Berlin: Springer-Verlag 1979; pp. 81-115.
- [12] Ørskov SL. Untersuchungen über den Einfluß von Kohlensäure und Blei auf die Permeabilität der Blutkörperchen für Kalium und Rubidium. *Biochem Z* 1935; 279: 250-61.
- [13] Wilbrandt W. A relation between the permeability of the red cell and its metabolism. *Trans Faraday Soc* 1937; 33: 956-9.
- [14] Gardos G. The function of calcium in the potassium permeability of human erythrocytes. *Biochim Biophys Acta* 1958; 30: 653-4.
- [15] Lassen UV, Pape L, Vestergaard-Bogind B, Bengtson O. Calcium-related hyperpolarization of the Amphiuma red cell membrane following micropuncture. *J Membr Biol* 1974; 18: 125-44.
- [16] Donlon JA, Rothstein A. The cation permeability of erythrocytes in low ionic strength media of various tonicities. *J Membr Biol* 1969; 1: 37-52.
- [17] Halperin JA, Brugnara C, Tosteson MT, Van Ha T, Tosteson DC. Voltage-activated cation transport in human erythrocytes. *Am J Physiol* 1989; 257: C986-96.
- [18] Richter S, Hamann J, Kummerow D, Bernhardt I. The monovalent cation "leak" transport in human erythrocytes: an electroneutral exchange process. *Biophys J* 1997; 73: 733-45.
- [19] Bernhardt I, Bogdanova AY, Kummerow D, Kiessling K, Hamann J, Ellory JC. Characterization of the K^+ (Na^+)/ H^+ monovalent cation exchanger in the human red blood cell membrane: effects of transport inhibitors. *Gen Physiol Biophys* 1999; 18: 119-37.
- [20] Kaestner L, Bollensdorff C, Bernhardt I. Non-selective voltage-activated cation channel in the human red blood cell membrane. *Biochim Biophys Acta* 1999; 1417: 9-15.
- [21] Bernhardt I, Weiss E, Robinson HC, Wilkins R, Bennekou P. Differential effect of HOE642 on two separate monovalent cation transporters in the human red cell membrane. *Cell Physiol Biochem* 2007; 20: 601-6.
- [22] Schwarz W, Grygorczyk R, Hof D. Recording single-channel currents from human red cells. *Methods Enzymol* 1989; 173: 112-21.
- [23] Hamill OP. Potassium channel currents in human red blood cells. *J Physiol* 1981; 319: 97P-8P.
- [24] Hamill OP. Potassium and chloride channels in red blood cells. In: Sakmann B, Neher E, Eds. *Single-Channel Recording*. New York, London: Plenum Press 1983; pp. 451-71.
- [25] Grygorczyk R, Schwarz W. Properties of the Ca^{2+} -activated K^+ conductance of human red cells as revealed by the patch-clamp technique. *Cell Calcium* 1983; 4: 499-510.
- [26] Grygorczyk R, Schwarz W, Passow H. Ca^{2+} -activated K^+ channels in human red cells. Comparison of single-channel currents with ion fluxes. *Biophys J* 1984; 45: 693-8.
- [27] Shields M, Grygorczyk R, Fuhrmann GF, Schwarz W, Passow H. Lead-induced activation and inhibition of potassium-selective channels in the human red blood cell. *Biochim Biophys Acta* 1985; 815: 223-32.
- [28] Fuhrmann GF, Schwarz W, Kersten R, Sdun H. Effects of vanadate, menadione and menadione analogs on the Ca^{2+} -activated K^+ channels in human red cells. Possible relations to membrane-bound oxidoreductase activity. *Biochim Biophys Acta* 1985; 820: 223-34.
- [29] Leinders T, van Kleef RG, Vijverberg HP. Distinct metal ion binding sites on $Ca(2+)$ -activated K^+ channels in inside-out patches of human erythrocytes. *Biochim Biophys Acta* 1992; 1112: 75-82.
- [30] Leinders T, van Kleef RG, Vijverberg HP. Single $Ca(2+)$ -activated K^+ channels in human erythrocytes: Ca^{2+} dependence of opening frequency but not of open lifetimes. *Biochim Biophys Acta* 1992; 1112: 67-74.
- [31] Bennekou P, Christophersen P. The occurrence of a low conductance channel in the human red cell. *Acta Physiol Scand* 1988; 13: S73.
- [32] Kaestner L, Bernhardt I. Ion channels in the human red blood cell membrane: their further investigation and physiological relevance. *Bioelectrochemistry* 2002; 55: 71-4.
- [33] Christophersen P, Bennekou P. Evidence for a voltage-gated, non-selective cation channel in the human red cell membrane. *Biochim Biophys Acta* 1991; 1065: 103-6.
- [34] Bennekou P. The voltage-gated non-selective cation channel from human red cells is sensitive to acetylcholine. *Biochim Biophys Acta* 1993; 1147: 165-7.
- [35] Kaestner L, Tabellion W, Lipp P, Bernhardt I. Prostaglandin E-2 activates channel-mediated calcium entry in human erythrocytes: an indication for a blood clot formation supporting process. *Thromb Haemost* 2004; 92: 1269-72.
- [36] Kaestner L, Tabellion W, Weiss E, Bernhardt I, Lipp P. Calcium imaging of individual erythrocytes: Problems and approaches. *Cell Calcium* 2006; 39: 13-19.
- [37] Huber SM, Gamper N, Lang F. Chloride conductance and volume-regulatory nonselective cation conductance in human red blood cell ghosts. *Pflugers Arch* 2001; 441: 551-8.
- [38] Duranton C, Huber SM, Lang F. Oxidation induces a $Cl(-)$ -dependent cation conductance in human red blood cells. *J Physiol* 2002; 539: 847-55.
- [39] Low TY, Seow TK, Chung MC. Separation of human erythrocyte membrane associated proteins with one-dimensional and two-dimensional gel electrophoresis followed by identification with matrix-assisted laser desorption/ionization-time of flight mass spectrometry. *Proteomics* 2002; 2: 1229-39.

- [40] Bruschi M, Seppi C, Arena S, *et al.* Proteomic analysis of erythrocyte membranes by soft Immobilized gels combined with differential protein extraction. *J Proteome Res* 2005; 4: 1304-9.
- [41] Logsdon NJ, Kang J, Togo JA, Christian EP, Aiyar J. A novel gene, hKCa4, encodes the calcium-activated potassium channel in human T lymphocytes. *J Biol Chem* 1997; 272: 32723-6.
- [42] Jensen BS, Strobaek D, Christophersen P, *et al.* Characterization of the cloned human intermediate-conductance Ca²⁺-activated K⁺ channel. *Am J Physiol* 1998; 275: C848-56.
- [43] Hoffman JF, Joiner W, Nehrke K, Potapova O, Foye K, Wickrema A. The hSK4 (KCNN4) isoform is the Ca²⁺-activated K⁺ channel (Gardos channel) in human red blood cells. *Proc Natl Acad Sci USA* 2003; 100: 7366-71.
- [44] Kaestner L, Christophersen P, Bernhardt I, Bennekou P. The non-selective voltage-activated cation channel in the human red blood cell membrane: reconciliation between two conflicting reports and further characterisation. *Bioelectrochemistry* 2000; 52: 117-25.
- [45] Kaestner L, Tabellion W, Weiss E, Bernhardt I, Lipp P. Calcium imaging of individual erythrocytes: problems and approaches. *Cell Calcium* 2006; 39: 13-19.
- [46] Foller M, Kasinathan RS, Koka S, *et al.* TRPC6 contributes to the Ca(2+) leak of human erythrocytes. *Cell Physiol Biochem* 2008; 21: 183-92.
- [47] Makhro A, Wang J, Vogel J, *et al.* Functional NMDA receptors in mammalian erythrocytes. *Am J Physiol Cell Physiol* 2010; 298: C1315-25.
- [48] Andrews DA, Yang L, Low PS. Phorbol ester stimulates a protein kinase C-mediated agatoxin-TK-sensitive calcium permeability pathway in human red blood cells. *Blood* 2002; 100: 3392-9.
- [49] Catterall WA, Perez-Reyes E, Snutch TP, Striessnig J. International Union of Pharmacology. XLVIII. Nomenclature and structure-function relationships of voltage-gated calcium channels. *Pharmacol Rev* 2005; 57: 411-25.
- [50] Giarratana MC, Kobari L, Lapillonne H, *et al.* *Ex vivo* generation of fully mature human red blood cells from hematopoietic stem cells. *Nat Biotechnol* 2005; 23: 69-74.
- [51] Jones EA, Baron MH, Fraser SE, Dickinson ME. Measuring hemodynamic changes during mammalian development. *Am J Physiol Heart Circ Physiol* 2004; 287: H1561-9.
- [52] Scheller N, Resa-Infante P, de la Luna S, *et al.* Identification of PatL1, a human homolog to yeast P body component Pat1. *Biochim Biophys Acta* 2007; 1773: 1786-92.
- [53] Kaestner L, Scholz A, Hammer K, Vecerde A, Ruppenthal S, Lipp P. Isolation and genetic manipulation of adult cardiac myocytes for confocal imaging. *J Vis Exp* 2009; 31: <http://www.jove.com/index/Details.stp?ID=1433>.
- [54] Viero C, Kraushaar U, Ruppenthal S, Kaestner L, Lipp P. A primary culture system for sustained expression of a calcium sensor in preserved adult rat ventricular myocytes. *Cell Calcium* 2008; 43: 59-71.
- [55] Kaestner L, Ruppenthal S, Schwarz S, Scholz A, Lipp P. Concepts for optical high content screens of excitable primary isolated cells for molecular imaging. In: Licha K, Lin CP, Eds. *SPIE biomedical optics*. Munich: SPIE 2009; pp. 737-45.
- [56] Mohandas N, Gallagher PG. Red cell membrane: past, present, and future. *Blood* 2008; 112: 3939-48.
- [57] Bennekou P, Barksman TL, Jensen LR, Kristensen BI, Christophersen P. Voltage activation and hysteresis of the non-selective voltage-dependent channel in the intact human red cell. *Bioelectrochemistry* 2004; 62: 181-5.
- [58] Rodighiero S, De Simoni A, Formenti A. The voltage-dependent nonselective cation current in human red blood cells studied by means of whole-cell and nystatin-perforated patch-clamp techniques. *Biochim Biophys Acta* 2004; 1660: 164-70.
- [59] Bennekou P, Barksman TL, Kristensen BI, Jensen LR, Christophersen P. Pharmacology of the human red cell voltage-dependent cation channel. Part II: inactivation and blocking. *Blood Cells Mol Dis* 2004; 33: 356-61.
- [60] Bennekou P, Barksman TL, Christophersen P, Kristensen BI. The human red cell voltage-dependent cation channel. Part III: Distribution homogeneity and pH dependence. *Blood Cells Mol Dis* 2006; 36: 10-14.
- [61] Yang L, Andrews DA, Low PS. Lysophosphatidic acid opens a Ca(++) channel in human erythrocytes. *Blood* 2000; 95: 2420-5.
- [62] Lang KS, Myssina S, Tanneur V, Wieder T, Huber SM, Lang F, Duranton C. Inhibition of erythrocyte cation channels and apoptosis by ethylisopropylamiloride. *Naunyn Schmiedebergs Arch Pharmacol* 2003; 367: 391-6.
- [63] Myssina S, Huber SM, Birka C, Lang PA, Lang KS, Friedrich B, Risler T, Wieder T, Lang F. Inhibition of erythrocyte cation channels by erythropoietin. *J Am Soc Nephrol* 2003; 14: 2750-7.
- [64] Abramowitz J, Birnbaumer L. Physiology and pathophysiology of canonical transient receptor potential channels. *FASEB J* 2009; 23: 297-328.
- [65] Bogdanova A, Makhro A, Goede J, *et al.* NMDA receptors in mammalian erythrocytes. *Clin Biochem* 2009; 42: 1858-9.
- [66] Yamakura T, Shimoji K. Subunit- and site-specific pharmacology of the NMDA receptor channel. *Prog Neurobiol* 1999; 59: 279-98.
- [67] Li Q, Jungmann V, Kiyatkin A, Low PS. Prostaglandin E2 stimulates a Ca²⁺-dependent K⁺ channel in human erythrocytes and alters cell volume and filterability. *J Biol Chem* 1996; 271: 18651-6.
- [68] Barksman TL, Kristensen BI, Christophersen P, Bennekou P. Pharmacology of the human red cell voltage-dependent cation channel; Part I. Activation by clotrimazole and analogues. *Blood Cells Mol Dis* 2004; 32: 384-8.
- [69] Grygorczyk R, Schwarz W. Ca²⁺-activated K⁺ permeability in human erythrocytes: modulation of single-channel events. *Eur Biophys J* 1985; 12: 57-65.
- [70] Grygorczyk R. Temperature dependence of Ca²⁺-activated K⁺ currents in the membrane of human erythrocytes. *Biochim Biophys Acta* 1987; 902: 159-68.
- [71] Kaestner L. Passive elektrogene Transportprozesse durch die Membran von Humanerythrozyten und Erythrozyten von gesunden und Malaria-infizierten Hühnern. Berlin: Logos Verlag 2001.
- [72] Bernhardt I, Erdmann A, Glaser R, Reichmann G, Bleiber R. Influence of lipid composition on passive ion transport of erythrocytes. In: Klein RA, Schmitz B, Eds. *Topics in lipid research from structural elucidation to biological function*. London: The Royal Society of Chemistry 1986; pp. 243-8.
- [73] Lassen UV, Pape L, Vestergaard-Bogind B. Effect of calcium on the membrane potential of Amphiuma red cells. *J Membr Biol* 1976; 26: 51-70.
- [74] Gardos G, Lassen UV, Pape L. Effect of antihistamines and chlorpromazine on the calcium-induced hyperpolarization of the Amphiuma red cell membrane. *Biochim Biophys Acta* 1976; 448: 599-606.

Received: December 15, 2009

Revised: July 04, 2010

Accepted: July 15, 2010

© Lars Kaestner; Licensee *Bentham Open*.This is an open access article licensed under the terms of the Creative Commons Attribution Non-Commercial License (<http://creativecommons.org/licenses/by-nc/3.0/>), which permits unrestricted, non-commercial use, distribution and reproduction in any medium, provided the work is properly cited.