Red Blood Cell Cationic conductances in Health and Disease

Guillaume Bouyer
Stéphane Egée
David Monedero Alonso
Laurent Pérès
Serge Thomas

&
Poul Bennekou
Vertebrates

- Gnathostomes
  - Osteichthyans
  - Tetrapodes
  - Agnathans
  - Chondrichtyans
  - Teleosts
  - Mammals
  - Amphibians

Gill/Lung

Tissues

O$_2$

Hb

RBC

Cl$^-$

HCO$_3^-$

CO$_2$

Biological context
Biological context

‘Homeostasis’

Volume

Elastic properties

- Choline
- Glucose
- Nucleoside
- Amino acids
- Mono-carboxylate

Pumps

- Na / K
- Ca

Exchangers

- Na / H
- Cl / HCO

Co-transporteurs

- Na – K
- 2C
- K
- Cl

Transporters

- Choline
- Glucose
- Nucleoside
- Amino acids
- Mono
- Carboxylate

Hb/O2

Acid-base

Volume

RBC

Ionic channels
Biological context: pump-leak concept

\[
\text{3Na}^+ - 2\text{K}^+ \text{ ATPase}
\]

\[
[\text{Na}^+] / [\text{K}^+] = 0.12 - 0.16
\]

\[
20 - 50 \text{ nmol.l}^{-1}
\]

\[
\text{Ca}^{2+} \text{ ATPase}
\]

\[
\text{Hb} \quad 7.2 \text{ mmol.l}^{-1}
\]

' Homeostasis '

Volume

Elastic properties
Biological context: pump-leak concept

‘Homeostasis’
- Volume
- Elastic properties

Pumps
- Na / K, Ca

Echangers
- Na / H, Cl / HCO

Co-transporteurs
- Na – K, 2C, K – Cl

Transporters
- choline, glucose, nucleoside, amino acids, mono carboxylate

Hb/O2, acid-base, volume

RBC

Ionic channels

Physiological role?

Thomas et al. Blood Cells Molecules and Diseases, 2011
Situations where “Pump-Leak” is challenged

‘Pump-Leak’ concept

Physiological situations
- membrane deformation
- senescence
- low ionic strength
- Erythropoiesis

Pathologies
- malaria
- sickle cell disease
- anemias

Physico-chemical environment
- temperature
- storage

\[
\frac{[\text{Ca}^{2+}]}{[\text{Ca}^{2+}]} > 1 \times 10^{5-6}
\]

\[
\frac{[\text{Na}^+]}{[\text{K}^+]} = 0.12-0.16
\]
Membrane fluxes as a system

At equilibrium

\[ r = \frac{[\text{Cl}^-]_o}{[\text{Cl}^-]_i} = \frac{[\text{HCO}_3^-]_o}{[\text{HCO}_3^-]_i} = \frac{[H^+]_i}{[H^+]_o} \]

\[ E = -\frac{RT}{F} \ln (r) \approx -10 \text{ mV} \]

Membrane fluxes as a system

Gárdos channel
A selective K⁺ channel dependent on [Ca^{2+}]₀

At equilibrium
\[
E = -\frac{RT}{F} \ln (r) \approx -10 \text{ mV}
\]

1) Isotonicity:
\[ f_{Hb} Q_{Hb} + Q_{Na} + Q_K + Q_{Mg} + Q_X = V_w (C_{Na}^m + C_K^m + C_A^m + C_B^m + C_Y^m) \]

2) Initial electroneutrality:
\[ Q_{Na}^* + Q_K^* + 2Q_{Mg}^* + Q_X^* - Q_A^* + n_{Hb}^* Q_{Hb} + n_X^* Q_X^* = 0 \]

3) Non ideal osmotic behaviour of hemoglobin:
\[ f_{Hb} = 1 + b \frac{Q_{Hb}}{V_w} + c \frac{Q_{Hb}^2}{V_w^2} \]

4) H⁺ buffer behavior of hemoglobin:
\[ n_{Hb} = a (pH^o - pI) \]

5) Fluxes equations
\[ \Phi_{Na} = \Phi_{Na}^p + \Phi_{Na}^L + \Phi_{Na}^G + \Phi_{Na}^{Co} \]
\[ \Phi_K = \Phi_K^p + \Phi_K^L + \Phi_K^G + \Phi_K^{Co} \]
\[ \Phi_A = \Phi_A^G + \Phi_A^{AH} + \Phi_A^{Co} \]
\[ \Phi_H = \Phi_A^{AH} \]

6) Maintenance of Electroneutrality
\[ \sum z_i \Phi_i = 0 \]

7) Computation of transients
\[ DQ_i = \Phi_i \cdot D_t \]

8) Redistribution of permeant solutes among both compartments
\[ C_i^m(t) = C_i^m(t-Dt) \left( 1 + \frac{H_t}{1 - H_t DV_w} \right) - \left( \frac{H_t}{1 - H_t} \right) DQ_i \]
Situations where “Pump-Leak” is challenged

‘Pump-Leak’ concept

Physiological situations
- membrane deformation
- senescence
- low ionic strength
- Erythropoiesis

Pathologies
- malaria
- sickle cell disease
- anemias

Physico-chemical environment
- temperature
- storage

[Na⁺]/[K⁺] = 0.12-0.16

[Ca²⁺]₀/[Ca²⁺]ᵢ > 1*10⁵⁻⁶

Ca²⁺ ATPase

Na⁺ / Ca²⁺

K⁺

Na⁻ K ATPase
A very long journey

Transit in capillaries

(500 ms / min) → PGCa

\[ \text{RBC} \]

\[ \text{Ca}^{2+} \]

\[ \text{K}^+ \]

\[ \text{A}^- \]

\[ \text{C}^+ \]


Li, J. et al. (2007) Cytoskeletal dynamics of human erythrocyte. PNAS 104 (12), 4937-4942
Aging challenges ionic balance

Em, [Ca]_, pH_, ROS…
rigidity, glycation, cytoskeleton…

\[
\frac{[\text{Na}^+]}{[\text{K}^+]} < 1
\]

1.11 g/ml

\[
\frac{[\text{Na}^+]}{[\text{K}^+]} > 1
\]

1.08 g/ml

[Na^+]/[K+] < 1
[Na^+]/[K+] > 1
100 days
Storage conditions speeds up aging

Morphological alterations

D’Alessandro et al., Transfusion. 2015 Jan;55(1):205-19
Roussel et al. Transfusion. 2017 Apr;57(4):1007-1018
Storage conditions speeds up aging

Storage Temperature: 4°C
Hematocrit: 45%

Model fits resting storage conditions

K+ + Na+
K+
Na+
mEq.lcw⁻¹

day, after sampling

0 1 2 3 7 14 21
Ca$^{++}$ Permeation pathway

ARTICLE
Piezo proteins are pore-forming subunits of mechanically activated channels

Membrane deformation

extracellular side

cytosolic side

Ca$^{++}$

Evidence for a mechanosensitive calcium influx into red cells
Michael C. Brain,* Carin Fäh, Laurie Robertson, and Christopher B. Brown
Department of Biochemistry and Molecular Biology, University of Calgary, Calgary, AB, Canada
Received 21 January 2004

Mutations in the mechanotransduction protein PIEZO1 are associated with hereditary xerocytosis
Ryan Zarychanski,*1,² Vincent R. Slutz,*² Brett L. Houston,³ Valeria Malkimova,⁴ Donald S. Houston,⁵ Brian Smith,⁴ Joseph Fantini,* and Patrick G. Galliher¹,²

blood 2012 129: 1928-1935
doi 10.1182/blood-2012-04-42253 early published online April 23, 2012
A23187, 10 µM

Em, mV

Time, min

Em → E_K

Ca^{2+}

‘Gardos’

K^+

Ca^{2+}
A23187, 10 µM

Em, mV

Time, min

Na+ / Ca2+ K+

'Gardos'

K+

Ca2+

A−
Modelisation implies Non Selective Cation channel activity

htc 4%
Modelisation implies NSC activity

A23187, 10 µM

Triton X
Modelisation implies NSC activity

\[ V_M, \text{ mV} \]

Initial values of permeability for \( K^+ \), \( Na^+ \) & \( Cl^- \)

A23187, 10 \( \mu \)M

Triton X
Modelisation implies NSC activity

Initial values of permeability for K^+, Na^+ & Cl^-  
Permeability for Na^+ and K^+ increased by 100 fold  

A23187, 10 µM  
Triton X
The graph shows the change in membrane voltage ($V_M$, mV) over time (s). There are two main events indicated: A23187, 10 µM at 0 s and Triton X at 600 s. The voltage changes are represented by the different colored lines, with red indicating the most significant change.
Aging challenges ionic balance

- **Day 0 of storage**
- **Day 6 of storage**

**Graphical Data:**
- **V<sub>M</sub>, mV**
- **Time, s**
- **A23187, 10 µM**
- **Triton X**

*Egée et al. In prep.*
Does in vitro ageing enhance NSC activity?

Htc 4%

\[ [\text{Na}^+]_i = 54 \text{ mM} \]
\[ [\text{K}^+]_i = 96 \text{ mM} \]

A23187, 10 µM

Triton X

Initial values of permeability for K\(^+\), Na\(^+\) & Cl\(^-\)
Does *in vitro* ageing enhance NSC activity?

Htc 4%

\[[\text{Na}^+]_i = 54 \text{ mM}\]

\[[\text{K}^+]_i = 96 \text{ mM}\]

A23187, 10 µM

Triton X

Initial values of permeability for K⁺, Na⁺ & Cl⁻

350 fold increase of Na⁺ permeability
Does *in vitro* ageing enhance NSC activity?

Initial values of permeability for $K^+$, $Na^+$ & $Cl^-$

350 fold increase of $Na^+$ permeability

350 fold increase of $Na^+$ & $K^+$ permeability

Htc 4%

$[Na^+]_i = 54 \text{ mM}$

$[K^+]_i = 96 \text{ mM}$
Does *in vitro* ageing enhance NSC activity?

Htc 4%

\[[\text{Na}^+]_i = 54 \text{ mM}\]

\[[\text{K}^+]_i = 96 \text{ mM}\]

A23187, 10 µM

Triton X

---

Initial values of permeability for \(\text{K}^+\), \(\text{Na}^+\) & \(\text{Cl}^-\)

350 fold increase of \(\text{Na}^+\) permeability

350 fold increase of \(\text{Na}^+\) & \(\text{K}^+\) permeability

350 fold increase of \(\text{Na}^+\) & \(\text{K}^+\) permeability & 5 fold of \(\text{Cl}^-\) permeability
Aging challenges ionic balance

Initial values of permeability for K\(^+\), Na\(^+\) & Cl\(^-\) determined at day 0

350 fold increase of Na\(^+\) & K\(^+\) permeability + 5 fold increase of Cl\(^-\) permeability

Htc 4%

\([\text{Na}^+]_i = 54 \text{ mM}\)

\([\text{K}^+]_i = 96 \text{ mM}\)

A23187, 10 \(\mu\text{M}\)

Triton X

Egée et al. In prep.
Situations where “Pump-Leak” is challenged

‘Pump-Leak’ concept

Physiological situations
- membrane deformation
- senescence
- low ionic strength
- Erythropoiesis

Pathologies
- malaria
- sickle cell disease
- anemias

Physico-chemical environment
- temperature
- storage

\[
\frac{[Ca^{2+}]}{[Ca^{2+}]} > 1 \times 10^{5-6}
\]

\[
\frac{[Na^+] }{[K^+]} = 0.12 - 0.16
\]

\[Na^+/Ca^{2+}\]
\[K^+\]
\[Na-K\ ATPase\]
\[Ca^{2+}\ ATPase\]
**ARTICLE**

Piezo proteins are pore-forming subunits of mechanically activated channels

**Ca++ Permeation pathway**

extracellular side

Membrane deformation

cytosolic side

**Ca++**

**blood**

2012;129:1905-1915
doi:10.1182/blood-2012-04-42253
electronically published online April 23, 2012

Mutations in the mechanotransduction protein PIEZO1 are associated with hereditary xerocytosis

Ryan Zarzour,*,1† Vincent R. Schulz,‡ Brett L. Houston,§ Yelena Maksimova,‖ Donald S. Houston,‖ Brian Smith,‖ Joseph H. McGahan,‡ and Patrick G. Gallagher,†,‡
"The important thing is to stay hydrated."