Blood Cells, Molecules and Diseases xxx (xxxx) xxx–xxx

Contents lists available at ScienceDirect

Blood Cells, Molecules and Diseases

journal homepage: www.elsevier.com/locate/bcmd

Review

Red blood cells, compasses and snap shots

Joseph F. Hoffman

Department of Cellular and Molecular Physiology, Yale University School of Medicine, New Haven, CT 06520, United States

ARTICLE INFO

Editor: Mohandas Narla

Keywords:
Red blood cells
Sodium
Gardos channel
Unusual cells and adaptations

1. Introduction

This article, similar to those before [1,2], presents a selection of nails for those red blood cell physiologists with hammers. The various topics are not necessarily lost for, like occupants on a carousel, they reappear periodically. Sometimes, however, some articles seem to have been written in invisible ink for the findings reappear de novo unaware of previous studies. This, of course, reflects a diminished interest in searching or familiarity with the past. The hope inherent with this article, with limited coverage of the mentioned topics, is to stimulate interest in thinking about important aspects of red cell properties, like those previously mentioned, which should, in my opinion, not be lost.

2. Control of red blood cell Na and associated properties

While the intracellular content of $Na^+ + K^+$ controls the water content and therefore red cell volume (e.g., [3]) it has yet to be established what cellular membrane mechanisms underlie this control. Excluding carnivores, red cell Na^+ in mammals is primarily modulated by the Na^+/K^+ pump balancing the influx of Na^+ that occurs by various means. Na^+ transport into the cell happens mainly by electrodiffusion but can also occur by Na^+/PO_4^{2-} transport [4], ion pair formation/cotransport [5], by Na^+/H^+ exchange [6,7] and by NaK2Cl co-transport [8,9]. It would be of interest to know just what the contribution/activity each of these mechanisms have to the intracellular Na^+ concentration under physiological circumstances, i.e., red cells in their normal gas-containing plasma at 37 °C. (see [10]). One wonders how Na^+ transport (and metabolism) might change or be influenced in diseased states, altered acid/base balance or variations in plasma Na^+. Alterations in plasma phosphate levels could alter the Na^+ pump activity either directly or by affecting the compartmental concentration of ATP contained in the membrane pool that fuels the Na^+ pump [11]. It is not known whether the cell's Ca^{2+} pump is also affected. Cell volume changes that occur during flow from arterial to venus circulation or through the renal papilla are presumably too transient to effectively alter intracellular Na^+.

A different level of complexity concerns the molecular mechanisms that define the number of Na^+/K^+ pumps in the mature red blood cell membrane. It is known that the number of pumps in immature cells (e.g. erythroid progenitors) exceeds the number found in mature cells but the genetic control mechanisms that are involved are unknown. So too are the mechanisms for Na^+/K^+ pump degradation during maturation of reticulocytes [12,13]. The number of red cell Na^+/K^+ pumps in normal individuals is known to vary by a factor of 3 to 4 that results in an inverse correlation with the cellular control of Na^+ ([14] p. 38). The contribution of the various factors mentioned above to the slope of this relationship is unknown.

3. Divisive topics

It is always curious when a misleading title of an article appears in the literature but none more flawed than one claiming the demise of the “perfect” osmometric properties of human red blood cells [15]. The authors claim that because exposure to certain agents induces a regulated volume decrease (RVD) that red cells can no longer be considered perfect osmometers. Unfortunately, their findings and arguments are in fact irrelevant because they fail to address the issue directly or indirectly, particularly with normal unaltered cells.

It is also interesting how terms creep into the literature such as “suicidal death” of red blood cells and “eryptosis” (e.g., [16,17]). Serious science has historically excluded the use of anthropomorphic terms and concepts. “Eryptosis” erroneously implies an analogy with

E-mail address: joseph.hoffman@yale.edu.

https://doi.org/10.1016/j.bcmd.2018.03.003
Received 20 March 2018; Accepted 20 March 2018

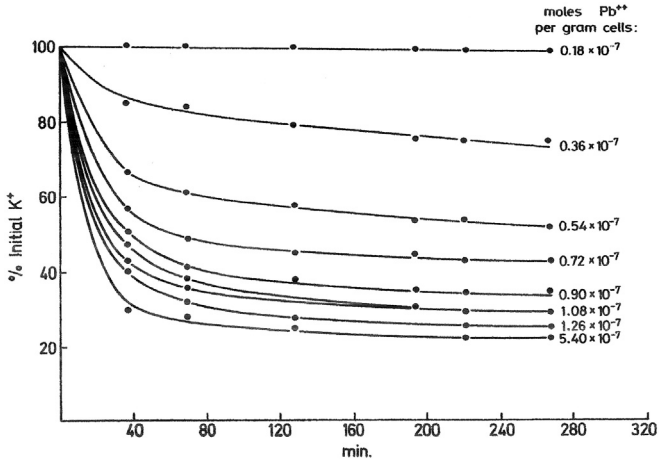

Fig. 1. The time-course of K^+ loss from intact red cells exposed to varying concentrations of Pb^{2+} at 25 °C (legend modified, details in ref. [20], p. 556). Here K^+ loss is either all-or-none in response to exposure to Pb^{2+}.

apoptosis, a nuclear and mitochondrial event. There is no need for presumed laboratory jargon to appear in the literature when relevant and accepted terms are historically already in use.

4. Some Gardos channel perplexities

While much is known about the Gardos channel (inside Ca^{2+}-activated K^+ channel) and it's characteristics in human red blood cells (e.g. [18,19]), there are a number of intriguing and unexplained phenomena that are in much need of study. One is that activation of the channel by Ca^{2+} or Pb^{2+} [20] displays under certain circumstances all-or-none kinetics. All-or-none characteristics are not new to red cells for it is known that in hypotonic hemolysis, under various conditions, hemoglobin either fails to escape from the cells or does so completely attaining diffusion equilibrium [21]. All-or-none loss of K^+ is illustrated in Fig. 1 (Fig. 7 in ref. [20]) for the Gardos channel where intact red cells have been exposed to varying concentrations of Pb^{2+}. Similar results were also seen in resealed ghosts containing different concentrations of Ca^{2+} (Fig. 9 in [20]). In the experiments with Pb^{2+} the cells were shown, by density separation, to either retain their original K^+ content or to lose their K^+ to equilibrium. While the mechanism is not known, this type result implies that different channels in the membrane have different affinities for the divalent ligands (see [20]).

Another unusual if not unique characteristic of the Gardos channel is its sensitivity to temperature [22]. Grygorczyk showed that in excised inside-out patches of human red cells that while the single channel conductance did not change, the open probability *increased* (reversibly) from about 0.1 at 37 °C to about 0.6 at 20 °C. The K^+ flux via the Gardos channel was also shown to increase as the temperature was lowered from 35 °C to 25 °C [18]. An explanation for this unusual if not unique sensitivity to temperature is needed.

Other unexplained properties of the Gardos channel include the effect of external K^+. Activation of the channel has an obligatory requirement for external K^+ [23]. Incubation of Ca^{2+}-loaded resealed ghosts in the absence of external K^+ renders them unresponsive to the subsequent exposure to external K^+. While external protons have been shown to compete with external K^+, protons cannot activate the Gardos channel in the absence of external K^+. The mechanism of action of substances that either stimulate the Gardos channel, such as prostaglandin E_2 [24] or inhibit, such as charybdotoxin or clotrimazole, are also unknown. Perhaps when the atomic structure of the Gardos channel protein has been solved, insight into the ways the effects discussed above may be revealed.

5. Life at high altitude

The physiology associated with adaptations to unusual environments is never more remarkable than those that provide the ability of the Bar-headed Goose (*Anser indicus*) and the Andean goose (*Chloephaga melanoptera*) to fly at very high altitudes. The former goose flies over the Himalayas while the latter goose flies over the Andes mountain ranges. Each species has, for instance, lung and muscle adaptations but none more curious than the mutations that have occurred in their respective hemoglobins (Hb). The Hbs in both species have changes that result in an increased affinity for O_2 which increases its utilization efficiency [25]. It is remarkable that the mutations in Hb of the Bar-headed goose are somewhat different than those in the Andean goose: namely, for instance, in the former the inter-subunit contact is Ala-α119 and Leu-ß55 while in the latter the contact is between Pro-α119 and Ser-ß55. Nevertheless the αß interaction in the two Hbs lies adjacent to each other on the different polypeptide chains. Similar interactions have been extensively studied in other types of birds that also show impressive instances of convergent evolution [26,27].

In addition to the geese, Rippon's griffon, an African vulture, that evidently can fly as high as 11 km, is said to have four types of Hbs, rather than the usual one or two of low flying birds, that provide O_2 loading and unloading functions over a wide range of tensions (see: [28], p. 112). Yaks, also living at high altitude, are said to have four types of HBs in their cells [29].

Emergent studies have recently shown that Sherpas living at high altitude have adaptations that make life at high altitude more compatible. Sherpas are known to have blood Hb concentrations about the same as lowlanders and Han populations [30]. This is different from what happens to lowlanders, Han and even Andean individuals when they adapt at high altitude for they develop a polycythemia that often results in chronic mountain sickness (CMS). Studies have identified in Sherpa populations a mutation in the EGLN1 gene that reduces the effectiveness of hypoxia-inducible factors (HIFs) with regard to their effects on erythropoiesis [31,32]. These are not the only changes associated with Sherpa adaptations at high altitude and there appears to be great interest in studying the various mechanisms involved [33]. Treatment for CMS as well as for acute-mountain disease includes going to a lower altitude (best) and/or the use of acetazolamide [34]. The physiological changes that occur in acute and CMS have yet to be defined as well as the mechanism(s) by which acetazolamide exerts its effect.

It is also of interest that cattle raised or kept at high altitude develop a high-altitude sickness often referred to as ‘Brisket Disease’. The characteristics are of course somewhat different from CMS but the genetic basis is related [35]. As with the human disorder the mechanism (s) involved in the origin of brisket disease is unknown.

Another group of animals that have members living both at low and high altitudes are the *camelidae*. This group is comprised of six species, namely, the bactrain, dromedary, llama, alpaca, guañaco and the vicuña. The first two live essentially at sea level (middle east, Africa and Asia) while the others live mainly in the Andean mountains. The Hbs of the high altitude species display higher O_2 affinities than those at sea level. In addition, all six species appear to have unique and different amino acid substitutions in their respective Hbs [29].

Interestingly, the recent cloning of Woolly mammoth Hb found that certain amino acid substitutions on their ß/δ-globin subunits provided at least one indication of how these ancient animals were able to cope/adapt in their Artic-Ice Age environment [36].

6. Fish without red blood cells

For some red cell aficionados, it may come as a surprise to find a group of vertebrates that live without red blood cells! Nevertheless, the group of fish called ‘ice-fish’ (family *Channichthyidae*), suborder *notothenioiri* represent a case in point [37]. These ice-fish (some 16