Regulatory Peptides 180 (2013) 58–61

Contents lists available at SciVerse ScienceDirect

Regulatory Peptides

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

Review

Erythrocyte-based analgesic peptides

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ARTICLE INFO

Article history:
Received 8 September 2012
Received in revised form 25 October 2012
Accepted 13 November 2012
Available online 7 December 2012

Keywords:
Erythrocrine
Analgesic peptide
Erythrocyte
Hemoglobin
Proteasomal degradation
Hemorphin

ABSTRACT

Human erythrocyte discards the major organelles in a bid to maximize cellular hemoglobin. Hemoglobin, approximately 98% of the intraerythrocytic protein, serves as the principle transport medium of gaseous conveyance. The accumulated data speaks in favor of erythrocyte not merely engaging in gas exchange, but building molecular signaling as a side job during its 4-month sojourn in blood circulation. The production mechanism of erythrocyte-based bioactive peptides is not clear. Recent studies indicate that proteasome and its subunits persist in mature erythrocyte. The intraerythrocytic proteasome is involved in the formation of hemoglobin-derived analgesic peptides and enables erythrocyte to exert the erythrocrine function. Erythrocrine describes erythrocyte for generation and excretion of signaling molecules and has the potential of shedding light on our understanding of novel actions of erythrocyte. Different types of erythrocrine analgesic peptides are originated from the intraerythrocytic degradation of hemoglobin and manifest the systemic influence in physiology and pathophysiology along its travel through the body via the bloodstream. Translational research from bench to bedside will expand our knowledge of erythrocrine concept and facilitate the development of therapeutic strategies for clinical pain.

Contents

1. Introduction

Erythrocyte or red blood cell originally serves as a specialized container of hemoglobin and is engaged in gaseous exchange. Human erythrocyte discards the majority of organelles and metabolic byproducts during maturation. In the absence of transcription and protein synthesis, the function and survival of erythrocytes totally rely on the preexistent proteins and enzymes. Hemoglobin constitutes approximately 98% of the intraerythrocytic protein [1]. A mounting body of evidence indicates that hemoglobin is not just a carrier for oxygen and carbon dioxide in erythrocytes, but known as the precursor of signaling molecules [2]. Proteasome acts as a molecular machine for the controlled proteolysis [3,4]. Recent studies indicate that proteasome and its subunits persist in mature erythrocyte [5]. The intraerythrocytic proteasome contributes to the generation of hemoglobin-derived bioactive peptides and enables erythrocyte to exert an erythrocrine function [6]. Many erythrocrine peptides have defined analgetic effects.

An adult human body harbors 100 trillion cells of about 260 different types [7]. Regarding over 25 trillion erythrocytes being able to reach

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http://dx.doi.org/10.1016/j.regpep.2012.11.003

most parts of our body by means of the blood circulation [8], the local effects and systemic influences of erythrocrine analgesic peptides cannot be disregarded.

2. Analgesic peptides originated from erythrocyte

Hemoglobin, a specialized protein in erythrocyte, acts as the principal transport medium of oxygen and carbon dioxide between the lungs and the tissues. Intriguingly, hemoglobin is involved in the production of signaling molecules which perform a holistic action through erythrocrine. Hemoglobin is copious within the cytoplasm of erythrocytes. One erythrocyte harbors 2.7×10^8 hemoglobin molecules [9]. About 50 intra-erythrocytic hemoglobin fragments have been identified [10]. The fraction of hemoglobin proteolytic segments consists about 0.5% of hemoglobin content in erythrocyte [11].

Hemorphin family contains a set of endogenous morphinomimetic peptides which derive from enzymatic hydrolysis of the β, γ, δ or ε chain of hemoglobin [6,12–14]. The spectrum of hemorphins differs from tripeptide to nonapeptide. The Tyr-Pro-Trp core sequence for all hemorphins resides in position 35–37 of the β, γ, δ or ε chain of human hemoglobin. A limited degradation of hemoglobin gives rise to a series of sequentially overlapping peptides with N- and/or C-terminal extensions of Tyr-Pro-Trp. Hemorphin-3 to -7 represent the C-terminal truncations. The hemorphin sub-families are relevant to N-terminal sequences and classify as hemorphins, V-hemorphins, VV-hemorphins and LVV-hemorphins [6,15]. As shown in Table 1, erythrocytes exhibit the preferential generation and excretion of V-hemorphin-3, V-hemorphin-5, hemorphin-7, and V-hemorphin-7 [8,13]. Hemorphins function as ligands of opiate receptors with their preference to different receptor subtypes [11,16]. Hemorphin-3 demonstrates the indispensable role in binding to μ- and δ-opiate receptors. Hemorphin-7 is in favor of μ-receptor. Hemorphin-5 binds the δ- and κ-opiate receptors and plays the roles of endogenous opiate peptide in vivo. Hemorphin-5 exhibits a dose-related antinociceptive effect in a mouse model of phasic nociception. In vitro hemorphin-7 stimulates β-endorphin and dynorphin release from pituitary tissue [17]. Furthermore, an observation shows that hemorphin-7 is capable of traversing the blood–brain barrier [18]. Accordingly, hemorphins can display analgesic effect mediated by activation of classical opioid peptide pathway [19,20].

Hemorphins interact with different opioid receptors to meet the complementary needs of regulation in analgesia, cognition and behavior [13]. Furthermore, the opioid responses are elicited by an increased level of blood plasma hemorphin-7 in human after long distance running, and thereby reveal euphoretic effects in the post exercise period [21].

In addition, the α-chain of hemoglobin serves as an endogenous precursor of neokyotorphin, a pentapeptide with identical sequence TSKYR to the 137–141 C-terminal segment [22,23] (Table 1). Neokyotorphin is secreted by human erythrocyte and exhibits analgesic activity as a non-classical neuropeptide. Its analgesic effect is not mediated through the opioid pathway since it is not blocked by naloxone, an antagonist of opioid receptor. Neokyotorphin is able to induce analgesia by inhibiting the release of γ-aminobutyric acid in brain [24]. Among the already known erythrocrine peptides, hemorphins and neokyotorphin are shown to engage in pain regulation.

Table 1
Hemoglobin-derived hemorphins and neokyotorphin excreted by human erythrocytes.

Peptide	Sequence	Location in hemoglobin
V-hemorphin-3	Val-Tyr-Pro-Trp	β34–37
V-hemorphin-5	Val-Tyr-Pro-Trp-Thr-Gln	β34–39
Hemorphin-7	Tyr-Pro-Trp-Thr-Gln-Arg-Phe	β35–41
V-hemorphin-7	Val-Tyr-Pro-Trp-Thr-Gln-Arg-Phe	β34–41
Neokyotorphin	Thr-Ser-Lys-Tyr-Arg	α137–141

3. Proteasomal degradation of hemoglobin within erythrocytes

Hemoglobin had been used as model protein in the early research of the ubiquitin–proteasome system. Hemoglobin deficient in native structure such as the unassembled chain in thalassemias is rapidly hydrolyzed by means of nonlysosomal digestion. The in-depth studies of hemoglobin degradation led to the discovery of the ubiquitin–proteasome pathway [3,25]. Proteasome plays a key role in various cellular processes including cell cycle, apoptosis and differentiation by degradation of a variety of regulatory proteins involved in the pathological progression of cancers. A successful translational research of proteasome brings on bortezomib, a reversible proteasome inhibitor, jump from bench to bedside for the clinical treatment of multiple myeloma [26].

Owing to lack of a transcriptome, erythrocyte does not synthesize any nascent hemoglobin. The study of erythrocyte proteome is helpful to uncover its protein metabolism. Proteasome and its subunits are still retained within the mature erythrocyte as identified by proteomic technologies [1,27,28]. Moreover, atomic-force microscopy and electron microscopy disclose the external morphology of intact 20S proteasomal particles in erythrocyte preparations [5]. The 20S proteasome is a conserved multicatalytic proteinase complex. Its catalytic β1, β2, and β5 subunit manifests caspase-like, trypsin-like, and chymotrypsin-like activities, respectively. Proteasome degrades proteins into peptides with length range from 3- to 22-residues [29]. The intraerythrocytic proteasome is the predominant proteolytic enzyme for degradation of oxidative hemoglobin [30]. As indicated in the previous investigations, the unstructured or damaged hemoglobins are degraded by the 20S proteasome without ubiquitination. Erythrocyte is under constant oxidative process due to the substantial flux of oxygen. However, intraerythrocyte proteasome is able to efficiently maintain the activity of proteolytic degradation even in oxidative stress conditions.

Bortezomib, a cell-permeable dipeptide boronate molecule, selectively and reversibly inhibits chymotrypsin-like activity of the proteasome. It has been approved by FDA as a therapeutic drug for treatment of hematologic malignancies and solid tumors [31]. Bortezomib can prevent the production of hemorphins in a dose-dependent manner [6]. The current formulation of bortezomib is merely amenable to the intravenous administration. The pharmacokinetics analysis indicates that bortezomib is preferentially taken by erythrocytes and rapidly metabolized from human plasma [32]. Bortezomib-induced pain is emerging as a main adverse event and a dose-limiting side effect in clinical practice. Even if the pain is invertible in the majority of cases, it can substantially affect the life quality of patients, or even leads to therapy withdrawal [33]. The molecular mechanism of bortezomib-induced pain is not clear. The erythrocrine behavior might provide a different insight into this neuropain. Intraerythrocytic proteasome takes part in the generation of hemoglobin-derived atypical opioid peptides [6]. The physiological yields of erythrocrine analgesic peptides are retarded in virtue of proteasomal inhibition by administration of bortezomib. Subsequently, the perception of bortezomib-induced pain appears.

Erythrocytes are incapable of self-repair by renewal and are unable to resynthesize any nascent hemoglobin for lack of transcriptome and translatome. Disposal of nonfunctional modified hemoglobin would preserve the cellular integrity and expand the lifespan of erythrocyte. Around 20% of the total hemoglobin gradually leaves the intact erythrocyte during its lifetime [34]. Hemoglobin is used as the substrate of intraerythrocytic proteasome. The analgesic peptides are generated via proteasomal cleavage of hemoglobin and released into extracellular milieu (Fig. 1).

Human erythrocytes excrete V-hemorphin-3, V-hemorphin-5, V-hemorphin-7, hemorphin-7 and neokyotorphin into extracellular