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Glutamate release from platelets: Exocytosis versus glutamate transporter reversal

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ABSTRACT

Platelets express neuronal and glial glutamate transporters EAAT 1–3 in the plasma membrane and vesicular glutamate transporters VGLUT 1,2 in the membrane of secretory granules. This study is focused on the assessment of non-exocytotic glutamate release, that is, the unstimulated release, heteroexchange and glutamate transporter reversal in platelets. Using the glutamate dehydrogenase assay, the absence of unstimulated release of endogenous glutamate from platelets was demonstrated, even after inhibition of glutamate transporters and cytoplasmic enzyme glutamine synthetase by DL-threo-β-benzyloxyaspartate and methionine sulfoximine, respectively. Depolarization of the plasma membrane by exposure to elevated [K^+] did not induce the release of glutamate from platelets that was shown using the glutamate dehydrogenase assay and radiolabeled L-[^{14}C]glutamate. Glutamate efflux by means of heteroexchange with transportable inhibitor of glutamate transporters DL-threo-β-hydroxyaspartate (DL-THA) was not observed. Furthermore, the protonophore cyanide-p-trifluoromethoxyphenyl-hydrazon (FCCP) and inhibitor of V-type H^+-ATPase bafilomycin A1 also failed to stimulate the release of glutamate from platelets. However, exocytotic release of glutamate from secretory granules in response to thrombin stimulation was not prevented by elevated [K^+], DL-THA, FCCP and bafilomycin A1.

In contrast to nerve terminals, platelets cannot release glutamate in a non-exocytotic manner. Heteroexchange, transporter-mediated and unstimulated release of glutamate are not inherent to platelets. Therefore, platelets may be used as a peripheral marker/model for the analysis of glutamate uptake by brain nerve terminals only (direct function of transporters), whereas the mechanisms of glutamate release are different in platelets and nerve terminals. Glutamate is released by platelets exclusively by means of exocytosis. Also, reverse function of vesicular glutamate transporters of platelets is rather ambiguous.
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1. Introduction

Nowadays, platelets are in the main stream of neurochemical research because they can play a specific role in a range of neurological disorders (Aliprandi et al., 2005; Behari and Shrivastava, 2013; do Nascimento et al., 2006; Rainesalo et al., 2003; Rolf et al., 1993; Yao et al., 2006; Zoia et al., 2004). Platelets are able: (1) to accomplish glutamate uptake by high-affinity Na^+-dependent glutamate transporters EAAT 1–3 in the plasma membrane, which use Na^+/K^+ gradient as a driving force (Begni et al., 2005; Hoogland et al., 2005; Kasatkina and Borisova, 2010; Mangano and Schwarcz, 1981; Rainesalo et al., 2003); (2) to accumulate glutamate in dense secretory granules by special vesicular glutamate transporters VGLUT 1 and 2, which utilize the proton electrochemical gradient generated by the vacuolar H^+-ATPase (Tremolizzo et al., 2006); (3) to release granular glutamate by means of exocytosis during activation (Borisova et al., 2011a; Tremolizzo et al., 2006); (4) to express NMDA, AMPA, kainate and mGlu 3,4 receptors, which are involved in the regulation of platelet aggregation/activation (Amisten et al., 2008; Franconi et al., 1998; Sun et al., 2009). Glutamate released from platelets acts in an autocrine manner promoting complete platelet activation, and in a paracrine manner increasing the agonist sensitivity of the recruited platelets (Gawaz, 2001).

A number of experimental data declare a tight interdependence between the pathological changes of glutamate transport in brain and consequent alterations in glutamate transport and activity/expression of glutamate metabolizing enzymes in platelets (Aliprandi et al., 2005; Behari and Shrivastava, 2013; do Nascimento

Abbreviations: GABA, gamma-aminobutyric acid; GDH, glutamate dehydrogenase; DL-THA, DL-threo-β-hydroxyaspartate; DL-TBOA, DL-threo-β-benzyloxyaspartate; NMDA, N-methyl-D-aspartate; AMPA, 2-amino-3-(5-methyl-3-oxo-1,2-oxazol-4-yl)propanoic acid; VGLUT, vesicular glutamate transporter; EAAT, excitatory amino-acid transporter; SBFI-AM, sodium-binding benzofuran isophthalate acetoxymethyl ester.

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et al., 2006; Rainesalo et al., 2003; Yao et al., 2006; Zoia et al., 2004). In infantile autism, platelet level of serotonin was significantly increased, whereas glutamate, glutamine, aspartate and GABA contents were considerably decreased in comparison with the control (Rolf et al., 1993). It is noteworthy that higher level of plasma glutamate and lower level of plasma glutamine accompanied high-functioning autism in children (Shimmura et al., 2011). Besides platelets per se, glutamate may also serve as a potent regulator of other cells in situ. Glutamate is recognized as an important signalling molecule in differentiation of megakaryoblastic cells (Genever et al., 1999), in support of self-renewal of pluripotent cells (Cappuccio et al., 2005), and in the interaction of antigen-presenting cells and T lymphocytes (Pacheco et al., 2006). Glutamate released from platelets also contributes to allograft rejection through glutamate receptor signalling (Swaim et al., 2010).

Na^+-dependent glutamate uptake, the exocytotic release of glutamate during platelet activation, as well as glutamate receptor-mediated regulation of platelet aggregation/activation are documented in the literature (Amisten et al., 2008; Borisova et al., 2011a; Franconi et al., 1998; Gawaz, 2001; Kasatkina and Borisova, 2010; Mangano and Schwarcz, 1981; Sun et al., 2009; Tremolizzo et al., 2006). In contrast, non-exocytotic release of glutamate, which is one of the most important characteristics of glutamate transport in presynaptic nerve terminals of the brain (Grewer et al., 2008; Jabaudon et al., 2000; Rossi et al., 2007), has not yet been assessed in platelets. The term "non-exocytotic release of glutamate" is used to describe unstimulated release, release by heteroexchange triggered by transportable inhibitor of glutamate transporters, and transporter-mediated release of glutamate, that is, glutamate transporter reversal.

In nerve terminals, the origin of unstimulated glutamate release has not yet been completely identified, however, it is suggested that the neurotransmitter enters the extracellular space in part due to spontaneous exocytosis, via swelling-activated anion channels, cystine-glutamate exchange and trans-membrane diffusion (Cavelier and Attwell, 2005; Jabaudon et al., 1999; Rutledge et al., 1998). A balance between unstimulated release and uptake of glutamate determines the extracellular level of the neurotransmitter, which is important for tonic activation of post- and presynaptic glutamate receptors. An increased extracellular level of glutamate over-activates glutamate receptors and causes neurotoxicity. In the synaptic cleft (without stimulation), the concentration of glutamate is less than 1 μM, whereas, it consists of ~30 μM in the human plasma (Divino Filho et al., 1998; Aliprandi et al., 2005) that may be considered as the extracellular level of glutamate for platelets. Therefore, the ambient glutamate concentration for platelets is at least ten times higher than that for nerve terminals.

The aim of this study is to assess whether or not non-exocytotic release of glutamate takes place in platelets. The availability of non-exocytotic glutamate release from platelets or lack thereof is of interest because of the following: (1) extracellular glutamate per se is a modulator of platelet function; (2) possible physiological role of platelets in the maintenance of extracellular glutamate homeostasis of the mammalian CNS via removing of excess extracellular glutamate from brain interstitial fluids to blood plasma (Gottlieb et al., 2003; Kuo et al., 2001; O'Kane et al., 1999; Wang et al., 2004); (3) possible contribution of glutamate released from platelets by glutamate transporter reversal to an increase in the plasma glutamate concentration in ischaemic stroke (Castillo et al., 1996) and other neurological disorders (Aliprandi et al., 2005) as well as at the site of injury and in elevated plasma $[K^+]$; and (4) the suggested similarities of platelets with nerve terminals that underlie the possible usage of platelets as a model of glutamate transport in the presynapse, and a peripheral marker for the analysis of the alterations in the functioning of glutamate transporters in the brain.

2. Materials and methods

2.1. Materials

EGTA, HEPES, L-glutamic acid, filipin, glutamate dehydrogenase, apyrase, D-glucose, NAD^+, amphotericin B, thrombin, allophycocyanin-conjugated anti-CD63-mAbs (SAB4700216), gramicidin A (50845) and analytical grade salts were purchased from Sigma-Aldrich (USA). L-[^{14}C]glutamate, aqueous counting scintillant (ACS) were from Amersham (UK). Acridine orange, rhodamine 6G and SBFI-AM (S-1263) were obtained from Molecular Probes (USA), Repel-Silane ES – from Pharmacia.

2.2. Ethics statement

Experiments were carried out in accordance with the European Guidelines and International Laws and Policies. Before starting the experiments, the protocols were approved by the Animal Care and Use Committee of the Palladin Institute of Biochemistry (Protocol from 18/04-2011). Blood sampling from healthy individuals conformed to the guidelines of the Palladin Institute of Biochemistry, National Academy of Sciences of Ukraine, Kyiv.

2.3. Isolation of blood platelets

Blood samples from healthy individuals, as well as rabbit and rat blood samples were processed according to (Borisova et al., 2011a). The pellet was re-suspended in standard salt solution containing (in mM): NaCl – 145.0, KCl – 4.0, $MgCl_2$ – 2.0, NaH_2PO_4 – 1.0, HEPES – 20.0, pH 7.4, EGTA – 2.0 and D-glucose – 10.0.

2.4. Isolation of rat brain nerve terminals (synaptosomes)

Synaptosomes were prepared by differential and Ficoll-400 density gradient centrifugation of rat brain homogenate according to the method in (Cotman, 1974) with slight modifications (Borisova et al., 2011b). Protein concentration was measured as described in Larson et al. (1986).

2.5. Confocal imaging of platelets

To visualize platelets, we used the fluorescent cholesterol-binding polyene antibiotic, filipin, as described in Borisova et al. (2011a), and allophycocyanin-conjugated anti-CD63-mAbs to detect release of dense secretory granules. Platelets were then labelled with anti-CD63-mAb (APC) at 4 °C for 30 min followed by two washing procedures. For confocal imaging, platelets were viewed under the confocal laser scanning microscope LSM 510 META, Carl Zeiss, with objective Plan-Apochromat 100×/1,4 Oil DIC using 405 nm excitation and >505 nm emission for filipin, and 633 and 660 nm for allophycocyanin, respectively. Platelets were evaluated in the thin layer squashed between the glass surfaces preliminarily treated with Repel-Silane ES (to avoid their aggregation/activation) and the images were captured with a digital integrated camera AxioCam HRc.

2.6. Flow cytofluorometry analysis of platelets

Flow cytometry analysis was performed on flow cytometer COULTER EPICS XL (Beckman Coulter, USA) as described in Kasatkina and Borisova (2010).

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