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Na^+/H^+ exchanger in the regulation of platelet activation and paradoxical effects of cariporide

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ABSTRACT

Platelets are anucleated cell fragments derived from mature megakaryocytes and function in hemostasis when the endothelium is injured. Hemostasis involving platelets can be divided into four phases: adhesion, activation, secretion, and aggregation. Platelet activation requires a rise in intracellular Ca^{2+} concentrations and results in both a morphological change and the secretion of platelet granule contents. Na^+/H^+ exchanger isoform 1 (NHE1) regulates the intracellular pH (pH_i) and the volume of platelets. In addition, NHE1 plays a large role in platelet activation. Thrombus generation involves NHE1 activation and an increase in $[Ca^{2+}]_i$, which results from NHE1-mediated Na^+ overload and the reversal of the Na^+/Ca^{2+} exchanger. Cariporide (HOE-642), a potent NHE1 inhibitor, has inhibitory effects on the degranulation of human platelets, the formation of platelet–leukocyte-aggregates, and the activation of the GPIIb/IIIa receptor (PAC-1). However, despite the demonstrated protection against myocardial infarction as mediated by cariporide in patients undergoing coronary artery bypass graft surgery, the EXPEDITION clinical trial revealed that cariporide treatment increased mortality due to thromboembolic stroke. These findings suggest that a better understanding of NHE1 and its effect on platelet function and procoagulant factor regulation is warranted in order to develop therapies using NHE inhibitors.

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Introduction

Na^+/H^+ exchanger isoform 1 (NHE1) is the most abundantly expressed isoform of a family of proteins with nine members, NHE1–NHE9 (Huber et al., 2012). NHE1 plays an important role in regulating H^+ homeostasis and cell volume under physiological conditions via H^+ extrusion and Na^+ influx (Sarigianni et al., 2010). NHE1 has emerged as a therapeutic target molecule for several diseases, which include cardiac ischemia–reperfusion injury after heart failure (Mentzer et al., 2008), myocardium ischemia (Avkiran, 1999), cerebral ischemia–reperfusion injury of ischemic stroke (Leng et al., 2014) and hypoxic ischemic injury of neonatal immature brain injury (Cengiz et al., 2011). The beneficial effects of the blockade of NHE1 function are attributed to the reduction of NHE1-mediated intracellular Na^+ overload, an increase in Ca^{2+} extrusion via Na^+/Ca^{2+} exchange, and a decrease in cell injury after ischemia and reperfusion (Avkiran, 1999; Leng et al., 2014; Mentzer et al., 2008).

In light of the roles of NHE1 in myocardial ischemic injury, several clinical trials with various NHE1 inhibitors have been conducted. A trial evaluating zoniporide in patients at risk for coronary disease undergoing non-cardiac surgery showed no benefit in reducing composite cardiovascular end point (Fleisher et al., 2005). ESCAMI (Evaluation of the Safety and Cardioprotective Effects of Eniporide in Acute Myocardial Infarction) evaluated the inhibitor, eniporide, in patients undergoing thrombolytic therapy or angioplasty surgery, which did not limit myocardium infarction (MI) size or improve clinical outcome (Zeymer et al., 2001). Two clinical trials were conducted to assess cariporide (HOE-642): GUARDIAN (Guard During Ischemia Against Necrosis) and EXPEDITION (Na^+/H^+ Exchange Inhibition to Prevent Coronary Events in Acute Cardiac Condition). In the GUARDIAN study, patients undergoing coronary artery bypass graft surgery (CABG) receiving doses of 120 mg of cariporide had a decreased rate of all-cause mortality and MI (Chaitman, 2003). In the EXPEDITION trial, patients undergoing CABG received cariporide in a 180 mg dose 1 h prior to CABG, 40 mg/h for 24 h after CABG, and 20 mg/h over the subsequent 24 h. Cariporide significantly decreased rates of MI in the treated group (Mentzer et al., 2008). However, despite cariporide's ability to reduce ischemia–reperfusion injury, the clinical trial was terminated because of a high mortality due to ischemic embolic stroke. The increase in ischemic stroke has been hypothesized to result from a reduced procoagulant response and the stimulation of platelet function after the administration of

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NHE1 inhibitor, cariporide, at a high dosage (Mentzer et al., 2008). The mechanisms underlying the adverse effects of cariporide are unknown. These findings prompt us to review the current research of platelet biology and regulation, in particular, the roles of NHE1 in the regulation of platelet function. A better understanding of NHE1's role in platelet function is warranted, which will benefit the development of new strategies to overcome the adverse effects of NHE inhibitors and future applications for the protection against ischemia–reperfusion injury.

Platelet function and regulation

Platelet biology and function

Platelets are anucleated cell fragments derived from mature megakaryocytes (MKs) found in the bone marrow (Schulze and Shivdasani, 2005). The main function of platelets is hemostasis when the endothelium is injured (Ruggeri and Mendolicchio, 2007). Even though the normal role of platelets is to stop bleeding, because of its role in forming blood clots, platelets are involved in various arterial diseases such as heart attack and stroke (Ruggeri, 2002).

In mature MK, microtubules extend the cytoplasm into long processes called "proplatelets" and it is at the tip of these processes that platelets are filled, assembled, and released. In order to increase the number of platelets generated per proplatelet, proplatelet shafts are bifurcated in an event mediated by actin to increase the number of available tips (Hartwig and Italiano, 2003). The mechanisms by which platelets are released from their proplatelet tips have not been completely elucidated, but an intermediate stage between proplatelets and platelets called the 'preplatelet' has been suggested (Thon et al., 2010). Despite our incomplete knowledge regarding this mechanism, it is known that a single MK can lead to the genesis of hundreds or even thousands of individual platelets which culminate in a suicidal event for the original MK (Schulze and Shivdasani, 2005).

Each platelet is about 7 μm^3 in volume and 300 nm in diameter and is discoid in shape (Cimmino and Golino, 2013). In its resting state, the platelet's shape is maintained by a cytoskeleton composed of tubulin and actin polymers (Hartwig and Italiano, 2003). Despite being only a cell fragment and not a complete cell, platelets have a complex structure. They possess a membrane and cytoskeleton, many types of surface receptors, mitochondria, and dense granules and α-granules, which are capable of secreting various compounds (Cimmino and Golino, 2013).

Platelet activation

Hemostasis involving platelets can be divided into four phases: adhesion, activation, secretion, and aggregation (Cimmino and Golino, 2013). Adhesion is the process in which platelets attach to damaged endothelial cells, and this process is mediated by von Willebrand factor (vWF) and collagen. When the vessel wall becomes damaged, collagen beneath endothelial cells becomes exposed to platelets. Collagen is crucial because it can bind to Glycoprotein VI (GPVI), a collagen receptor on platelets, and it is also necessary for immobilizing vWF. Under high shear conditions, vWF is necessary for platelet adhesion via the Glycoprotein Ib-V–IX complex, but this receptor is incapable of binding plasma vWF; it can only bind vWF immobilized by collagen (Ruggeri and Mendolicchio, 2007). Adhesion occurs 1–3 s after injury and results in a monolayer of activated platelets (Kickler et al., 2006).

Platelet activation results in a morphological change and the secretion of platelet granule contents. With the help of actin and myosin in the cytoplasm, the platelet's shape changes from a disc to a compact sphere, which causes the granules to become centralized. This centralization is thought to lead to a more efficient secretion of granule contents (George, 2000). Platelets contain dense granules and α-granules. Dense granules secrete small non-protein molecules such as ADP, serotonin, and Ca^{2+}, while α-granules secrete larger proteins such as vWF and fibrinogen (George, 2000). While activation is often initiated by subendothelial collagen, other agonists include serotonin, thrombin, thromboxane A2 (TXA_2), and ADP (Angiolillo et al., 2010). Since many of the agonists are also secreted by the platelets upon activation, there is a positive feedback loop in clot formation.

Platelet activation also leads to a rise in intracellular Ca^{2+} concentrations ($[Ca^{2+}]_i$). Ca^{2+} is an essential second messenger, and the increase in $[Ca^{2+}]_i$ contributes to both the change in morphology and the secretion of the granule contents. Upon activation, the platelet can increase $[Ca^{2+}]_i$ via Ca^{2+} entry through the plasma membrane or intracellularly via the release of compartmentalized Ca^{2+}. In platelets, the majority of Ca^{2+} is stored in the endoplasmic reticulum (Varga-Szabo et al., 2009).

The final step in hemostasis is the aggregation of platelets. Whereas adhesion is the attachment of platelets to the damaged vessel wall, aggregation is the attachment of platelets to other platelets. Platelet activation activates the Glycoprotein IIb/IIIa (GPIIb/IIIa) receptor, the most abundant receptor on the platelet surface, which is necessary for aggregation. The activated GPIIb/IIIa on one platelet can bind a molecule of fibrinogen. The free end of the fibrinogen molecule is then available to bind to GPIIb/IIIa on another platelet. This results in a fibrinogen link connecting two platelets (Kickler et al., 2006).

Clot formation is essential in hemostasis but would be problematic if it were not controlled. However, the intact endothelium produces various substances that prevent clot formation. Nitric oxide (NO) is one such product, and it can prevent platelet activation in several ways. As mentioned, TXA_2 is a platelet agonist involved in platelet activation, and NO can inhibit the TXA_2 receptor. It does so by activating cGMP-dependent protein kinase, which serves as a catalyst for the phosphorylation of the TXA_2 receptor. Once the receptor is phosphorylated, TXA_2 can no longer participate in platelet activation (Wang et al., 1998). NO can also diminish activation by lowering $[Ca^{2+}]_i$ which is essential in platelet activation. NO causes an increase in NO-stimulated guanylyl cyclase which inhibits intracellular Ca^{2+} release, decreases Ca^{2+} entry, and increases the extrusion rate of Ca^{2+}. Furthermore, NO can also decrease platelet aggregation by reducing the number of GPIIb/IIIa receptors on the surface of platelets (Aszodi et al., 1999).

Another product made by endothelial cells is prostacyclin (PGI_2). Similar to the effects of NO, PGI_2 can prevent aggregation and can reduce Ca^{2+}. When the PGI_2 receptor becomes activated, production of cyclic adenosine monophosphate (cAMP) increases which causes the inhibition of both Ca^{2+} mobilization and granule release. Additionally, increased cAMP leads to the phosphorylation of vasodilator-stimulated phosphoprotein (VASP). The phosphorylation of VASP influences intracellular actin which helps to regulate platelet structure, and it also inactivates the GPIIb/IIIa receptor. The structural change and the inhibition of Ca^{2+} and GPIIb/IIIa receptors not only prevents platelet activation but also returns activated platelets to their resting state (Jin et al., 2005).

Endothelial cells also produce plasminogen activators which convert circulating plasminogen into plasmin. Plasmin, a serine protease prevents platelet aggregation via the proteolysis of platelet surface GPIIb/IIIa receptors and the fibrinogen molecules themselves (Jin et al., 2005). Finally, endothelial cells possess CD39, a membrane glycoprotein. By neutralizing ADP secreted by activated platelets, CD39 has an inhibitory effect on ADP-induced platelet aggregation (Jin et al., 2005).

The paradoxical effect of LDL on NHE and platelet function

High concentrations of low density lipoproteins (LDLs) have commonly been linked to increased platelet sensitivity to agonists, which enhances the platelets' response to stimuli and promotes the positive feedback loop (Nofer et al., 2006). Oversensitivity leads to an increased risk of atherosclerosis and stroke (Hackeng et al., 1999). Native LDLs are thought to work benignly, but elevated levels of native LDLs act as risk factors when defective apoB/E receptors fail to remove them from circulation, which allows them to become oxidized. Once oxidized in sufficient concentrations, LDLs are known to accumulate within the walls

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