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The Na^+/H^+ Exchanger-1 as a New Molecular Target in Stroke Interventions

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1. Introduction

Loss of ion homeostasis plays an important role in the pathogenesis of ischemic cell damage. Ischemia induces accumulation of intracellular Na^+ ($[Na^+]_i$) and Ca^{2+} ($[Ca^{2+}]_i$), and subsequent activation of proteases, phospholipases, and formation of oxygen and nitrogen free radicals. The Na^+/H^+ exchanger (NHEs) family is a group of secondary active membrane transport proteins that catalyze the electroneutral exchange of Na^+ for H^+ and is important in restoring intracellular pH (pH_i) after ischemia-induced intracellular acidosis. Nine isoforms of NHE (NHE1-9) have been identified in mammalian tissues (Orlowski & Grinstein, 2004). These isoforms differ in their tissue expression, subcellular distribution, kinetic properties, inhibitor sensitivity, and physiological functions. NHE-1 is ubiquitously expressed on the plasma membrane of virtually all mammalian cell types (Sardet et al., 1989). NHE-2-4 are expressed on the plasma membrane, predominantly in the epithelia of the kidney and gastrointestinal tract (Orlowski & Grinstein, 2004). NHE-3 is the only isoform known to recycle between the plasma membrane and the endosomal compartment (D'Souza et al., 1998). NHE-5 expression is concentrated in neurons (Attaphitaya et al., 1999) and may modulate the pH of synaptic vesicles (Szaszi et al., 2002). NHE-6 and NHE-9 are expressed predominantly in endosomal vesicles (Nakamura et al., 2005) and NHE-7 localizes to the trans-Golgi network and associated endosomes (Numata & Orlowski, 2001). NHE-8 has been localized to the plasma membrane of renal proximal tubule epithelial cells, and to endosomal vesicles and the trans-Golgi network (Goyal et al., 2003; Nakamura et al., 2005).

NHE-1 is the most extensively studied isoform, and the most abundant isoform in the CNS (Ma & Haddad, 1997; Orlowski et al., 1992). Research over the past two decades has expanded our understanding of the role of NHE-1 beyond that of simply maintenance of ion homeostasis and cell volume, to an emerging picture of a regulator of many cell functions. NHE-1 plays a role in regulation of cell proliferation, migration (Bussolino et al., 1989), and the microglial respiratory burst (Liu et al., 2010). NHE-1 protein consists of 815 amino acids with a calculated molecular weight of 85 kDa. However, NHE-1 has an apparent size of ~110 kDa due to its N- and O- linked glycosylation in the extracellular loop 1. NHE-1 has

two large functional domains, the highly conserved amphipathic N-terminal domain (~500 amino acids), which is responsible for cation translocation, and a less conserved hydrophilic cytoplasmic C-terminal domain (~315 amino acids), which is crucial for modulating NHE-1 activity (Putney et al., 2002). Activation of NHE-1 has been shown to be a pivotal event in cell damage induced by ischemia and reperfusion in the brain (Horikawa et al., 2001; Hwang et al., 2008; Luo et al., 2005), heart (Liu et al., 1997; Murphy et al., 1991; Wang et al., 2003), liver (Gores et al., 1989), and lungs (Rios et al., 2005). Here we will review recent findings implicating NHE-1 activation as a critical event in the pathogenesis of cellular dysfunction after cerebral ischemia, and the growing evidence supporting the use of NHE inhibitors as neuroprotective agents following cerebral ischemia.

2. Na^+/H^+ Exchanger isoform-1 (NHE-1) in cerebral ischemia

Ischemia and reperfusion injury is a complex and incompletely understood phenomenon. Ischemia deprives the cell of the energy required for normal cell function and leads to loss of ionic homeostasis within the cell due to opening of ionotropic glutamate receptors (Nishizawa, 2001) and acid sensing non-glutamate-dependent channels (Xiong et al., 2004), as well as activation of ion transport proteins such as NHE-1, $Na^+/K^+/Cl^-$ cotransporter (NKCCl) (Chen et al., 2005), and the Na^+/Ca^{2+} exchanger (NCXs) (Hoyt et al., 1998). Reperfusion triggers a cascade of intracellular events including release of reactive oxygen species (ROS) and inflammatory mediators, which exacerbate injury and promote cell death. Ischemia and reperfusion induces intracellular acidosis due to a shift from aerobic to anaerobic glycolysis, and leads to an increase in $[Na^+]_i$ and $[Ca^{2+}]_i$ by mechanisms that include the activation of acid responsive ion transporters (Yao & Haddad, 2004). Recent findings from our group and others highlight the important role of NHE-1 in pH_i regulation after cerebral ischemia and reperfusion.

2.1 NHE-1 mediated intracellular pH regulation

To regulate and maintain constant pH_i, eukaryotic cells express plasma membrane ion transporters such as NHE-1 that protect cells from internal acidification by exchanging extracellular Na^+ for intracellular H^+ (Luo et al., 2005). At physiological pH_i, NHE-1 is essentially inactive, despite the large inward Na^+ gradient established by Na^+/K^+- ATPase. However, upon exposure to intracellular acidification, NHE-1 is rapidly activated and uses the electrochemical gradient of Na^+ to pump H^+ out of the cell and restore pH_i. Upon restoration of pH_i, NHE-1 activity returns to steady state levels (Pedersen, 2006). Extracellular acidification (low pH_o) or removal of extracellular sodium suppresses this gradient-driven Na^+/H^+ exchange (Bobulescu et al., 2005). While NHE-1 serves to maintain homeostasis in the face of normal pH_i fluctuations (which result from changes in metabolic activity), profound acidosis after anoxia can induce a NHE-1 mediated parodoxic alkalinization, a so-called "overshoot" of pH_i restoration. We reported that post-anoxia alkalinization is ablated by pharmacological inhibition of NHE-1 and removal of extracellular sodium (Kintner et al., 2005). Protein kinase inhibitors attenuate this alkalinization, suggesting that activation of NHE-1 involves protein phosphorylation and multiple up-stream regulatory pathways such as extracellular signal-regulated kinases (ERK 1/2), protein kinase A (PKA), and protein kinase C (PKC) (Kintner et al., 2005; Luo et al., 2007; Yao et al., 2001).

2.2 Ionic homeostasis and brain cell function

Secondary active ion transport proteins are important in maintaining steady-state intracellular ion concentrations. NHE-1 plays an important role in regulation of many cellular processes in addition to pH_i and cell volume regulation, such as cell growth, proliferation and differentiation, cell migration and adhesion, cellular immunity, and as cytoskeletal scaffolding for the assembly of intracellular signaling complexes (De Vito, 2006; Luo & Sun, 2007; Luo et al., 2005; Meima et al., 2007; Orlowski & Grinstein, 2004; Pedersen et al., 2006; Xue & Haddad, 2010). Due to their high metabolic rate and rapid changes in metabolic demand, neurons are exposed to frequent fluctuations in pH_i, making efficient acid extrusion mechanisms essential for normal neuronal function. Neurons and astrocytes from mice deficient in the NHE-1 protein (NHE-$1^{-/-}$) demonstrate decreased basal pH_i and are unable to recover from an acid load (Luo et al., 2005). NHE-1 is the predominant NHE isoform in the CNS (Ma & Haddad, 1997; Orlowski et al., 1992), and as evidence of its importance in normal neurologic function, NHE-$1^{-/-}$ mice exhibit severe neurologic defects and seizures (Bell et al., 1999; Gu et al., 2001).

2.3 Role of NHE-1 in cellular dysfunction and cerebral injury during *in vivo* ischemia

Results from *in vivo* experimental studies support the importance of ion transport proteins in ischemia-mediated loss of ion homeostasis. NHE-1 activity in astrocytes (Cengiz et al., 2010), neurons (Manhas et al., 2010), and microglia (Shi et al., 2011) is stimulated following cerebral ischemia. Excessive stimulation of NHE-1 leads to intracellular Na^+ overload, and in turn causes a rise in intracellular Ca^{2+} due to increased Ca^{2+} influx via reversal of the Na/Ca exchanger. Thus, NHE-1 activity contributes to cerebral ischemic damage in part by disruption of intracellular Na^+ and Ca^{2+} homeostasis, an event which is characterized by rapid influx of Ca^{2+} and subsequent cell death.

2.3.1 Global ischemia

Global cerebral ischemia entails diminution in cerebral blood flow (CBF) over the entire brain, and is encountered clinically in cardiac arrest. On restoration of CBF, a secondary reperfusion brain injury may occur due to altered ionic homeostasis, increases in ROS, cerebral edema, and inflammatory cascades (Schaller & Graf, 2004). The contribution of NHE-1 activity to global cerebral ischemia has been reported in a number of animal models. In a gerbil model of transient forebrain ischemia, NHE-1 immunoreactivity was markedly increased in CA1 pyramidal neurons as well as in glial cells 4 days following injury, and inhibition of NHE protected CA1 pyramidal neurons and attenuated the activation of astrocytes and microglia (Hwang et al., 2008). Yorkshire-Duroc pigs treated with cariporide (HOE 642), a potent and selective inhibitor of NHE-1, at the onset of a 90 min deep hypothermic circulatory arrest demonstrated improved neurologic recovery (Castellá et al., 2005). Similarly, inhibition of NHE-1 with N-[aminoiminomethyl]-1-methyl-1H-indole-2-carboxamide methanesulfonate (SM-20220) improved neurologic function in a gerbil model of transient global cerebral ischemia (Kuribayashi et al., 2000). Administration of the NHE-1 inhibitor ethylisopropylamiloride (EIPA) prior to bilateral carotid artery occlusion in gerbils resulted in decreased hippocampal neuronal cell death and improved neurologic function (Phillis et al., 1999).

2.3.2 Focal ischemia

Unlike global cerebral ischemia, focal cerebral ischemia entails reduction in regional CBF in a specific vascular territory and is usually encountered clinically as an "ischemic stroke" due to thromboembolic or vaso-occlusive disease. An abundance of *in vivo* studies support the importance of NHE-1 in focal ischemia. The NHE-1 inhibitor SM-20220 reduces infarct size in both transient and permanent focal ischemia models (Kuribayashi et al., 1999). Another NHE inhibitor, Sabiporide, reduces infarct size and edema volume when administered before or after ischemia (Park et al., 2005).

Our investigations into the role of NHE-1 in cerebral ischemia have used both genetic and pharmacologic inhibition of NHE-1 in a mouse transient middle cerebral artery occlusion model (MCAO). Mice treated with HOE 642, a potent and selective inhibitor of NHE-1, prior to MCAO demonstrated a 35% reduction in infarct volume compared to vehicle treated controls. NHE-1 heterozygous mice (NHE-$1^{+/-}$), which demonstrate a ~ 70% reduction in NHE-1 protein expression, exhibited a similar reduction in infarct volumes, establishing the importance of NHE-1 over other NHE isoforms in the CNS (Luo et al., 2005; Wang et al., 2008). With T2-weighted and Diffusion Weighted MRI, we further confirmed that NHE-$1^{+/+}$ mice treated with HOE 642 immediately prior to reperfusion or 60 minutes post-reperfusiion, exhibited a significant reduction in infarct volume compared to NHE-$1^{+/+}$ vehicle control mice. NHE-$1^{+/-}$ mice demonstrated a significant reduction in infarct volume on T2 MRI at 72 hours after injury (Ferrazzano et al., 2011). These findings suggest that elevated NHE-1 activity contributes to neuronal injury following ischemia and reperfusion. We subsequently revealed that focal cerebral ischemia triggers a transient stimulation of the extracellular signal-regulated kinase/p90 ribosomal S6 kinase (ERK/$p90^{RSK}$) pathway that contributes to ischemic damage in part via phosphorylation of NHE-1 protein (Manhas et al., 2010). The NHE-1-mediated $[Na^+]_i$ overload causes reverse function of the Na^+/Ca^{2+} exchanger, elevating $[Ca^{2+}]_i$ and enhancing the p38 mitogen-activated protein kinase (MAPK) and/or nuclear factor kappa-light-chain-enhancer of activated B cells (NF-kB) (Liu et al., 2010). NHE-1 activity also plays a detrimental role in mitochondrial Ca^{2+} overload and mitochondrial dysfunction after ischemia as evidenced by attenuation of ischemia-induced cytochrome C release from mitochondria after NHE-1 inhibition (Wang et al., 2008). Interestingly, when NHE-1 activity is blocked either pharmacologically or by genetic knockdown, microglia activation and proinflammatory cytokine formation is significantly reduced in ischemic brains after MCAO (Shi et al., 2011). Taken together, these results strongly support that NHE-1 is activated after cerebral ischemia and worsens ischemic brain injury.

2.3.3 Hypoxia/Ischemia

Hypoxia-ischemia (HI) is a common cause of brain injury in neonates (Ferriero, 2004). We recently investigated the role of NHE-1 using a mouse model of neonatal hypoxia-ischemia as described by Vannucci (Vannucci & Vannucci, 2005). In these studies, post-natal day 9 mice (P9) underwent unilateral carotid artery ligation and subsequent exposure to 55 minutes of 8% O_2. Following carotid ligation, mice were treated with HOE 642 either immediately before or 10 minutes following exposure to hypoxia (Cengiz et al., 2010). Following HI, vehicle-treated control brains exhibited astrogliosis in the ipsilateral hippocampus, and reactive astrocytes expressed an abundant level of NHE-1. Inhibition of NHE-1 before or after HI resulted in decreased neurodegeneration in striatum, thalamus

and hippocampus and improved performance on tests of motor learning and memory (Cengiz et al., 2010). These findings suggest that NHE-1 mediated disruption of ionic homeostasis can contribute to CA1 pyramidal neuronal injury after neonatal HI**.** Moreover, T2 weighted and Diffusion Tensor (DTI) MRI revealed that NHE-1 inhibition with HOE 642 after HI resulted in improved white matter injury in the corpus callosum, which correlated with improvements in memory and learning (Cengiz et al., 2011).

2.4 Role of NHE-1 in cellular dysfunction during *in vitro* ischemia

Extensive *in vitro* studies have established that ischemia stimulates NHE-1 by reduction in pH_i or via signaling pathways such as ERK-$p90^{rsk}$, PKA or PKC (Dunbar & Caplan, 2001; Herrera et al., 1994; Kintner et al., 2007a; Li et al., 2004). The role of NHE-1 in cerebral ischemia has been mainly examined in two types of *in vitro* ischemic models, oxygen glucose deprivation/reoxygenation (OGD/REOX) or the hypoxic, acidic, ion-shifted Ringers's solution (HAIR). Superfused brain slices also represent a useful preparation to study acid-base disturbance that occurs in the mammalian brain during *in vitro* ischemic conditions.

2.4.1 Cell cultures

NHE-1 activity is stimulated during *in vitro* ischemia and subsequent reoxygenation and contributes substantially to neuronal and glial cell injury. Acutely isolated CA1 neurons exhibit a tri-phasic response to 5 minutes of anoxia. During anoxia, an initial acidification progresses to alkalinization that is followed by further alkalinization on exposure to reoxygenation. This alkalinization is attenuated by reduction of external pH, removal of extracellular sodium, or inhibition of NHE-1 (Sheldon & Church, 2002; Yao et al., 2001). Additionally, inhibition of PKA can block post-anoxia alkalinization, suggesting cAMP-dependent signaling pathways for NHE-1 activation (Sheldon & Church, 2002).

We have demonstrated that NHE-1 is essential in pH_i regulation using an internal acid load in cultured cortical neurons (Luo et al., 2005). Additionally, we found that activation of NHE-1 after OGD/REOX results in a significant increase in neuronal $[Na^+]_i$. This rise in $[Na^+]_i$ following OGD is significantly attenuated in HOE 642-treated or NHE-$1^{-/-}$ neurons, and cell death is reduced (Luo et al., 2005). In a separate study, we demonstrated that NHE-1-mediated Na^+ entry leads to reverse activation of the Na^+/Ca^{2+} exchanger (NCX_{rev}) and rise in $[Ca^{2+}]_i$, which contribute to the selective dendritic vulnerability to *in vitro* ischemia (Kintner et al., 2010). Taken together, our studies suggest that NHE-1 activity in neurons is significantly stimulated in response to the metabolic acidification associated with an ischemic insult. This ischemia-induced increase in NHE-1 activity causes intracellular Na^+ and Ca^{2+} overload, and eventually leads to cell death.

In another series of studies, we examined the role of NHE-1 in ischemic astrocyte damage using OGD/REOX in cultured cortical astrocytes and found that NHE-1 is the primary pH regulatory mechanism after ischemia. Astrocyte NHE-1 activity is increased by ~ 1.8 fold during REOX (Kintner et al., 2004), and depends on ERK1/2 signaling pathways (Kintner et al., 2005). OGD/REOX results in a drop in pH_i by 0.29 pH units (Kintner et al., 2004), and inhibition of NHE-1 results in a further decrease of pH_i. Additionally, we observed that OGD/REOX triggers a ~5-fold increase in $[Na^+]_i$ and 26% increase in astrocyte cell volume. This increase in $[Na^+]_i$ and cell swelling are significantly reduced either with HOE 642 treatment or in NHE-$1^{-/-}$ astrocytes (Kintner et al., 2004). Using the HAIR model in

astrocytes, we found a similar increase in $[Na^+]_i$ which could be abolished by the NHE-1 inhibitor HOE 642 (Kintner et al., 2007b). It has been reported that the expected rise in $[Ca^{2+}]_i$ after HAIR exposure is inhibited by NHE-1 inhibition with HOE 694 (Bondarenko et al., 2005). Taken together, these results indicate that NHE-1 activity raises $[Na^+]_i$ which fosters reversal of the Na^+/Ca^+ exchanger leading to increased intracellular Ca^{2+} and astrocyte cell death.

More recently, new evidence supports a role of NHE-1 in microglial pH_i regulation. Microglia activation by lipopolysaccharide (LPS), phorbol myristate acetate (PMA), or OGD/REOX triggers a concurrent stimulation of NHE-1 and NADPH oxidase (Liu et al., 2010). The elevation in NHE-1-mediated H^+ extrusion prevents intracellular acidosis, allowing for sustained NADPH oxidase function (Liu et al., 2010). Moreover, the coupling of NHE-1 activation with NCX_{rev} activates $[Na^+]_i$ and $[Ca^{2+}]_i$ dependent signaling, which promotes the microglial respiratory burst and production of proinflammatory cytokines (Liu et al., 2010).

2.4.2 Brain slice

Few studies have used brain slice preparations to examine acid-base homeostatic disturbances during ischemia. In hippocampal slices, hypoxia induces a significant drop in both pH_i and pH_o, and a brief alkaline peak is also occasionally observed (Fujiwara et al., 1992; Melzian et al., 1996; Roberts & Chih, 1997). In slice preparations from various brain regions, hypoxia causes acidosis with an approximately 0.8-1.2 pH_i unit drop (Ballanyi et al, 1996; Knopfel et al., 1998; Pirttila & Kauppinen, 1994). Cytosolic calcium changes are observed during ischemia in cortical brain slices that can be only partially inhibited by combined blockade of ion channels (Bickler and Hansen, 1994). Only one report shows a direct involvement of NHE mediated pH_i regulation in slice preparations. In brainstem slices from neonatal rats exposed to 10 minutes of anoxia, intracellular pH drops by 0.1-0.3 pH units in neurons. Inhibition of NHE with amiloride increases this anoxia-induced intracellular acidification (Chambers-Kersh et al., 2000).

2.5 NHE-1 inhibitors and potential therapies

Despite decades of research, the effective treatment and prevention of cerebral ischemic injury remains challenging. Inhibition of NHE-1 with either pharmacological agents or genetic ablation has been demonstrated to significantly reduce brain damage after ischemic insult, in both *in vitro* and *in vivo* models. These encouraging findings suggest the potential use of NHE inhibitors as neuroprotective therapies after cerebral ischemia.

2.5.1 Pharmacological approach

Two major classes of pharmacological agents are currently used to inhibit NHE-1 activity (Putney et al., 2002). The first class of drugs includes amiloride and its 5' alkyl-substituted derivatives (Counillon et al., 1993; Yu et al., 1993), such as ethylisopropylamiloride (EIPA), dimethylamiloride (DMA), 5-*N* (methylpropyl)amiloride (MPA), 5-(N-methyl-N-isobutyl)-amiloride (MIBA), and 5-(N, N-hexamethylene) amiloride (HMA). These agents are more effective inhibitors of NHE-1 than amiloride but have relatively weak selectivity toward NHE-1. The simultaneous replacement of the pyrazine ring by a phenyl and of the 6-chloro by sulfomethyl leads to another class of inhibitors that includes the benzoylguanidines and derivatives such as HOE 694 (Counillon et al., 1993) and HOE 642 (cariporide) (Scholz et al.,

1995). Both classes are more specific for NHE-1 than NHE-3, with the amiloride compounds demonstrating ~10^2-fold increased specificity and the HOE compounds ~10^3- to 10^5-fold more NHE-1 specificity. The HOE compounds are viewed as the most promising agents for treatment of ischemia-reperfusion injury due to their selectivity for NHE-1, and excellent solubility, resorption, and bioavailability profiles (Scholz et al., 1999; Baumgarth et al., 1997; Xue & Haddad, 2010). HOE compounds are competitive inhibitors of Na^+ binding at the extracellular cation-binding site (Baumgarth et al., 1997; Counillon et al., 1993; Kinsella & Aronson, 1981; Mahnensmith & Aronson, 1985), while the amiloride derivatives also act non-competitively (Warnock et al., 1988). More recently, several new molecules have been designed as potential NHE blockers based on the bicyclic template, including SM-20220, SM-20550, BMS-284640, T-162559, and TY-12533, which have also shown promising results in *in vivo* studies of cerebral ischemia (Kitayama et al., 2001). The IC_{50} for the human NHE-1 are as follows: Amiloride = 10.7 μM, Cariporide = 0.08 μM, T-165229 = 13 nM. Importantly, the NHE inhibitors HOE 642 and SM-20220 not only reduce cell death and edema, but also improve neurological function in *in vivo* ischemia models, and have demonstrated benefits when administered after ischemia (Kintner et al., 2007b; Kuribayashi et al., 2000).

2.5.2 Transgenic approach

While pharmacological studies indicate that NHE-1 plays a central role in cerebral ischemia-reperfusion injury, the use of pharmacologic inhibitors to study ion transport function raises questions regarding dosing, absorption, species specific T1/2, and non-specific effects. For this reason, confirmation by an alternative method using NHE-1 knockdown mice is warranted. NHE-$1^{-/-}$ mice exhibit neurologic abnormalities, seizures, ataxia, and growth retardation, and do not survive into adulthood (Bell et al., 1999; Gu et al., 2001). Therefore, NHE-$1^{-/-}$ mice are useful for cultures of NHE-1 null neurons, astrocytes and microglia, but cannot be used for *in vivo* studies. NHE-$1^{+/-}$ mice express <50% of NHE-1 protein levels, and are useful for *in vivo* studies of the role of NHE function after cerebral ischemia. A marked decrease of infarct volume, microglial activation and proinflammatory cytokine formation is found in NHE-$1^{+/-}$ mice after MCAO (Luo et al., 2005). NHE-$1^{+/-}$ and NHE-$1^{-/-}$ cortical neurons and astrocytes demonstrate decreased cell death after OGD/REOX (Luo et al., 2005).

The fact that NHE-1 inhibitors applied during or after cerebral ischemia protect the brain against ischemic damage is now well established in animal studies. Despite the uniformity of results from animal models, a number of challenges remain before NHE-1 inhibitors can be translated into clinical use. Questions regarding safety, optimal dose, and timing of administration remain to be addressed, and large animal studies demonstrating improved functional outcomes are still lacking.

3. Conclusion

NHE-1 plays a pivotal role in maintaining tissue ionic homeostasis under normal physiological conditions. However, excessive stimulation of NHE-1 appears to be a major contributor to cellular damage in ischemic conditions. The proposed mechanism for injury induced by NHE-1 activation includes accumulation of $[Na^+]_i$, subsequent $[Ca^{2+}]_i$ overload via reverse activation of the Na^+/Ca^{2+} exchanger, and eventual cell death. Additionally, activation of MAPKs, and release of excitatory amino acids and ROS also contribute to cell

damage and death after ischemia. NHE-1 inhibitors have been demonstrated to be neuroprotective in both *in vitro* and *in vivo* ischemia models, making NHE-1 an attractive therapeutic target for cerebral ischemia. Thus, mechanisms of NHE-1 activation in ischemia continue to present an interesting focus for future research in this field.

4. Acknowledgment

This work was supported by NIH grants R01NS48216 and R01NS38118 (D. Sun), 1UL1RR025011 from the Clinical and Translational Science Award (CTSA) program of the National Center for Research Resources (NCRR) (P. Ferrazzano), and P30HD03352 (Waisman Center).

5. References

Attaphitaya, S., Park, K. & Melvin, J.E. (1999). Molecular cloning and functional expression of a rat Na^+/H^+ exchanger (NHE5) highly expressed in brain. *Journal of Biological Chemistry,* 274, 4383-4388.

Ballanyi, K., Doutheil, J. & Brockhaus, J. (1996). Membrane potentials and microenvironment of rat dorsal vagal cells in vitro during energy depletion. *Journal of Physiology,* 495, 769–784.

Baumgarth, M., Beier, N. & Gericke, R. (1997). (2-Methyl-5-(methylsulfonyl)benzoyl) guanidine Na^+/H^+ antiporter inhibitors. *Journal of Medicinal Chemistry,* 40, 2017–2034.

Bell, S.M., Schreiner, C.M., Schultheis, P.J., Miller, M.L., Evans, R.L., Vorhees, C.V., Shull, G.E. & Scott, W.J. (1999). Targeted disruption of the murine *Nhe1* locus induces ataxia, growth retardation, and seizures. *American Journal of Physiology,* 276, C788 – C795.

Bickler, P.E. & Hansen, B.M. (1994). Causes of calcium accumulation in rat cortical brain slices during hypoxia and ischemia: role of ion channels and membrane damage. *Brain Research,* 665, 269-276.

Bobulescu, I.A., Di Sole, F. & Moe, O.W. (2005). Na^+/H^+ exchangers: physiology and link to hypertension and organ ischemia. *Current Opinion in Nephrology and Hypertension,* 14: 485-494.

Bondarenko, A., Svichar, N. & Chesler, M. (2005). Role of Na^+-H^+ and Na^+-Ca^{2+} exchange in hypoxia-related acute astrocyte death. *Glia,* 49, 143-152.

Bussolino, F., Wang, J.M., Turrini, F., Alessi, D., Ghigo, D., Costamagna, C., Pescarmona, G., Mantovani, A. & Bosia, A. (1989). Stimulation of the Na^+/H^+ exchanger in human endothelial cells activated by granulocyte- and granulocyte- macrophage-colony-stimulating factor. Evidence for a role in proliferation and migration. *Journal of Biological Chemistry,* 264, 18284–18287.

Castellá, M., Buckberg, G.D. & Tan, G. (2005). Neurologic preservation by Na^+-H^+ exchange inhibition prior to 90 minutes of hypothermic circulatory arrest. The *Annals of Thoracic Surgery,* 79, 646-654.

Cengiz, P., Kleman, N., Uluc, K., Kendigelen, P., Hagemann, T., Akture, E., Messing, A., Ferrazzano, P. & Sun, D. (2010). Inhibition of Na^+/H^+ exchanger isoform 1 is

neuroprotective in neonatal hypoxic ischemic brain injury. *Anitoxidants and Redox Signaling,* 14, 1803-1813.

Cengiz, P., Uluc, K., Kendigelen, P., Akture, E., Hutchinson, E., Song, C., Zhang, L., Lee, J., Budoff, G., Meyerand, E., Sun, D. & Ferrazzano, P. (2011). Chronic neurological deficits in mice after perinatal hypoxia and ischemia correlate with hemispheric tissue loss and white matter inury detected by MRI. *Developmental Neuroscience,* (in press).

Chambers-Kersh, L., Ritucci, N.A., Dean, J.B. & Putnam, R.W. (2000). Response of intracellular pH to acute anoxia in individual neurons from chemosensitive and nonchemosensitive regions of the medulla. *Advances in Experimental Medicine and Biology*, 475, 453–464.

Chen, H., Luo, J., Kintner, D.B., Shull, G.E. & Sun, D. (2005). Na+-dependent chloride transporter (NKCC1)-null mice exhibit less gray and white matter damage after focal cerebral ischemia. *Journal of Cerebral Blood Flow and Metabolism,* 25, 54-66.

Counillon, L., Scholz, W., Lang, H.J. & Pouyssegur, J. (1993). Pharmacological characterization of stably transfected Na^+/H^+ antiporter isoforms using amiloride analogs and a new inhibitor exhibiting antiischemic properties. *Molecular Pharmacology,* 44, 1041–1045.

De Vito, P. (2006). The sodium/hydrogen exchanger: a possible mediator of immunity. *Cell Immunology*, 240, 69–85.

D'Souza, S., Garcia-Cabado, A., Yu, F., Teter, K., Lukacs, G., Skorecki, K., Moore, H.P., Orlowski, J. & Grinstein, S. (1998). The epithelial sodiumhydrogen antiporter Na^+/H^+ exchanger 3 accumulates and is functional in recycling endosomes. *Journal of Biological Chemistry,* 273, 2035-2043.

Dunbar, L.A. & Caplan, M.J. (2001). Ion pumps in polarized cells: sorting and regulation of the Na^+, K^+- and H^+, K^+-ATPases. *Journal of Biological Chemistry,* 276, 29617–29620.

Ferrazzano, P., Shi, Y., Manhas, N., Wang, Y., Hutchinson, B., Chen, X., Chanana, V., Gerdts, J., Meyerand, M.E. & Sun, D. (2011). Inhibiting the Na^+/H^+ exchanger reduces reperfusion injury: a small animal MRI study. *Frontiers in BioScience,* 3, 81-88.

Ferriero, D.M. (2004). Neonatal brain injury. *The New England Journal of Medicine,* 351, 1985-1995.

Fujiwara, N., Abe, T., Endoh, H., Warashina, A. & Shimoji, K. (1992). Changes in intracellular pH of mouse hippocampal slices responding to hypoxia and/or glucose depletion. *Brain Research,* 572, 335–339.

Gores, G.J., Nieminen, A.L., Wray, B.E., Herman, B. & Lemasters, J.J. (1989). Intracellular pH during "chemical hypoxia" in cultured rat hepatocytes. Protection by intracellular acidosis against the onset of cell death. *Journal of Clinical Investigation*, 83, 386–396.

Goyal, S., Heuvel, V.G. & Aronson, P.S. (2003). Renal expression of novel Na^+/H^+ exchanger isoform NHE8. *American Journal of Physiology Renal Physiology,* 284, F467-F473.

Gu, X.Q., Yao, H. & Haddad, G.G. (2001). Increased neuronal excitability and seizures in the Na^+/H^+ exchanger null mutant mouse. *American Journal of Physiology Cell Physiology,* 281, C496–C503.

Herrera, V.L., Cova, T., Sassoon, D. & Ruiz-Opazo, N. (1994). Developmental cell-specific regulation of $Na^{(+)}$-$K^{(+)}$-ATPase alpha 1-, alpha 2-, and alpha 3-isoform gene expression. *American Journal of Physiology*, 266, C1301–C1312.

Horikawa, N., Nishioka, M., Itoh, N., Kuribayashi, Y., Matsui, K. & Ohashi, N. (2001) The Na^+/H^+ exchanger SM-20220 attenuates ischemic injury in *in vitro* and *in vivo* models. *Pharmacology*, 63, 76–81.

Hoyt, K.R., Arden, S.R., Aizenman, E. & Reynolds, I.J. (1998). Reverse Na^+/Ca^{2+} exchange contributes to glutamate-induced intracellular Ca^{2+} concentration increases in cultured rat forebrain neurons. *Molecular Pharmacology,* 53, 742-749.

Hwang, I.K., Yoo, K.Y., An, S.J., Li, H., Lee, C.H., Choi, J.H., Lee, J.Y., Lee, B.H., Kim, Y.M., Kwon, Y.G. & Won, M.H. (2008). Late expression of Na^+/H^+ exchanger 1 (NHE1) and neuroprotective effects of NHE inhibitor in the gerbil hippocampal CA1 region induced by transient ischemia. *Experimental Neurology*, 212, 314-323.

Kinsella, J.L. & Aronson, P.S. (1981). Amiloride inhibition of the Na^+-H^+ exchanger in renal microvillus membrane vesicles. *American Journal of Physiology,* 241, F374–F379.

Kintner, D.B., G. Su, G., Lenart, B., Ballard, A.J., Meyer, J.W., Ng, L.L., Shull, G.E. & Sun, D. (2004). Increased tolerance to oxygen and glucose deprivation in astrocytes from Na^+/H^+ exchanger isoform 1 null mice. *American Journal of Physiology Cell Physiology,* 287, C12-C21.

Kintner, D.B., Look, A., Shull, G.E. & Sun, D. (2005). Stimulation of astrocyte Na^+/H^+ exchange activity in response to *in vitro* ischemia in part depends on activation of extracellular signal-regulatory kinase. *American Journal of Physiology Cell Physiology,* 289, C934-C945.

Kintner, D.B., Luo, J., Gerdts, J., Ballard, A.J., Shull, G.E. & Sun, D. (2007a). Role of Na^+-K^+-Cl^- cotransport and Na^+/Ca^{2+} exchange in mitochondrial dysfunction in astrocytes following *in vitro* ischemia. *American Journal of Physiology Cell Physiology,* 292, C1113–C1122.

Kintner, D.B., Wang, Y. & Sun, D. (2007b). The role of membrane ion transport proteins in cerebral ischemic damage. *Frontiers in Bioscience,* 12, 762-770.

Kintner, D.B., Chen, X., Currie, J., Chanana, V., Ferrazzano, P., Baba, A., Matsuda, T., Cohen, M., Orlowski, J., Chiu, S.Y., Taunton, J. & Sun, D. (2010). Excessive Na^+/H^+ exchange in disruption of dendritic Na^+ and Ca^{2+} homeostasis and mitochondrial dysfunction following *in vitro* ischemia. *Journal of Biological Chemistry*, 285, 35155-35168.

Kitayama, J., Kitazono, T., Yao, H., Ooboshi, H., Takaba, H., Ago, T., Fujishima, M. & Ibayashi, S. (2001). Inhibition of Na^+/H^+ exchanger reduces infarct volume of focal cerebral ischemia in rats. *Brain Research,* 922, 223-228.

Knopfel, T. Tozzi, A., Pisani, A., Calabresi, P. & Bernardi, G. (1998). Hypoxic and hypoglycaemic changes of intracellular pH in cerebral cortical pyramidal neurons. *Neuroreport,* 9, 1447–1450.

Kuribayashi, Y., Horikawa, N., Itoh, N., Kitano, M. & Ohashi, N. (1999). Delayed treatment of Na^+/H^+ exchange inhibitor SM-20220 reduces infarct size in both transient and

permanent middle cerebral artery occlusion in rats. *International Journal of Tissue Reactions,* 21, 29-33.

Kuribayashi, Y., Itoh, N., Horikawa, N. & Ohashi, N. (2000). SM-20220, a potent Na^+/H^+ exchange inhibitor, improves consciousness recovery and neurological outcome following transient cerebral ischaemia in gerbils. *Journal of Pharmacy and Pharmacology,* 52, 441-444.

Li, C., Grosdidier, A., Crambert, G., Horisberger, J.D., Michielin, O. & Geering, K. (2004). Structural and functional interaction sites between Na,K-ATPase and FXYD proteins. *Journal of Biological Chemistry,* 279, 38895–38902.

Liu, H., Cala, P.M. & Anderson, S.E. (1997). Ethylisopropylamiloride diminishes changes in intracellular Na, Ca and pH in ischemic newborn myocardium. *Journal of Molecular and Cellular Cardiology*, 29, 2077–2086.

Liu, Y., Kintner, D.B., Chanana, V., Algharabli, J., Chen, X., Gao, Y., Chen, J., Ferrazzano, P., Olson, J.K. & Sun, D. (2010). Activation of microglia depends on Na^+/H^+ exchange-mediated H^+ homeostasis. *Journal of Neuroscience,* 30, 15210–15220.

Luo, J. & Sun, D. (2007). Physiology and Pathophysiology of Na^+/H^+ Exchange Isoform 1 in the Central Nervous System. *Current Neurovascular Research,* 4, 205-215.

Luo, J., Chen, H., Kintner, D.B., Shull, G.E. & Sun, D. (2005). Decreased neuronal death in Na^+/H^+ exchanger isoform 1-null mice after *in vitro* and *in vivo* ischemia. *Journal of Neuroscience,* 25, 11256-11268.

Luo, J., Kintner, D.B., Shull, G.E. & Sun, D. (2007). ERK1/2-$p90^{RSK}$-mediated Phosphorylation of Na+/H+ Exchanger Isoform 1, a role in ishcemic neuronal death. Journal of Biological Chemistry, 282, 28274-28284.

Ma, E. & Haddad, G.G. (1997). Expression and localization of Na^+/H^+exchangers in rat central nervous system. *Neuroscience*, 79, 591–603.

Mahnensmith, R.L. & Aronson, P.S. (1985). Interrelationships among quinidine, amiloride, and lithium as inhibitors of the renal Na^+-H^+ exchanger. *Journal of Biological Chemistry,* 260, 586–592.

Manhas, N., Shi, Y., Taunton, J. & Sun, D. (2010). $p90^{(RSK)}$ activation contributes to cerebral ischemic damage via phosphorylation of $Na^{(+)}/H^{(+)}$ exchanger isoform 1. *Journal of Neurochemistry*, 114, 1476-1486.

Meima, M.E., Mackley, J.R. & Barber, D.L. (2007). Beyond ion translocation: structural functionsof the sodium-hydrogen exchanger isoform-1. *Current Opinion in Nephrology and Hypertension,* 16, 365–372.

Melzian, D., Scheufler, E., Grieshaber, M. & Tegtmeier, F. (1996). Tissue swelling and intracellular pH in the CA1 region of anoxic rat hippocampus. *Journal of Neuroscience Methods,* 65, 183–187.

Murphy, E., Perlman, M., London, R.E. & Steenbergen, C. (1991). Amiloride delays the ischemia-induced rise in cytosolic free calcium. *Circulation Research*, 68, 1250–1258.

Nakamura, N., Tanaka, S., Teko, Y., Mitsui, K. & Kanazawa, H. (2005). Four Na^+/H^+ exchanger isoforms are distributed to golgi and post-golgi compartments and are involved in organelle pH regulation. *Journal of Biological Chemistry,* 280, 1561-1572.

Nishizawa, Y. (2001). Glutamate release and neuronal damage in ischemia. *Life Sciences*, 69, 369-381.

Numata, M. & Orlowski, J. (2001). Molecular cloning and characterization of a novel $(Na^+,K^+)/H^+$ exchanger localized to the trans-Golgi network. *Journal of Biological Chemistry,* 276, 17387-17394.

Orlowski, J. & Grinstein, S. (2004). Diversity of the mammalian sodium/proton exchanger SLC9 gene family. *Pflugers Arch- European Journal of Physiology,* 447, 549-565.

Orlowski, J., Kandasamy, R.A. and Shull, G.E. (1992). Molecular cloning of putative members of the Na/H exchanger gene family. cDNA cloning, deduced amino acid sequence, and mRNA tissue expression of the rat Na/H exchanger NHE-1 and two structurally related proteins. *Journal of Biological Chemistry,* 267, 9331–9339.

Park, H.S., Lee, B.K., Park, S., Kim, S.U., Lee, S.H., Baik, E.J., Lee, S., Yi, K.Y., Yoo, S.E., Moon, C.H. & Jung, Y.S. (2005). Effects of sabiporide, a specific Na^+/H^+ exchanger inhibitor, on neuronal cell death and brain ischemia. *Brain Research,* 1061, 67-71.

Pedersen, S.F. (2006). The Na^+/H^+ exchanger NHE1 in stress-induced signal transduction: implications for cell proliferation and cell death. *Pflugers Archiv - European Journal of Physiology*, 452, 249–259.

Pedersen, S.F., O'Donnell, M.E., Anderson, S.E. & Cala, P.M. (2006). Physiology and pathophysiology of Na^+/H^+ exchange and Na^+-K^+-$2Cl^-$ cotransport in the heart, brain, and blood. *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology,* 291, R1–R25.

Phillis, J.W., Estevez, A.Y., Guyot, L.L. & O'Regan, M.H. (1999). 5-(N-Ethyl-N-isopropyl)-amiloride, an Na^+-H^+ exchange inhibitor, protects gerbil hippocampal neurons from ischemic injury. *Brain Research,* 839, 199-202.

Pirttila, T.R. & Kauppinen, R.A. (1994). Regulation of intracellular pH in guinea pig cerebral cortex ex vivo studied by ^{31}P and ^{1}H nuclear magnetic resonance spectroscopy: role of extracellular bicarbonate and chloride. *Journal of Neurochemistry,* 62, 656–664.

Putney, L.K., Denker, S.P. & Barber, DL. (2002). The changing face of the Na^+/H^+ exchanger, NHE1: structure, regulation, and cellular actions. *Annual Reviw of Pharmacology and Toxicology,* 42, 527-552.

Rios, E.J., Fallon, M., Wang, J. & Shimoda, L.A. (2005). Chronic hypoxia elevates intracellular pH and activates Na^+/H^+ exchange in pulmonary arterial smooth muscle cells. *American Journal of Physiology Lung Cellular and Molecular Physiology*, 289, L867–L874.

Roberts Jr., E.L. & Chih, C.P. (1997). The influence of age of pH regulation in hippocampal slices before, during, and after anoxia. *Journal of Cerebral Blood Flow and Metabolism,* 17, 560–566.

Sardet, C., Franchi, A. & Pouyssegur, J. (1989). Molecular cloning, primary structure, and expression of the human growth factor-activatable Na^+/H^+ antiporter. *Cell,* 56, 271-280.

Schaller, B. & Graf, R. (2004). Cerebral ischemia and reperfusion: The pathophysiologic concept as a basis for clinical therapy. *Journal of Cerebral Blood Flow & Metabolism,* 24, 351-371.

Scholz, W., Albus, U., Counillon, L., Gogelein, H., Lang, H.J. , Linz, W., Weichert, A. & Scholkens, B.A. (1995). Protective effects of HOE642, a selective sodium-hydrogen exchange subtype 1 inhibitor, on cardiac ischaemia and reperfusion. *Cardiovascular Research,* 29, 260–268.

Scholz, W., Jessel, A. & Albus, U. (1999). Development of the Na^+/H^+ exchange inhibitor cariporide as a cardioprotective drug: from the laboratory to the GUARDIAN trial. *Journal of Thrombosis and Thrombolysis,* 8, 61–70.

Sheldon, C. & Church, J. (2002). Intracellular pH response to anoxia in acutely dissociated adult rat hippocampal CA1 neurons. *Journal of Neurophysiology,* 87, 2209-2224.

Shi, Y., Chanana, V., Watters, J.J., Ferrazzano, P. & Sun, D. (2011). Role of sodium/hydrogen exchanger isoform 1 in microglial activation and proinflammatory responses in ischemic brains. *Journal of Neurochemistry,* (in press).

Szaszi, K., Paulsen, A., Szabo, E.Z., Numata, M., Grinstein, S. & Orlowski, J. (2002). Clathrin-mediated endocytosis and recycling of the neuronspecific Na^+/H^+ exchanger NHE5 isoform. Regulation by phosphatidylinositol 3'-kinase and the actin cytoskeleton. *Journal of Biological Chemistry,* 277, 42623-42632.

Vannucci, R.C. & Vannucci, S.J. (2005). Perinatal hypoxic-ischemic brain damage: evolution of an animal model. *Developmental Neuroscience,* 27, 81-86.

Wang, Y., Meyer, J.W., Ashraf, M. & Shull, G.E. (2003). Mice with a null mutation in the NHE1 Na^+/H^+ exchanger are resistant to cardiac ischemia-reperfusion injury. *Circulation Research*, 93, 776–782.

Wang, Y., Luo, J., Chen, X., Chen, H., Cramer, S.W. and Sun, D. (2008). Gene inactivation of Na^+/H^+ exchanger isoform 1 attenuates apoptosis and mitochondrial damage following transient focal cerebral ischemia. *European Journal of Neuroscience,* 28, 51–61.

Warnock, D.G., Yang, W.C., Huang, Z.Q. & Cragoe, E.J. Jr. (1988). Interactions of chloride and amiloride with the renal Na^+/H^+ antiporter. *Journal of Biological Chemistry,*263, 7216–7221.

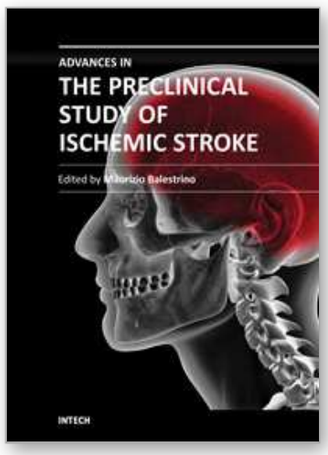
Xiong, Z.G., Zhu, X.M., Chu, X.P., Minami, M., Hey, J., Wei, W.L., MacDonald, J.F., Wemmie, J.A., Price, M.P., Welsh, M.J. & Simon, R.P. (2004). Neuroprotection in ischemia: blocking calcium-permeable acid-sensing ion channels. *Cell,* 118, 687-698.

Xue, J. & Haddad, G.G. (2010). The Na^+/H^+ exchanger: A target for therapeutic intervention in cerebral ischemia, In: *New strategies in stroke intervention: Ionic transporters, pumps, and new channels,* Annunziato, L. pp. (113-128), Human press, a part of Springer Science, ISBN 978-1-60761-279-7, New York, USA

Yao, H. & Haddad, G.G. (2004). Calcium and pH homeostasis in neurons during hypoxia and ischemia. *Cell Calcium,* 36, 247-255.

Yao, H., Gu, X.Q., Douglas, R.M. & Haddad, G.G. (2001). Role of Na^+/H^+ exchanger during O_2 deprivation in mouse CA1 neurons. *American Journal of Physiology Cell Physiology,* 281, C1205-C1210.

Yu, F.H., Shull, G.E. & Orlowski, J. (1993). Functional properties of the rat Na/H exchanger NHE-2 isoform expressed in Na/H exchanger-deficient Chinese hamster ovary cells. *Journal of Biological Chemistry,* 268, 25536–25541.



Advances in the Preclinical Study of Ischemic Stroke
Edited by Dr. Maurizio Balestrino

ISBN 978-953-51-0290-8
Hard cover, 530 pages
Publisher InTech
Published online 16, March, 2012
Published in print edition March, 2012

This book reports innovations in the preclinical study of stroke, including - novel tools and findings in animal models of stroke, - novel biochemical mechanisms through which ischemic damage may be both generated and limited, - novel pathways to neuroprotection. Although hypothermia has been so far the sole "neuroprotection" treatment that has survived the translation from preclinical to clinical studies, progress in both preclinical studies and in the design of clinical trials will hopefully provide more and better treatments for ischemic stroke. This book aims at providing the preclinical scientist with innovative knowledge and tools to investigate novel mechanisms of, and treatments for, ischemic brain damage.

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