**Review**

Vivek Verma, Anjana Bali, Nirmal Singh and Amteshwar Singh Jaggi*

# Implications of sodium hydrogen exchangers in various brain diseases

DOI 10.1515/jbcpp-2014-0117
Received October 30, 2014; accepted April 22, 2015; previously published online May 28, 2015

**Abstract:** $Na^+/H^+$ exchangers (NHEs) are the transporter proteins that play an important role in intracellular pH ($pH_i$) regulation, cell differentiation and cell volume and that mediate transepithelial $Na^+$ and $HCO_3^-$ absorption on the basis of chemical gradients across the plasma membrane. Its activation causes an increase in intracellular $Na^+$, which further leads to $Ca^+$ overload and cell death. The pharmacological inhibition of these transporter proteins prevents myocardial infarction and other heart diseases like congestive heart failure in experimental animal models as well as in clinical situations. The more recent studies have implicated the role of these exchangers in the pathophysiology of brain diseases. Out of nine NHE isoforms, NHE-1 is the major isoform present in the brain and regulates the trans-cellular ion transport through blood-brain barrier membrane, and alteration in their function leads to severe brain abnormalities. NHEs were shown to be involved in pathophysiologies of many brain diseases like epilepsy, Alzheimer's disease, neuropathic pain and ischemia/reperfusion-induced cerebral injury. $Na^+/H^+$-exchanger inhibitors (e.g., amiloride and cariporide) produce protective effects on ischemia/reperfusion-induced brain injury (e.g., stroke), exhibit good antiepileptic potential and attenuate neuropathic pain in various animal models. The present review focuses on the pathophysiological role of these ion exchangers in different brain diseases with possible mechanisms.

**Keywords:** Alzheimer's disease; brain; epilepsy; $Na^+/H^+$ exchangers; neuroprotection.

***Corresponding author: Dr. Amteshwar Singh Jaggi,** Department of Pharmaceutical Sciences and Drug Research, Punjabi University, Patiala-147002, Punjab, India, E-mail: amteshwarjaggi@yahoo.co.in
**Vivek Verma, Anjana Bali and Nirmal Singh:** Department of Pharmaceutical Sciences and Drug Research, Punjabi University, Patiala, India

## Introduction

$Na^+/H^+$ exchangers (NHEs) are ubiquitous integral membrane ion transporters and mediate the electroneutral exchange of $H^+$ with $Na^+$ or $K^+$ to regulate pH [1], osmolarity, cell volume and cell proliferation [2] during physiological as well as pathophysiological conditions. Nine mammalian NHE isoforms (NHE-1–9) have been identified so far, and these are categorized into two types: plasma membrane NHEs (NHE-1–5) and organellar NHEs (NHE-6–9). The major role of NHE-1 has been described in pathologies of heart as its activation is involved in the development of cardiac hypertrophy, heart failure and ischemia-reperfusion injury [3, 4], and inhibition of NHE-1 is shown to attenuate cardiovascular diseases [5–7]. Since both heart and brain are prone to ischemia-induced injury, the role of NHEs in brain pathologies, particularly in cerebral ischemia, has also been investigated. The majority of studies have suggested that the activation of NHE-1 is associated with development of mental disorders like epilepsy, Alzheimer disease (AD), stroke and neuropathic pain, and accordingly, its pharmacological inhibition has been shown to exert beneficial effects in the different brain diseases [8, 9]. The recent studies have shown that mutations in organellar NHEs lead to development of several neurological diseases and mental disorders probably due to NHE malfunctioning-induced defects in intracellular trafficking [10]. The mutation of NHE-6 proteins (deletion of Glu255 and Ser256 amino acid residues in its transmembrane segment) has been associated with development of Angelman syndrome-like X-linked mental retardation, characterized by mental retardation, microcephaly, seizures, ataxia and absence of speech [11]. The mutations in NHE 9 isoform have also been associated with development of neurological disorders such as family-based autisms [12] and attention deficient hyperactivity disorder [13]. The present review discusses the pathophysiological role of different NHE isoforms, particularly NHE-1, in the development of various brain diseases.

## NHE subtypes and localization in brain

The studies from the rodent brains have revealed that NHE-1 is the most abundant and widely dispersed isoform, whereas other family members (i.e., NHE-2–4) show a more restricted distribution [14]. Functionally, NHE-1 has received more attention due to its high abundance in the brain as compared to other isoforms. In the brain, its role in microglial and astrocyte activation and subsequently in abnormal neuronal functioning has been very well documented. The importance of NHE-1 in neuronal function has been demonstrated by employing transgenic NHE-1 knock out or spontaneously mutated mice, which develop neurodegeneration, ataxia, epileptic-like seizures and significant mortality [15, 16]. NHE-1 is the major isoform present in the brain, predominantly in the hippocampus and cortex regions [17], and at the cellular levels, these are highly expressed on the astrocytes and glial cells [18]. NHE-2 and NHE-3 are predominantly located in the apical membrane of the epithelia and are highly expressed in the kidney and intestine [19, 20]. Within the brain, NHE-3 isoform has been detected only in cerebellar Purkinje cells [14]. Their targeted disruptions in mice have not shown neurodegenerative or other neurological symptoms suggesting their lesser significant role in the nervous system functioning as compared to other isoforms [21, 22]. NHE-4 is the most abundant isoform in the stomach. However, it is also expressed in other organs including the intestine, kidney, brain, uterus and skeletal muscles [19]. NHE-5 is predominantly expressed in discrete regions of the brain, including the dentate gyrus, cerebral cortex and hippocampus [23, 24]. NHE-6 expression is highest in the heart, brain and skeletal muscles and is localized to early recycling endosomes [23, 25, 26]. The location and role of NHE-7 and NHE-8 isoforms in the brain has not been reported yet; however, very high expression of NHE-8 is demonstrated in the skeletal muscles and kidney. The more recently identified NHE-9 isoform is localized on late recycling endosomes [25] and also detected in the brain [12].

The role of organellar NHE isoforms in maintaining the optimum pH in the lumen of recycling endosomes for proper apical membrane recycling and maintenance of cell polarity has been described [26]. The four organellar NHE isoforms, 6 and 9 isoforms show a comparable localization in sorting/recycling endosomes, whereas 7 and 8 isoforms are mainly localized on the trans-Golgi network and mid/trans-Golgi stacks, respectively [25, 27]. A prominent distinction between organellar and plasma membrane NHEs, other than their subcellular localization and phylogenic relationship, is their selectivity for alkaline cations. The organellar NHEs operate as both $K^+/H^+$ and $Na^+/H^+$ exchangers, whereas plasma membrane NHEs solely mediate $Na^+/H^+$ exchange [28, 29]. The organellar NHEs have similar $K_M$ and $V_{max}$ values for $Na^+$ and $K^+$ [25, 26], and under physiological conditions, they mainly operate as $K^+/H^+$ exchangers due to high cytoplasmic $K^+$ concentration (~140 mM) as compared to $Na^+$ (~10 mM). Structurally, all mammalian NHEs possess 12 transmembrane helices in their N-termini that constitute the ion translocation domain, while the cytoplasmic hydrophilic C-terminus plays an important role in the binding to various regulatory proteins [30]. As compared to the C-terminal cytoplasmic tail, the amino acid sequences of the N-terminal transmembrane segments (which directly mediate the trans-membrane crossing of ions) are similar in plasma membrane and organellar NHEs suggesting that the actual mechanism of ion translocation in both NHE families is similar.

## NHE and brain disorders

### Dementia

AD is a progressive neurodegenerative disorder and is the most common form of dementia which is characterized by β-amyloid protein deposits (plaques) in brain. Its pathophysiology primarily involves the brain, but some studies have also revealed its systemic manifestations like an altered lymphoblast activity in AD patients [31]. Abnormal functioning of NHEs in the brain is found to be associated with AD pathology. Urcelay and coworkers correlated abnormal lymphoblast proliferation in AD patients with NHE and altered pH homeostasis. The lymphoblasts in AD patients showed higher rates of proliferation in response to serum-derived growth factors (cell activating agents) as compared to normal individuals. However, the increased lymphoblast proliferation and accompanied intracellular alkalization was prevented in the presence of NHE inhibitors suggesting a key role of this exchanger in eliciting the abnormal cellular responses in lymphocytes of AD patients. Moreover, calmodulin inhibition with calmidazolium or W-7 also exhibited the similar response in lymphoblast proliferation like NHE inhibitors [32]. The other studies have also suggested that calmodulin may activate NHE-1 through $Ca^{2+}$-calmodulin-depentent kinases mediated phosphorylation of the anti-porter [33]. The cytosolic tail of NHE-1 contains the binding domains

for calmodulin, and thus, $Ca^{2+}$/calmodulin may directly bind to this anti-porter to promote its activation. Accordingly, it has been hypothesized that $Ca^{2+}$/calmodulin-mediated NHE-1 activation may contribute significantly in abnormal lymphoblast proliferation in AD proliferation.

Apart from NHE-1, the role of other isoforms including NHE-4 and NHE-5 has been shown in neuronal excitation and long-term potentiation (LTP). Treatment of ethyl-isopropyl amiloride (EIPA), a non-selective NHE blocker, has been shown to modulate neuronal plasticity and LTP in the hippocampal slices isolated from the young (8 weeks) and senescent (30 months) rat with respect to tetanic stimuli, and it was suggested that NHE activity serves as a negative feedback mechanism to control neuronal excitability and plasticity in both young and senescent animals [34]. Along with NHE-1, NHE-4 and NHE-5 are the predominant isoforms in hippocampal neurons [14]. Therefore, these effects of a non-selective NHE blocker have been attributed to inhibition of all the three isoforms [34]. An involvement of organellar NHE-6 isoform in abnormal expression and deposition of tau leading to taupathy and, consequently, AD has also been suggested. The wild-type 'solute carrier family 9 isoform A6' (SLC9A6) gene encodes for NHE-6, which is localized on endosomal vesicles and participates in targeting intracellular vesicles and recycling synaptic vesicles. Neuropathologically, the X-linked in-frame 9 base pair deletion in SLC9A6 gene causes abnormal tau deposition in the hippocampal CA4 region, cerebellar cortex, substantia nigra, putamen, globus pallidus and dentate nucleus of the cerebellum leading to neuronal loss and gliosis as observed in various forms of taupathies [35]. Tau-positive inclusions are the defining neuro-pathological characteristics of a number of adult-onset neurodegenerative disorders including AD [36, 37]. Tau phosphoproteins play an important role in axonal transport and in maintaining axonal integrity by promoting their assembly and stability [37, 38]. The abnormal tau deposition in family's syndrome due to SLC9A6 mutation demonstrates that NHE and cytoskeletal elements interact with each other to maintain the axonal and vesicular transport, and disturbance in these interactions (due to NHE mutation) may disrupt the membrane transport system leading to development of taupathies and AD [35].

The human immunodeficiency virus (HIV)-associated cognitive impairment and AIDS-associated dementia is very common and afflicts over 50% of patients. Benos and coworkers linked gp-120 (core protein part of HIV) and cytokines-induced astrocyte proliferation with activation of NHE localized on astrocytes [39]. The elevation in IL-1, IL-6, IFN-γ, TNF-α and transforming growth factor-β (TGF-β) levels in the cerebrospinal fluid and the brains of AIDS patients is very well documented [40]. It is demonstrated that the elevated levels of cytokines during HIV infection such as TNF-α, interferon (IFN)-gamma and interleukin (IL)-lβ (but not TGF, IL-2 and IL-6) stimulate the NHE. Furthermore, IFN-γ and gp-120-induced NHEs activation and astrocyte proliferation is ameliorated in the presence of tyrosine kinase inhibitors (herbimycin A and genistein) suggesting that increased levels of cytokines during HIV infection activates astrocyte-located NHE in a tyrosine kinase-dependent manner. It has been proposed that gp120 or cytokines-induced NHE activation with resultant increase in intracellular pH may change the membrane permeability properties of the astrocytes to imbalance the $K^+$ and glutamate microenvironment of the neurons. It may eventually raise the intra-neuronal $Ca^{2+}$ leading to neuronal dysfunction/damage and dementia [39]. This hypothesis was supported by Holden and coworkers by demonstrating that the pressure application of recombinant gp120 on to cultured human fetal neurons and astrocytes results in a significant increase in intracellular calcium levels, and treatment with non-selective NHE blocker 5-(N-methyl-N-isobutyl)-amiloride significantly attenuated gp 120-mediated increase in intracellular calcium in the neurons and astrocytes. It suggests that the gp 120 may trigger the activation of these exchangers on the neurons to raise intracellular calcium ion and produce neurodegeneration along with dementia [41]. Ali and coworkers demonstrated the memory-improving actions of amiloride in mice model of epilepsy [42]. Recently, administration of selective and potent NHE-1 inhibitor, HOE-642, in P9 mice has been shown to attenuate ischemia-induced neurodegeneration and improve spatial learning [9] (Figure 1).

## Epilepsy

The role of NHEs has also been investigated in modulating the electrical properties of neurons and seizure activity in various in vitro as well as in vivo models. The functional defects in NHEs alter the neuronal excitatory properties leading to their over-excitation and CNS diseases like epilepsy. It has been shown that the genetic modification of the NHE-1 gene in mice (NHE null mutant mice) produces non-functional NHE-1, which leads to development of epileptic seizures, ataxia, decreased growth and high mortality within the age of 2–3 weeks [16]. It suggests the functional role of NHE-1 during embryogenesis as its non-functionality is associated with decreased growth rate and development of epilepsy. Gu and coworkers reported the higher neuronal excitability of hippocampal

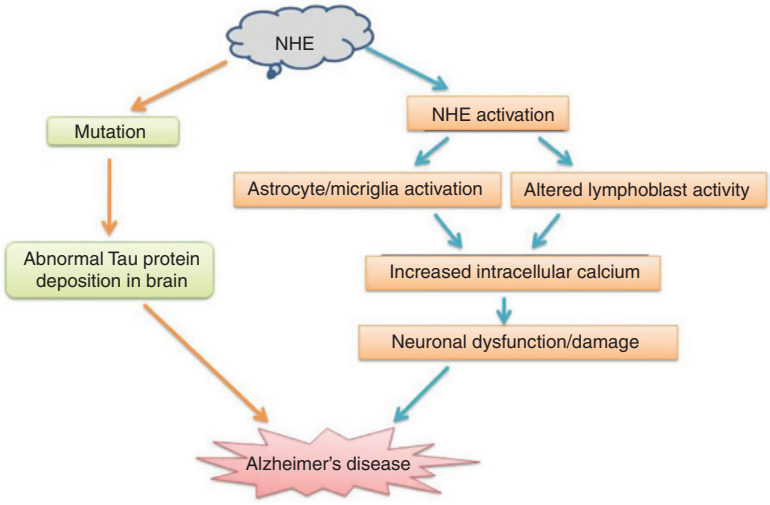


**Figure 1:** The involvement of NHE mutation/activation in the development of AD.
The mutation in *NHE* gene results in abnormal tau protein deposition in brain which participates in AD, whereas its activation leads to astrocyte and microglia activation to alter the lymphoblast activity which ultimately results in AD through $Ca^{2+}$ overload mediated neuronal dysfunctions.

CA1 neurons in NHE-1 null mutant mice. Furthermore, the electrophysiological studies revealed the elevation in $Na^+$ current density in these NHE-1 mutants as compared to corresponding wild type suggesting that functional loss of NHE-1 increases the $Na^+$ current density, which may be responsible for abnormal neuronal excitation and epilepsy [43]. Using neurophysiological, autoradiographic and immunoblotting techniques, Xia and coworkers demonstrated increased $Na^+$-channel density, $Na^+$ current and membrane excitability in both CA1 and cortical neurons in NHE-1 null mice [17]. Accordingly, it has been proposed that endogenous NHEs regulate $Na^+$ channels in different brain areas including the hippocampus and cortex to control the neuronal excitability, and its non-functioning may be associated with abnormal neuronal excitability leading to development of epilepsy. The development of excessive neuronal excitability and seizure development in NHE-1 null mice may also be attributed to decreased inhibitory GABA neurotransmission due to non-functional NHE in the brain. Jang and coworkers provided the evidence that NHE is a major pH regulator in GABAergic presynaptic nerve terminals synapsing onto CA3 pyramidal neurons [44]. The acidification of presynaptic terminals increased the frequency of GABAergic miniature inhibitory postsynaptic currents (mIPSCs) from the depolarized presynaptic terminals and EIPA abolished acidification-induced inhibitory GABA tone. The studies have shown that neuronal NHE expression for most isoforms is greatest in inhibitory neurons [44, 45]. Dietrich and Morad further provided the evidence that GABAergic synapses onto cultured rat cerebellar granule cells are acidified by proton extrusion via NHE, which leads to enhancement of mIPSCs. The inhibition of NHE by amiloride was shown to reduce mIPSCs in a manner similar to alkalization. Based on these, it has been postulated that development of seizures (increased neuronal excitation) due to NHE dysfunction may be secondary to reduced inhibitory GABAergic neurotransmission [46]. Conversely, overexpression of NHE may lead to enhanced inhibition resulting in decreased respiratory drive in rabbits [47] and sudden infant death syndrome in clinics [48].

On the contrary, the role of NHE activation in epileptogenesis in different animal models of epilepsy has also been documented. Ali and coworkers demonstrated the anticonvulsant effect of amiloride in electroshock and pentylenetetrazole (PTZ)-induced seizures in mice in terms of increase in seizure threshold in a dose-dependent manner [49]. The same group of workers also demonstrated that administration of amiloride (2 h before PTZ, in doses of 0.65 and 1.3 mg/kg, p.o.) significantly prolongs the onset of kindling and reduces the incidence and severity of seizures in PTZ (25 mg/kg, i.p., once every 2 days for 5 weeks)-induced kindling model in mice in a dose-dependent manner. The anticonvulsant effects of amiloride (1.3 mg/kg) were comparable to diazepam (3 mg/kg) and observable even after 15 or 30 days of last treatment [49]. The anticonvulsant effects of amiloride or cariporide have been related indirectly to inhibition of the glutamatergic system and decrease in intracellular calcium overload via intracellular acidification [50–55].

Furthermore, the acidification of neurons is very well documented to decrease the epileptiform discharge [55, 56], and accordingly, the NHE inhibition-induced intracellular acidification may also directly inhibit the neuronal excitability and decrease seizure threshold and frequency. The other studies have also shown that NHE inhibition-induced acidification is a critical factor in inhibiting the neuronal excitability in CA3 neurons [57, 58].

The epileptogenic modulatory effects in NHE knockout mice are directly comparable to its pharmacological inhibition in various epilepsy models. The possible contradiction may be explained on the basis of differential function of NHEs under different cellular conditions. The non-functioning of NHE in mutant mice may cause alteration of physiological homeostasis in the neurons to induce secondary changes in the ion channel density and expression, especially $Na^+$ channels, which ultimately leads to hyper-excitation of neurons and associated epileptic seizures. On the other hand, during pathological state, the role of NHE activation in generating epilepsy has been attributed directly to pH-mediated increase in neuronal excitability or indirectly to NHE-mediated increase in intracellular $Na^+$, reversal of $Na^+/Ca^{2+}$ exchange function, intracellular $Ca^{2+}$ overload and enhanced glutamate release. Furthermore, the pharmacological inhibition of NHE is also shown to produce biphasic effects on the membrane excitability of the CA3 hippocampal neurons. The application of NHE inhibitor amiloride or cariporide initially increases the frequency of action potential and burst followed by progressive suppression in bicuculline and other excitatory agents-induced neuronal epileptiform burst activity. However, the more quick and complete acidification by inhibiting NHE and other acid-regulating ion exchangers produces only the monophasic response, i.e., suppression of the neuronal activity [57, 58]. The variable responses of NHE inhibition may also be a possible contributing factor in contradictory effects regarding epileptogenesis in studies employing NHE-null mice or NHE pharmacological inhibitors. However, more studies are warranted to understand the complexity of physiological and pathophysiological function of NHE in neuronal excitation mediated mental diseases like epilepsy (Figure 2).

## Cerebral ischemia

Studies have shown that NHE is essential for intracellular pH ($pH_i$) regulation in both physiological and pathological conditions in the brain [1, 59]. Furthermore, the studies from NHE-1 null mutant mice have revealed that NHE-1 is the major intracellular pH-controlling NHE isoform in the mice cortical astrocytes [53]. NHEs are also present on the blood-brain barrier (BBB) [60], and about 50% of transcellular transport of sodium from the blood occurs through NHE and sodium channels [61]. Studies have shown an increased NHE activity on the BBB in response to cerebral ischemia [60], which may participate in increased sodium transport (intracellular $Na^+$ overload) leading to edema in cerebral microvascular endothelial cells [62]. Administration of SM-20220 (highly specific NHE inhibitor) has been shown to prevent hypoxia-induced BBB disruption

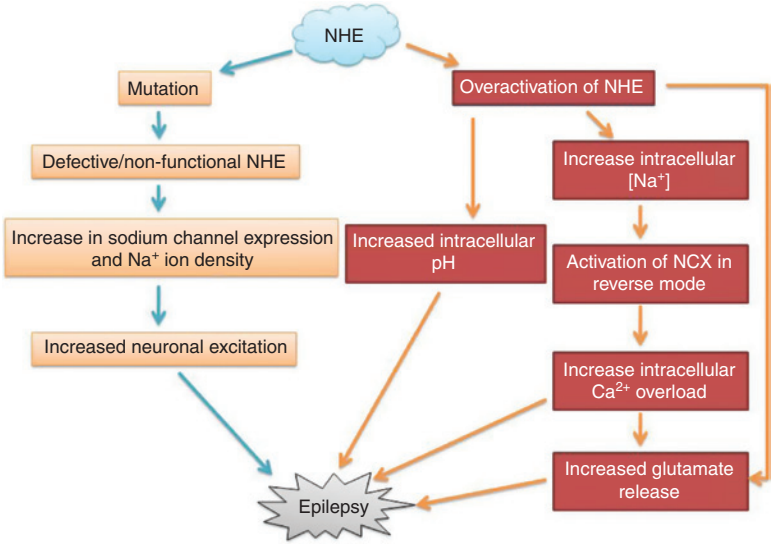


**Figure 2:** The abnormal functioning of NHE either as mutated non-functional or excessive activation results in epileptic seizures through increased intracellular $Na^+$, $Ca^{2+}$ and glutamate levels.

and attenuate post-ischemic hypoperfusion to prevent hypoxic endothelial cell injury [63].

Various preclinical studies have shown the deleterious effects of NHE activation during cerebral ischemia-reperfusion, and pharmacological inhibition of these exchangers or genetic knock out of these exchangers is associated with neuroprotective effects [64, 65]. Kendall and coworkers demonstrated that N-methyl-isobutyl-amiloride (MIA) (NHE inhibitor) reduces brain injury in a model of neonatal hypoxia and ischemia (HI), and its administration 30 min before HI was shown to produce significant neuroprotective effects on the forebrain and the hippocampus [66]. The selective inhibition of NHE-1 using dimethylamiloride has also been associated with attenuation of ischemia-induced cerebral infarct volume in spontaneous hypertensive rats following middle cerebral artery occlusion [67]. In a more recent study, treatment with MIA after severe perinatal asphyxia in piglets (aged <24 h) has been shown to produce neuroprotective effects [68].

A recent study has shown a significant rise in NHE-1 immunostaining in the hippocampal CA1 neurons and the reactive astrocytes of mice at 72 h after hypoxia-ischemia [9]. An earlier study has also demonstrated the elevation of NHE-1 expression in reactive astrocytes in CA1 regions of adult gerbil brains after a transient global ischemia. These suggest a point that hippocampal neurons and astrocytes respond to hypoxia-induced intracellular acidosis by up-regulating NHE-1 expression and function [9]. Moreover, administration of HOE-642 (cariporide), NHE-1 inhibitor, was shown to exert neuro-protection, prevent neurodegeneration and preserve the morphologic hippocampal structures [9, 69]. $IC_{50}$ of HOE-642 for NHE-1 is 0.01 μM; NHE-2, 1.6 μM and NHE-3, 1000 μM, thereby considered as a potent and highly selective NHE-1 inhibitor. The pretreatment of adult gerbils with EIPA is also shown to significantly reduce the extent of CA1 pyramidal neuronal loss at 6 days after global ischemia [70]. The pretreatment of neonatal mice with the nonspecific NHE inhibitor (MIA) 30 min before the induction of hypoxia-ischemia is reported to increase the forebrain tissue survival from 44% to 67% [66]. The in vitro experiments on the cultured microglia have also shown that NHE-1 plays a significant role in activating microglia as inhibition of NHE-1 activity with cariporide (HOE-642) inhibits various deleterious cellular events like $pH_i$ elevation, increase in $[Na^+]_i$ and $[Ca^{2+}]_i$, production of superoxide anion and cytokines in the microglia following lipopolysaccharide or oxygen and glucose deprivation exposure [71]. Shi and coworkers also demonstrated that the NHE-1 protein is abundantly expressed on the activated microglia and investigated the role of these exchangers in microglial activation following focal cerebral ischemia in mice. Furthermore, inhibition of NHE-1 with HOE-642 or its genetic knockout was shown to significantly attenuate the activation of microglia in the peri-infarct area following 2–7 days ischemia-reperfusion and significantly reduce pro-inflammatory cytokines in ischemic brains [72].

Cengiz and coworkers reported the conversion of astrocytes into reactive astrogliosis in the hippocampus, which was characterized by upregulation of GFAP and NHE-1 expression in the regions of ipsilateral hippocampus at 72 h after hypoxia. Accordingly, it has been speculated that NHE-1 activity in reactive astrocytes is detrimental to neuronal survival, and HOE-642-mediated neuroprotection is imparted through inhibition of NHE-1 activity in reactive astrocytes and protecting hippocampal neurons by reducing the detrimental effects of reactive astrocytes. It has been hypothesized that reduced NHE-1-mediated $Na^+$ influx may increase the driving force for $Na^+$-dependent glutamate transporter and promote glutamate uptake from the synaptic interstitial fluid. The decreased synaptic availability of glutamate may be responsible for neuroprotection observed with NHE inhibitors (discussed below). The decrease in NHE-1 and $Na^+/Ca^{2+}$ exchange may also prevent $Ca^{2+}$ elevation in reactive astrocytes and reduce gliotransmitter release and pro-inflammatory cytokine release [9]. The activation of NHEs present on astrocytes has also been shown to enhance glutamate release [41], and the deleterious effects of NHE activation during cerebral hypoxia/ischemia may also be due to increased glutamate release [73] and, subsequently, increased calcium overload [65]. Administration of cariporide has also been shown to prevent both glutamate-induced necrotic and apoptotic neuronal cell death accompanied with reduced mitochondrial $Ca^{2+}$ and reactive oxygen species production [54]. The studies showing that both NHE inhibitor (SM-20220) and the N-methyl-D-aspartate receptor (NMDA) receptor antagonist (MK-801) attenuate glutamate-induced neuronal death in cultured rat cortical neurons [74] support the hypothesis that the deleterious effects of NHE activation are mediated indirectly through glutamate release. On the other hand, glutamate is also involved in NHE activation in the brain leading to induction of a vicious cycle. Glutamate causes neuronal calcium overload, mitochondrial dysfunction and disruption of ATP synthesis, which in turn increases intracellular pH and activates the NHE. The increased intracellular $Na^+$ due to activation of NHE causes reversal of $Na^+/Ca^+$ exchanger activity and hence results in intracellular $Ca^+$ overload leading to release of proinflammatory cytokines

that produce cellular damage through necrotic and/or apoptotic pathways [73, 75, 76].

Manhas and coworkers demonstrated a transient increase in phosphphorylated NHE-1 (*p*-NHE-1) expression in the ischemic brain tissues during early reperfusion (3–10 min) following transient ischemia in mice, accompanied with a concurrent elevation of extracellular signal-regulated kinase (ERK)/90-kDa ribosomal S6 kinase ($p90^{RSK}$) expression. Immunofluorescence staining analysis revealed a robust increase of p-$p90^{RSK}$, a known NHE-1 kinase, expression in the ischemic striatal neurons and cortical neurons at 3–10 min reperfusion. Stimulation of $p90^{RSK}$ in ischemic neurons was downstream of ERK activation because inhibition of MEK1 (MAP kinase/ERK kinase) with its inhibitor U0126 blocked phosphorylation of $p90^{RSK}$. Moreover, direct inhibition of $p90^{RSK}$ by its selective inhibitor fluoromethyl ketone (FMK) not only reduced p-NHE-1 expression but also reduced ischemic infarct volume by 60% and number of degenerated neurons by approximately 80%. Taken together, it revealed that reperfusion triggers a transient stimulation of the ERK/$p90^{RSK}$ pathway, and $p90^{RSK}$ activation further contributes to cerebral ischemic damage in part via activation of NHE-1 protein [77].

On the contrary, Douglas and coworkers have shown the decreased expression of acid-base transporter proteins like NHE and $Na^+/HCO_3^-$ cotransporter in response to chronic intermittent hypoxia (CIH) in the mouse brain. The NHE-1 and NHE-3 isoforms protein expression was found to be decreased in all the parts of the CNS especially in the cerebellum and hippocampus, which are more prone to hypoxia-induced damage. The decrease in acid-base protein expression resulting from CIH has been defined as generalized cellular strategy (adaptive cellular response) to decrease the cellular protein synthesis and minimize the expenditure of metabolic energy reserves during hypoxic exposure. However, this down-regulation in acid-extruding capacity also renders the neurons more prone to acidity and to injury during CIH mainly in the cerebellum and hippocampus [78] (Figure 3).

## Neuropathic pain

Neuropathic pain pathologies have shown the involvement of altered ion homeostasis due to changes in the multiple ion channels and exchangers including NHEs. By employing the immune-histochemical analytical methods, Castañeda-Corral and coworkers revealed the significantly reduced expression of NHE-1 in both ipsilateral and contra-lateral dorsal root ganglia and dorsal horn of the spinal cord of rat in formalin-induced pain model suggesting the active participation of NHEs in the pain processing pathway at both the peripheral and the spinal levels [79–81]. Administration of partially selective NHE-1 inhibitors such as dimethyl amiloride (DMA), EIPA (0.3–30 μM/rat) and a selective NHE-1 inhibitor zoniporide (0.03–3 μM/rat) was shown to significantly increase formalin-induced nociception in a dose-dependent manner during both phases I and II [80]. On the contrary, the report from our own laboratory has shown the ameliorative effects of amiloride (15 mg/kg) administration for 10 consecutive days in chronic constriction-induced (CCI) neuropathic

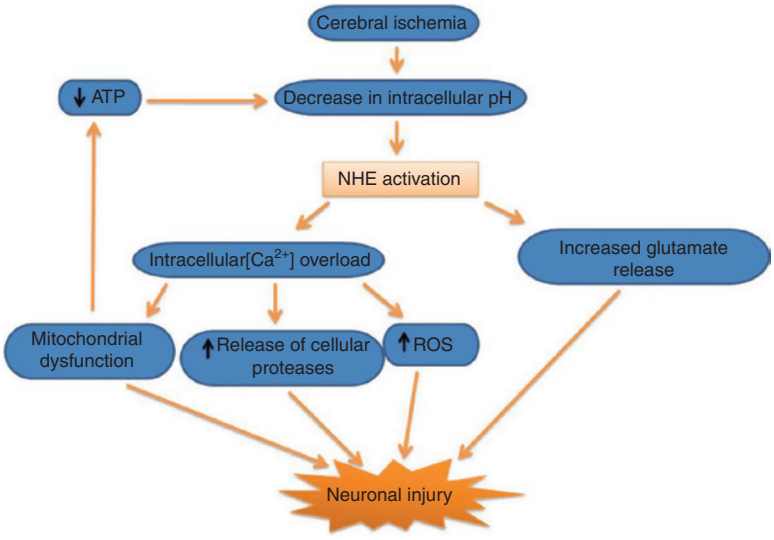


**Figure 3:** The involvement of NHE activation in cerebral ischemia-induced neuronal injury through increased glutamate and $Ca^{2+}$ mediated mitochondrial dysfunction, increased proteases and increased reactive oxygen species production.

pain and vincristine models in terms of axonal degeneration and pain-related behavioral changes. It was also shown to attenuate CCI and vincristine-induced rise in neuronal calcium levels and oxidative stress thereby, suggesting its protective effects secondary to decrease in $Ca^{2+}$ overload and oxidative stress [82].

## Conclusions

The role of NHE activation in the development of various pathophysiological diseases of brain such as epileptic seizures, neurodegenerative disorders like AD and cerebral ischemia-induced neuronal injury has been defined. Despite having their significant role in mental abnormalities, their role in mental diseases like depression, anxiety, stress and psychosis is yet to be explored. Although the preclinical reports suggest the importance of NHE inhibitors in improving learning and memory, reducing seizures and protecting ischemia/reperfusion-induced cerebral damage, their clinical relevance in these brain diseases is still under question.

**Acknowledgments:** The authors are grateful to the Department of Pharmaceutical Sciences and Drug Research, Punjabi University, Patiala, India, for supporting this study.
**Author contributions:** All the authors have accepted responsibility for the entire content of this submitted manuscript and approved submission.
**Research funding:** None declared.
**Employment or leadership:** None declared.
**Honorarium:** None declared.
**Competing interests:** The funding organization(s) played no role in the study design; in the collection, analysis and interpretation of data; in the writing of the report; or in the decision to submit the report for publication.

## References

1. Yao H, Ma E, Gu XQ, Haddad GG. Intracellular pH regulation of CA1 neurons in Na/H isoform 1 mutant mice. J Clin Invest 1999;104:637–45.
2. Slepkov ER, Rainey JK, Sykes BD, Fliegel L. Structural and functional analysis of the $Na^+/H^+$ exchanger. Biochem J 2007;401:623–33.
3. Baartscheer A, Schumacher CA, van Borren MM, Belterman CN, Coronel R, Fiolet JW. Increased $Na^+/H^+$ exchange activity is the cause of increased $[Na^+]i$ and underlies disturbed calcium handling in the rabbit pressure and volume overload heart failure model. Cardiovasc Res 2003;57:1015–24.
4. Yokoyama H, Gunasegaram S, Harding SE, Avkiran M. Sarcolemmal $Na^+/H^+$ exchanger activity and expression in human ventricular myocardium. J Am Coll Cardiol 2000;36:534–40.
5. Young M, Funder J. Mineralocorticoid action and sodium-hydrogen exchange: studies in experimental cardiac fibrosis. Endocrinology 2003;144:3848–51.
6. Aker S, Snabaitis AK, Konietzka I, Van De Sand A, Bongler K, Avkiran M. Inhibition of the $Na^+/H^+$ exchanger attenuates the deterioration of ventricular function during pacing-induced heart failure in rabbits. Cardiovasc Res 2004;63:273–81.
7. Chen S, Khan ZA, Karmazyn M, Chakrabarti S. Role of endothelin-1, sodium hydrogen exchanger-1 and mitogen activated protein kinase (MAPK) activation in glucose-induced cardiomyocyte hypertrophy. Diabetes Metab Res Rev 2007;23:356–67.
8. Park SL, Lee DH, Yoo SE, Jung YS. The effect of Na(+)/H(+) exchanger-1 inhibition by sabiporide on blood-brain barrier dysfunction after ischemia/hypoxia in vivo and in vitro. Brain Res 2010;1366:189–96.
9. Cengiz P, Kleman N, Uluc K, Kendigelen P, Hagemann T, Akture E, et al. Inhibition of Na+/H+ exchanger isoform 1 is neuroprotective in neonatal hypoxic ischemic brain injury. Antioxid Redox Signal 2011;14:1803–13.
10. Ohgaki R, van IJzendoorn SC, Matsushita M, Hoekstra D, Kanazawa H. Organellar Na+/H+ exchangers: novel players in organelle pH regulation and their emerging functions. Biochemistry 2011;50:443–50.
11. Gilfillan GD, Selmer KK, Roxrud I, Smith R, Kyllerman M, Eiklid K, et al. SLC9A6 mutations cause X-linked mental retardation, microcephaly, epilepsy, and ataxia, a phenotype mimicking Angelman syndrome. Am J Hum Genet 2008;82:1003–10.
12. Morrow EM, Yoo SY, Flavell SW, Kim TK, Lin Y, Hill RS, et al. Identifying autism loci and genes by tracing recent shared ancestry. Science 2008;321:218–23.
13. Markunas CA, Quinn KS, Collins AL, Garrett ME, Lachiewicz AM, Sommerd JL, et al. Genetic variants in SLC9A9 are associated with measures of attention-deficit/hyperactivity disorder symptoms in families. Psychiatr Genet 2009;20:73–81.
14. Ma E, Haddad GG. Expression and localization of Na+/H+ exchangers in rat central nervous system. Neuroscience 1997;79:591–603.
15. Cox GA, Lutz CM, Yang CL, Biemesderfer D, Bronson RT, Fu A, et al. Sodium/hydrogen exchanger gene defect in slow-wave epilepsy mutant mice. Cell 1997;91:139–48.
16. Bell SM, Schreiner CM, Schultheis PJ, Miller ML, Evans RL, Vorhees CV, et al. Targeted disruption of the murine NHE1 locus induces ataxia, growth retardation, and seizures. Am J Physiol 1999;276:C788–95.
17. Xia Y, Zhao P, Xue J, Gu XQ, Sun X, Yao H, et al. Na+ channel expression and neuronal function in the Na+/H+ exchanger 1 null mutant mouse. J Neurophysiol 2003;89:229–36.
18. Pizzonia JH, Ransom BR, Pappas CA. Characterization of $Na^+/H^+$ exchange activity in cultured rat hippocampal astrocytes. J Neurosci Res 1996;44:191–8.
19. Orlowski J, Kandasamy RA, Shull GE. Molecular cloning of putative members of the $Na^+/H^+$exchanger gene family. J Biol Chem 1992;267:9331–9.
20. Noel J, Roux D, Pouyssegur J. Differential localization of $Na^+/H^+$ exchanger isoforms (NHE1 and NHE3) in polarized epithelial cell lines. J Cell Sci 1996;109:929–39.

21. Schultheis PJ, Clarke LL, Meneton P, Harline M, Boivin GP, Stemmermann G, et al. Targeted disruption of the murine Na+/H+ exchanger isoform 2 gene causes reduced viability of gastric parietal cells and loss of net acid secretion. J Clin Invest 1998;101:1243–53.
22. Schultheis PJ, Clarke LL, Meneton P, Miller ML, Soleimani M, Gawenis LR, et al. Renal and intestinal absorptive defects in mice lacking the NHE3 Na+/H+ exchanger. Nat Genet 1998;19:282–5.
23. Baird NR, Orlowski J, Szabó EZ, Zaun HC, Schultheis PJ, Menon AG, et al. Molecular cloning, genomic organization, and functional expression of Na+/H+ exchanger isoform 5 (NHE5) from human brain. J Biol Chem 1999;274:4377–82.
24. Attaphitaya S, Park K, Melvin JE. Molecular cloning and functional expression of a rat Na+/H+ exchanger (NHE5) highly expressed in brain. J Biol Chem 1999;274:4383–8.
25. Nakamura N, Tanaka S, Teko Y, Mitsui K, Kanazawa H. Four $Na^+/H^+$ exchanger isoforms are distributed to Golgi and post-Golgi compartments and are involved in organelle pH regulation. J Biol Chem 2005;280:1561–72.
26. Ohgaki R, Matsushita M, Kanazawa H, Ogihara S, Hoekstra D, van Ijzendoorn SC. The $Na^+/H^+$ exchanger NHE6 in the endosomal recycling system is involved in the development of apical bile canalicular surface domains in HepG2 cells. Mol Biol Cell 2010;21:1293–304.
27. Brett CL, Wei Y, Donowitz M, Rao R. Human $Na^+/H^+$ exchanger isoform 6 is found in recycling endosomes of cells, not in mitochondria. Am J Physiol Cell Physiol 2002;282:C1031–41.
28. Numata M, Orlowski J. Molecular cloning and characterization of a novel (Na+, K+)/H+ exchanger localized to the trans-Golgi network. J Biol Chem 2001;276:17387–94.
29. Hill JK, Brett CL, Chyou A, Kallay LM, Sakaguchi M, Rao R, et al. Vestibular hair bundles control pH with $(Na^+, K^+)/H^+$ exchangers NHE6 and NHE9. J Neurosci 2006;26:9944–55.
30. Malo ME, Fliegel L. Physiological role and regulation of the $Na^+/H^+$ exchanger. Can J Physiol Pharm 2006;84:1081–95.
31. Gibson GE, Toral-Barza L. Cytosolic free calcium in lymphoblasts from young, aged and Alzheimer subjects. Mech Ageing Dev 1992;63:1–9.
32. Urcelay E, Ibarreta D, Parrilla R, Ayuso MS, Martín-Requero A. Enhanced proliferation of lymphoblasts from patients with Alzheimer dementia associated with calmodulin-dependent activation of the $Na^+/H^+$ exchanger. Neurobiol Dis 2001;8: 289–98.
33. Fliegel L, Walsh MP, Singh D, Wong C, Barr A. Phosphorylation of the C-terminal domain of the Na+/H+ exchanger by Ca2+/calmodulin-dependent protein kinase II. Biochem J 1992;282:139–45.
34. Rönicke R, Schröder UH, Böhm K, Reymann KG. The $Na^+/H^+$ exchanger modulates long-term potentiation in rat hippocampal slices. Naunyn Schmiedebergs Arch Pharmacol 2009;379:233–9.
35. Garbern JY, Neumann M, Trojanowski JQ, Lee VM, Feldman G, Norris JW, et al. A mutation affecting the sodium/proton exchanger, SLC9A6, causes mental retardation with tau deposition. Brain 2010;133:1391–402.
36. Sima AA, Defendini R, Keohane C, D'Amato C, Foster NL, Parchi P. The neuropathology of chromosome 17-linked dementia. Ann Neurol 1996;39:734–43.
37. Lee VM, Goedert M, Trojanowski JQ. Neurodegenerative tauopathies. Annu Rev Neurosci 2001;24:1121–59.
38. Buee L, Bussiere T, Buee-Scherrer V, Delacourte A, Hof PR. Tau protein isoforms, phosphorylation and role in neurodegenerative disorders. Brain Res Rev 2000;33:95–130.
39. Benos DJ, McPherson S, Hahn BH, Chaikin MA, Benveniste EN. Cytokines and HIV envelope glycoprotein gp120 stimulate Na+/H+ exchange in astrocytes. J Biol Chem 1994;269:13811–6.
40. Gallo P, Frei K, Rordorf C, Lazdins J, Tavolato B, Fontana A. Human immunodeficiency virus type 1 (HIV-1) infection of the central nervous system: an evaluation of cytokines in cerebrospinal fluid. J Neuroimmunol 1989;23:109–16.
41. Holden CP, Haughey NJ, Nath A, Geiger JD. Role of Na+/H+ exchangers, excitatory amino acid receptors and voltage-operated Ca2+ channels in human immunodeficiency virus type 1 gp120-mediated increases in intracellular Ca2+ in human neurons and astrocytes. Neuroscience 1999;91:1369–78.
42. Ali A, Ahmad FJ, Pillai KK, Vohora D. Evidence of the antiepileptic potential of amiloride with neuropharmacological benefits in rodent models of epilepsy and behavior. Epilepsy Behav 2004;5:322–8.
43. Gu XQ, Yao H, Haddad GG. Increased neuronal excitability and seizures in the Na/H exchanger null mutant mouse. Am J Physiol Cell Physiol 2001;281:C496–503.
44. Jang IS, Brodwick MS, Wang ZM, Jeong HJ, Choi BJ, Akaike N. The Na+/H+ exchanger is a major pH regulator in GABAergic presynaptic nerve terminals synapsing onto rat CA3 pyramidal neurons. J Neurochem 2006;99:1224–36.
45. Douglas RM, Schmitt BM, Xia Y, Bevensee MO, Biemesderfer D, Boron WF, et al. Sodium-hydrogen exchangers and sodium bicarbonate co-transporters: ontogeny of protein expression in the rat brain. Neuroscience 2001;102:217–28.
46. Dietrich CJ, Morad M. Synaptic acidification enhances GABAA signaling. J Neurosci 2010;30:16044–52.
47. Kiwull-Schöne H, Kiwull P, Frede S, Wiemann M. Role of brainstem sodium/proton exchanger 3 for breathing control during chronic acid base imbalance. Am J Respir Crit Care Med 2007;176:513–9.
48. Wiemann M, Frede S, Tschentscher F, Kiwull-Schöne H, Kiwull P, Bingmann D, et al. NHE3 in the human brainstem: implication for the pathogenesis of the sudden infant death syndrome (SIDS)? Adv Exp Med Biol 2008;605:508–13.
49. Ali A, Ahmad FJ, Pillai KK, Vohora D. Amiloride protects against pentylenetetrazole-induced kindling in mice. Br J Pharmacol 2005;145:880–4.
50. Xiong ZQ, Saggau P, Stringer JL. Activity-dependent intracellular acidification correlates with the duration of seizure activity. J Neurosci 2000;20:1290–6.
51. Giffard RG, Monyer H, Christine CW, Choi DW. Acidosis reduces NMDA receptor activation, glutamate neurotoxicity, and oxygen-glucose deprivation neuronal injury in cortical cultures. Brain Res 1990;506:339–42.
52. Puka M, Lehmann A. In vivo acidosis reduces extracellular concentrations of taurine and glutamate in the rat hippocampus. J Neurosci Res 1994;37:641–6.
53. Kintner DB, Su G, Lenart B, Ballard AJ, Meyer JW, Ng LL, et al. Increased tolerance to oxygen and glucose deprivation in astrocytes from Na(+)/H(+) exchanger isoform 1 null mice. Am J Physiol Cell Physiol 2004;287:C12–21.
54. Zhao X, Gorin FA, Berman RF, Lyeth BG. Differential hippocampal protection when blocking intracellular sodium and calcium entry during traumatic brain injury in rats. J Neurotrauma 2008;25:1195–205.

55. Lee BK, Jung YS. The Na+/H+ exchanger-1 inhibitor cariporide prevents glutamate-induced necrotic neuronal death by inhibiting mitochondrial $Ca^{2+}$ overload. J Neurosci Res 2012;90:860–9.
56. Dulla CG, Frenguelli BG, Staley KJ, Masino SA. Intracellular acidification causes adenosine release during states of hyper-excitability in the hippocampus. J Neurophysiol 2009;102:1984–93.
57. Bonnet U, Bingmann D, Wiemann M. Intracellular pH modulates spontaneous and epileptiform bioelectric activity of hippocampal CA3-neurones. Eur Neuropsychopharmacol 2000;10:97–103.
58. Bonnet U, Leniger T, Wiemann M. Alteration of intracellular pH and activity of CA3-pyramidal cells in guinea pig hippocampal slices by inhibition of transmembrane acid extrusion. Brain Res 2000;872:116–24.
59. Yao H, Gu XQ, Douglas RM, Haddad GG. Role of $Na^+/H^+$ exchanger during $O_2$ deprivation in mouse CA1 neurons. Am J Physiol Cell Physiol 2001;281:C1205–10.
60. Helmy MM, Ruusuvuori E, Watkins PV, Voipio J, Kanold PO, Kaila K. Acid extrusion via blood-brain barrier causes brain alkalosis and seizures after neonatal asphyxia. Brain 2012;135:3311–9.
61. Ennis SR, Ren XD, Betz AL. Mechanisms of sodium transport at the blood-brain barrier studied with in situ perfusion of rat brain. J Neurochem 1996;66:756–63.
62. Chang E, O'Donnell ME, Barakat AI. Shear stress and 17-estradiol modulate cerebral microvascular endothelial Na-K-Cl co-transporter and Na/H exchanger protein levels. Am J Physiol Cell Physiol 2008;294:C363–71.
63. Horikawa N, Kuribayashi Y, Itoh N, Nishioka M, Matsui K, Kawamura N, et al. Na+/H+ exchange inhibitor SM-20220 improves endothelial dysfunction induced by ischemia-reperfusion. Jpn J Pharmacol 2001;85:271–7.
64. Luo J, Chen H, Kintner DB, Shull GE, Sun D. Decreased neuronal death in Na+/H+ exchanger isoforms 1-null mice after in vitro and in vivo ischemia. J Neurosci 2005;25:11256–68.
65. Wang Y, Luo J, Chen X, Chen H, Cramer SW, Sun D. Gene inactivation of Na+/H+ exchanger isoform 1 attenuates apoptosis and mitochondrial damage following transient focal cerebral ischemia. Eur J Neurosci 2008;28:51–61.
66. Kendall GS, Robertson NJ, Iwata O, Peebles D, Raivich G. N-methyl-isobutyl-amiloride ameliorates brain injury when commenced before hypoxia ischemia in neonatal mice. Pediatr Res 2006;59:227–31.
67. Hom S, Fleegal MA, Egleton RD, Campos CR, Hawkins BT, Davis TP. Comparative changes in the blood-brain barrier and cerebral infarction of SHR and WKY rats. Am J Physiol Regul Integr Comp Physiol 2007;292:R1881–92.
68. Robertson NJ, Kato T, Bainbridge A, Chandrasekaran M, Iwata O, Kapetanakis A, et al. Methyl-isobutyl amiloride reduces brain Lac/NAA, cell death and microglial activation in a perinatal asphyxia model. J Neurochem 2013;124:645–57.
69. Ferrazzano P, Shi Y, Manhas N, Wang Y, Hutchinson B, Chen X, et al. Inhibiting the Na+/H+ exchanger reduces reperfusion injury: a small animal MRI study. Front Biosci 2011;3:81–8.
70. Phillis JW, Estevez AY, Guyot LL, O'Regan MH. 5-(NEthyl-N-isopropyl)-amiloride, an Na+/H+ exchange inhibitor, protects gerbil hippocampal neurons from ischemic injury. Brain Res 1999;839:199–202.
71. Liu Y, Kintner DB, Chanana V, Algharabli J, Chen X, Gao Y, et al. Activation of microglia depends on Na+/H+ exchange-mediated H+ homeostasis. J Neurosci 2010;30:15210–20.
72. Shi Y, Chanana V, Watters JJ, Ferrazzano P, Sun D. Role of sodium/hydrogen exchanger isoform 1 in microglial activation and proinflammatory responses in ischemic brains. J Neurochem 2011;119:124–35.
73. Randall RD, Thayer SA. Glutamate-induced calcium transient triggers delayed calcium overload and neurotoxicity in rat hippocampal neurons. J Neurosci 1992;12:1882–95.
74. Matsumoto Y, Yamamoto S, Suzuki Y, Tsuboi T, Terakawa S, Ohashi N, et al. Na+/H+ exchanger inhibitor, SM-20220, is protective against excitotoxicity in cultured cortical neurons. Stroke 2004;35:185–90.
75. Dalkara T, Tan E, Erdemli G, Onur R, Zileli T. Electrophysiological evidence for activation of NMDA receptors and its antagonism by MK-801 in cerebral ischemia. Brain Res 1990;532:101–6.
76. Wang GJ, Randall RD, Thayer SA. Glutamate-induced intracellular acidification of cultured hippocampal neurons demonstrates altered energy metabolism resulting from $Ca^{2+}$ loads. J Neurophysiol 1994;72:2563–9.
77. Manhas N, Shi Y, Taunton J, Sun D. p90 activation contributes to cerebral ischemic damage via phosphorylation of Na+/H+ exchanger isoform 1. J Neurochem 2010;114:1476–86.
78. Douglas RM, Xue J, Chen JY, Haddad CG, Alper SL, Haddad GG. Chronic intermittent hypoxia decreases the expression of Na/H exchangers and HCO3-dependent transporters in mouse CNS. J App Physiol 2003;95:292–9.
79. Rocha-González HI, Castañeda-Corral G, Araiza-Saldaña CI, Ambriz-Tututi M, Caram-Salas NL, Torres-López JE, et al. Identification of the Na+/H+ exchanger 1 in dorsal root ganglion and spinal cord: its possible role in inflammatory nociception. Neuroscience 2009;160:156–64.
80. Castañeda-Corral G, Rocha-González HI, Godínez-Chaparro B, Jiménez-Andrade JM, Granados-Soto V. Role of the spinal Na+/H+ exchanger in formalin-induced nociception. Neurosci Lett 2011;501:4–9.
81. Castañeda-Corral G, Rocha-González HI, Araiza-Saldaña CI, Vidal-Cantú GC, Miguel Jiménez-Andrade J, Murbartián J, et al. Blockade of peripheral and spinal Na+/H+ exchanger increases formalin-induced long-lasting mechanical allodynia and hyperalgesia in rats. Brain Res 2012;1475:19–30.
82. Muthuraman A, Jaggi AS, Singh N, Singh D. Ameliorative effects of amiloride and pralidoxime in chronic constriction injury and vincristine induced painful neuropathy in rats. Eur J Pharmacol 2008;587:104–11.