REVIEW ARTICLE

The Role of Na$^+$/Ca^{2+} Exchanger Subtypes in Neuronal Ischemic Injury

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Abstract The Na$^+$/Ca^{2+} exchanger (NCX) plays an important role in the maintenance of Na$^+$ and Ca^{2+} homeostasis in most cells including neurons under physiological and pathological conditions. It exists in three subtypes (NCX1-3) with different tissue distributions but all of them are present in the brain. NCX transports Na$^+$ and Ca^{2+} in either Ca^{2+}-efflux (forward) or Ca^{2+}-influx (reverse) mode, depending on membrane potential and transmembrane ion gradients. During neuronal ischemia, Na$^+$ and Ca^{2+} ionic disturbances favor NCX to work in reverse mode, giving rise to increased intracellular Ca^{2+} levels, while it may regain its forward mode activity on reperfusion. The exact significance of NCX in neuronal ischemic and reperfusion states remains unclear. The differential role of NCX subtypes in ischemic neuronal injury has been extensively investigated using various pharmacological tools as well as genetic models. This review discusses the mode of action of NCX in ischemic and reperfusion states, the differential roles played by NCX subtypes in these states as well as the role of NCX in pre- and postconditioning. NCX subtypes carry variable roles in ischemic injury. Furthermore, the mode of action of each subtype varies in ischemia and reperfusion states. Thus, therapeutic targeting of NCX in stroke should be based on appropriate timing of the administration of NCX subtype-specific strategies.

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Introduction

Ca^{2+} signaling is a prerequisite of many cellular physiological activities. Precise control of intracellular Ca^{2+} (Ca$^{2+}_i$) is therefore critical for proper regulation of cellular functions. Intracellular organelles, including endoplasmic reticulum, mitochondria, and secretory vesicles have Ca^{2+} uptake mechanisms to remove Ca^{2+} from the cytosol and bring Ca$^{2+}_i$ concentration back to the resting level. In the plasma membrane, the Ca^{2+} pump and the Na$^+$/Ca^{2+} exchanger (NCX) are the two main mechanisms for exporting Ca^{2+} out of the cell. Using these mechanisms, Ca^{2+} concentration in various regions of the cell may be differentially modulated [1–3]. The idea of NCX dates back to the 1960s, when investigators identified a countertransport mechanism between Na$^+$ and Ca^{2+} across the plasma membrane [4]. Purification and cloning of NCX have resulted in major advances in our understanding of this system [5–8]. NCX plays an important role in the maintenance of Ca$^{2+}_i$ homeostasis and is detected in the plasma membrane of most cells, including neurons and glia [4, 9]. It plays a dominant role in removing Ca^{2+} from the cytosol in cells that require extracellular Ca^{2+} for their physiological activities, such as neurons and cardiac muscle cells. NCX is a bidirectional membrane ion transporter. The direction of transport depends on the prevailing Na$^+$ and Ca^{2+} electrochemical gradients (see below) [10, 11].

NCX Subtypes

Two families of Na^+/Ca^{2+} exchangers were identified, the K^+-independent NCX (SLC8) and the K^+-dependent NCKX (SSLC8b1). Both exchangers are encoded by multigene families. Three genes, *ncx1-3*, code for the NCX subtypes NCX1-3 [12, 13], and at least four genes, *nckx1-4*, code for the NCKX subtypes NCKX1-4 [14, 15]. NCX1 was first cloned from canine cardiac sarcolemma [16], and later the corresponding human gene, *SLC8A1*, was mapped to chromosome 2p22.1 [17]. The NCX2 gene, *SLC8A2*, is located on chromosome 19q13.2 [18], while the *SLC8A3* gene, encoding NCX3, maps to chromosome 14q24.2 [19]. The recently discovered crystal structure of archaebacterial *Methanococcus jannaschii* (NCX_Mj) represents a long-awaited breakthrough [20]. Two variants of NCX3 exist in mice: NCX-B, existing in the brain, and NCX-AC, predominant in the skeletal muscles. NCX3-AC has a higher Na^+ sensitivity [21]. Although all three members arise from separate genes on different chromosomes, they share high amino acid identity (about 70 %), especially in the hydrophobic regions [5–7, 16]. A specific 20-amino-acid stretch (219–238), known as XIP (endogenous exchange inhibitory peptide), belonging to the so-called f-loop of the exchanger, exerts an autoinhibitory action thus limiting NCX activity. Many splice variants arise from a combination of six small exons (A, B, C, D, E, and F), whereas a mutually exclusive exon (either A or B) appears in each given splice variant. At least 17 NCX1 and 5 NCX3 proteins are produced through alternative splicing, whereas no splice variants of NCX2 have yet been identified.

Although NCX subtypes are found in almost every type of cell, the expression profiles of these isoforms vary among different tissues and change during development [22]. NCX1 is expressed in multiple organs, including the brain, heart, kidney, skeletal muscle, eye, and blood cells. NCX2 is found only in the brain whereas NCX3 is found in both brain and skeletal muscle [23, 24]. In addition to neurons, NCX is also expressed in glial cells, playing many crucial roles, including the regulation in Na and Ca homeostasis in astrocytes, neurotransmitter release, oligodendrocyte maturation, and myelin formation [25–27].

Mode of Action

NCX acts in a reversible and electrogenic way with a stoichiometry of 3 Na^+ ions/1 Ca^{2+} ion [9]; however, more recently, in addition to the major 3:1 transport mode, it has been demonstrated that ion flux ratio can vary from 1:1 to a maximum of 4:1, depending on the intracellular concentration of Na^+ (Na^+_i) and Ca^{2+} ions [28] (for a comprehensive detailed review, see Khananshvili [29]).

The equilibrium potential of the NCX (E_{NCX}) was calculated to be −73 mV. At membrane potentials (E_m) more negative than the E_{NCX}, NCX will operate in the direction of Ca^{2+} efflux (forward mode). Conversely, at E_m more positive than the E_{NCX}, NCX will operate in the direction of Ca^{2+} influx (reverse mode). As the resting potential of the cell is −80 mV ($E_{NCX} > E_m$), NCX will operate in the Ca^{2+} efflux mode, and it has been demonstrated that Na^+/Ca^{2+} exchange is the major Ca^{2+}-efflux mechanism especially in the heart [4, 30]. When the electrochemical gradient for Na^+ is reversed, such as during membrane depolarization or the opening of gated Na^+ channels, the exchanger will work in reverse mode, i.e., it will transport Na^+ out of the cell and Ca^{2+} into the cell [9, 31].

The activity of NCX is indirectly dependent on the energy-dependent plasma membrane pumps. Under anoxic and ischemic conditions, the functions of Na^+/K^+ ATPase and Ca^{2+} ATPase are impaired, with consequent dysregulation in Na^+_i and Ca^{2+}_i homeostasis [32], which is consequently reflected in the mode of activity of NCX (see below). Study of the physiological functions of NCX has been facilitated by the development of pharmacological tools modifying its activity [4]; the most relevant of which are summarized in Table 1. However, the reliability of data has been greatly challenged by the specificity of the pharmacological agents used, as most of them have actions on other ion transport systems.

Role of NCX in Neuronal Ischemia

Under normal physiological conditions, it is generally accepted that neuronal NCX is primarily involved in extruding Ca^{2+}_i. Under ischemic conditions, energy-producing machinery fails and production of ATP is low. Anaerobic glycolysis and ATP degradation produce H^+, activating the Na^+/H^+ exchanger and causing Na^+ influx [50]. Furthermore, Na^+ efflux is attenuated because the Na^+/K^+ ATPase is inhibited during ischemia. Because the release of glutamate is increased during ischemia [51], overstimulated *N*-methyl-D-aspartate/α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (NMDA/AMPA) receptors result in Na^+ and Ca^{2+} influx into the neurons, and depending on the duration and severity of the excitotoxic event, acute or delayed neuronal death follows. The process of elevation of Ca^{2+}_i will contribute to neuronal death by activation of calpains, endonucleases, nitric oxide synthase, and phospholipases; free radical production; and mitochondrial dysfunction [52–54]. The rise in Na^+_i is also catastrophic, causing cellular swelling and cytoskeletal dysfunction. The rise in Na^+_i in the neurons will allow the $Na^+/$ glutamate carrier to operate in the opposite direction, allowing excess release of glutamate and increasing excitotoxicity [55]. The net increase in Na^+_i, which will increase the E_m above the E_{NCX}, causes NCX to work in the reverse or Ca^{2+}-influx mode. On this basis, it is thought that the NCX, working in

Table 1 Various pharmacological agents affecting NCX

Agent	Pharmacological actions	Mode affected: forward/reverse	References
Inhibitors			
Amiloride, DMB, CB-DMB	Amiloride inhibits both the epithelial Na^+ channel and Na^+/H^+ exchanger. Amiloride derivatives (DMB, CB-DMB) are more specific for NCX	Both	[33, 34]
Amiodarone	Blocks NCX and many other ion channels	Both	[35]
Bepridil, aprindine	Inhibit NCX and other ion channels including L and T-Ca^{2+} channels and delayed rectifier K^+ channels	For bepridil: forward manner is more inhibited than in the reverse manner	[35, 36]
Cibenzoline	Inhibits voltage-operated Na^+ and Ca^{2+}, and K^+ channels Inhibits K^+ currents elicited by muscarinic receptors	Undetermined	[15]
Endogenous exchange inhibitory peptides (XIP: XIP1–3)	XIP1 is a good inhibitor of NCX1 activity XIP2 and XIP3 exhibit only a weak inhibitory property on NCX1	Both	[13, 37]
KB-R7943	NCX3 inhibition requires concentrations that are 3-fold lower than those necessary to inhibit NCX1 and NCX2 Inhibitory effect on L-type VGCC NMDAR blocker	Inhibits reverse mode	[38–39]
Mn^{2+}, Ni^{2+}, and Co^{2+}	NCX3 is 10-fold less sensitive to Ni^{2+} or Co^{2+} inhibition than NCX1 and NCX2	Both	[38]
Nd^{3+}, Tm^{3+}, and Y^{3+}	Block NCX as well as Ca operating systems	Both	[41]
Pralidoxime, 2,3-buthenedione monoxime	Inhibit VGCC and transient outward K^+ currents	Both	[42]
Quinacrine	May stimulate NCX activity under Na^+ deprivation states	Both	[43]
SEA0400	Predominantly blocks NCX1 (lower affinity for NCX2 and no effect on NCX3)	Inhibits reverse mode	[44]
SM-15811	Undetermined	Undetermined	[45]
Verapamil	NCX and L-Ca^{2+} channels	Undetermined	[46]
Activators			
Li^+	Extent of stimulation is lower in NCX1 than in NCX2 and NCX3	Stimulates reverse mode	[47]
Oxidizing agents, such as Fe^{3+}, H_2O_2, GSSG, and O_2	Fe^{3+} is a component of sodium nitroprusside	Undetermined, but Fe^{3+} was shown to stimulate reverse mode	[48, 49]

DMB dimethylbenzamylamiloride, *CB-DMB* 3′,4′-dichlorobenzamyl and α-phenylbenzamyl, N-(4-chlorobenzyl)]2,4-dimethylbenzamy, *GSSG* reduced glutathione, *KB-R7943* a 2-[2-[4-(4-nitrobenzyloxy) phenyl] ethyl] isothiourea methanesulfonate derivative, *NMDAR* N-methyl-D-aspartate receptor, *SEA0400* 2-[4-[(2,5-difluorophenyl)-methoxyl-phenoxy]-5-ethoxyaniline, *SM-15811* 4-phenyl-3-[(N-benzyl)-4-pyperidine]-3,4-dihydro-2(1H)-quinazolinone, *VGCC* voltage-gated calcium channels

the reverse mode under ischemic conditions (Figs. 1 and 2), contributes to increase in Ca^{2+}_i in these states.

From this perspective, the NCX while working in its reverse mode may have two opposing roles in ischemic injury: adding to ischemic insult by increasing Ca^{2+}_i and reducing neuronal damage by reducing Na^+_i. Various in vivo and in vitro studies (Table 2) have been conducted to address these questions and to delineate the exact role played by NCX in order to evaluate its therapeutic potential. Despite the conflicting results, most in vivo studies using the focal cerebral ischemia model indicate that blocking NCX activity is neurodamaging whereas increasing NCX activity is neuroprotective [63].

The activation of NCX to work in reversal (Ca^{2+}-influx mode) using extracellular Na^+ depletion model could prevent neuronal damage and death, and this protective effect was inhibited by amiloride (an NCX inhibitor) [64]. A similar protective effect was shown by stimulation of the Ca^{2+}-influx mode by sodium nitroprusside, which stimulates the NCX owing to its Fe^{3+} content [62]. Sodium nitroprusside increased Ca^{2+} following chemical hypoxia, an effect that was blocked by the NCX blockers bepridil and CB-DMB. Amoroso et al.

Fig. 1 The alternation in NCX mode of activity before, during, and after ischemic insult
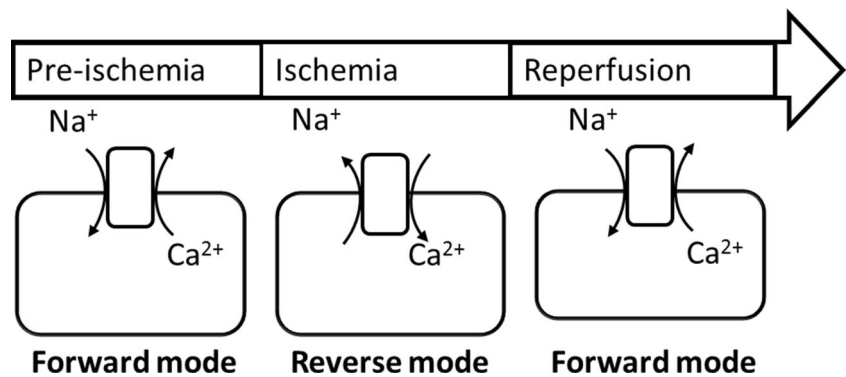
have provided evidence that inhibiting NCX is harmful in rat hippocampal slices exposed to chemical hypoxia and glucopenia [58]. Levels of aspartate (an excitatory amino acid) released were higher when several inhibitors of NCX were present at the beginning of hypoxia, indicating a more severe excitatory insult when NCX reversal mode is inhibited, allowing more Na^+ accumulation and release of excitatory neurotransmitters.

The neuroprotective effect of NCX working in the reverse mode (Fig. 2) could be due to a variety of factors. The NCX by promoting Ca^{2+} influx to the cell will regenerate the Ca^{2+} stored in the endoplasmic reticulum depleted by the ischemic state [65]. By eliciting a decrease in Na^+_i overload, NCX prevents cell swelling and death [66], and by reducing Na^+_i it decreases the release of glutamate, reducing the excitotoxic insult exerted by glutamate under ischemic conditions. Li^+, which stimulates the reverse mode of NCX, was shown to

be protective against glutamate-induced damage; however, this required a dose far higher than that needed to stimulate NCX [47].

Another set of studies yielded different conclusions. KB-R7943, which preferentially inhibits the reversal mode in low doses and the forward mode in high doses, was neuroprotective only in low doses (0.1 μM), whereas high doses (30 μM) exacerbated neuronal damage [59]. The same compound was also found to be neuroprotective by reducing Ca^{2+}_i loads [67]. However, some have suggested that the neuroprotective mechanism of KB-R7943 is unrelated to NCX inhibition, including effects on store-operated Ca^{2+} channels and NMDA receptors [68] as inhibition of the mitochondrial permeability transition pore [69] and activity on mitochondrial Ca^{2+} uniporters [70].

S1EA0400, a more potent inhibitor of NCX (IC_{50} equals 33 nM), also showed a neuroprotective effect both

Fig. 2 The role of NCX working in reverse mode during an ischemic attack. Does the cell *trade* removal of excess Na^+_i by accepting more Ca^{2+}_i? *Dotted arrow* indicates slowed ion transport; *thick solid line* indicates enhanced ion transport. *Dashed arrows* indicate negative effect; *thin solid line* indicates positive effect
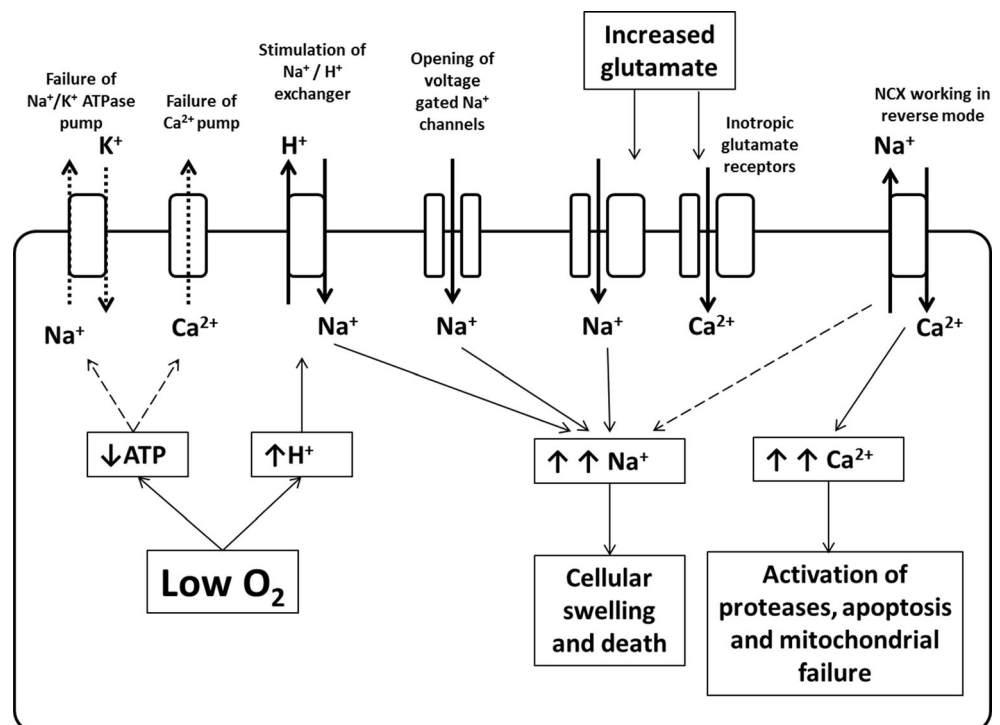
Table 2 Effect of pharmacological agents affecting NCX on neuronal insults

Type of study	Agents affecting the NCX	Neurons studied	Effects	References
In vitro	Bepridil	Rat optic nerve	Protective against anoxic injury	[56]
In vitro	Bepridil and KB-R7943	Cultured cortical neurons prolonged oxygen glucose deprivation	No effect	[57]
In vitro	KB-R7943	Hippocampal slices	Protective against hypoxia and hypoglycemia	[59]
In vitro	Li$^+$	Cerebellar granule cells	Protective against excitotoxicity during hypoglycemic injury	[60]
In vitro	SEA0400	Cultured rat astrocytes	Protective against Ca^{2+}-paradox-induced injury	[39]
In vivo	SEA0400	Transient middle cerebral artery occlusion	Reduced infarction size	[39]
In vivo	CB-DMB, bepridil, GLU-XIP and KB-R7943	Permanent middle cerebral artery occlusion	Increased infarction volume	[40]
In vivo	KB-R7943	Global ischemia/reperfusion	Reduced infarction size	[61]
In vivo	Bepridil and GLU-XIP glycosylated exchange inhibitory peptide	Cortical neurons after middle cerebral artery occlusion	Increased infarct volume	[62]

in vivo and in vitro in a series of experiments conducted by Matsudo et al. [39], a finding also confirmed by Iwamoto et al. [44]. However, the matter is complicated by data suggesting that SEA0400 lacks specificity and affects Ca^{2+} transport through other pathways [71]. Moreover, the mode of action of SEA0400 on NCX seems to be more selective for NCX1 [44, 72], suggesting the need to study the differential roles of individual NCX subtypes, which was facilitated by genetic models (see below). It is worth mentioning that some reports have noted a neutral effect of NCX in neuronal ischemic states, stating that the NCX activity may play no significant role in protecting or damaging ischemic neurons [57].

Role of NCX in Reperfusion States

Following the recovery of ATPase activity, including Na^+/K^+ ATPase, secondary to replenishment of ATP in reperfusion, NCX operates in a forward mode [59]. Although NCX contributes to Ca^{2+}_i overload by working in reverse mode under ischemic or excitotoxic states, NCX is protective in excitotoxic states when it works in its forward Ca^{2+} extrusion capacity [73]. Failure of NCX to work in its forward mode contributes to excitotoxic Ca^{2+} overload. Knockdown of NCX decreased the lethal dose of glutamate and overexpression of NCX decreased glutamate-induced Ca^{2+} overload and rescued the cells. Bano et al. also proposed that destruction of NCX by calpains contributes to excitotoxic damage [74]. This greatly highlighted the importance of the forward working NCX during reperfusion injury.

However, it is known that the Ca^{2+} extrusion system is critically affected during ischemia by destruction of proteins involved in Ca^{2+} homeostasis [75], which raises questions about the role of NCX during reperfusion. It seems, however, that not all NCX subtypes are destroyed by Ca^{2+}-dependent proteolysis through ischemia/reperfusion injury. ncx1 messenger RNA (mRNA) level was reduced by 42.1 and 27.8 %, respectively, after 2 and 6 h of reperfusion (after transient middle cerebral artery occlusion (MCAO) for 120 min) and was restored to normal levels after 12 and 24 h of reperfusion. NCX1 protein was decreased after 2 h of reperfusion and regain to normal level at 24 h although other reports show it may remain downregulated for 72 h after reperfusion [76, 77]. The mRNA and protein levels of NCX2 and NCX3 did not change significantly over time [76], but NCX3 protein was reduced indicating that it might be destroyed by proteolytic system during reperfusion [77]. Some have proposed that after the energy state of the cell improves, e.g., during reperfusion, NCX operates in a rectifying Ca^{2+}-efflux (forward) mode, clearing excess Ca^{2+}_i accumulated during the ischemic episode [63]. This has also been supported by the recent work of Cross et al., who showed that neuronal and HEK293 cell survival following oxygen glucose deprivation was dramatically improved following overexpression of NCX2 and NCX3 (but not NCX1) by improving Ca^{2+}_i management, i.e., by working in forward mode [78] (Fig. 1). However, the protective effect of the forward mode is based mainly on excitotoxic models, and further studies are needed to clarify how much NCX contributes in modulating reperfusion injury. This will require conditioned knockdown studies conducted during reperfusion states.

Differential role of the NCX Subtypes in Neuronal Ischemic Injury

NCX1

Using transient MCAO, the cerebral infarcts in NCX1$^{+/-}$ mice were significantly smaller than those in wild-type mice. SN-6, pharmacological blocker for the NCX1, significantly reduced the infarct volume [72]. These findings may explain the protective effect of SEA0400, as a selective NCX1 inhibitor, found in in vivo and in vitro studies (see before). However, these conclusions do not accord with the data reported by Pignataro et al. [79], who showed that intracerebroventricular infusion of oligodeoxynucleotides (ODNs) against NCX1 in rats for 24 and 48 h prior to permanent MCAO caused an increase in the infarction volume. However, the models were different between the two groups. The infarction volume was evaluated 24 h after reperfusion in the former study and 24 h after permanent ischemia in the latter, suggesting that NCX1 may contribute to neuronal damage during ischemia/reperfusion, while having a neuroprotective effect in permanent ischemic states. This also suggests that downregulation of NCX1 during reperfusion [76, 77] might be a physiological protective response of neurons although further investigation is required to test this hypothesis. It is worth mentioning that SN-6 have multiple inhibitory effects on other mechanisms which should be considered while evaluating its effects [80].

The role of NCX1 in mediating cell damage was further evaluated by its role in mediating NMDAR-induced retinal damage. NCX1$^{+/-}$ mice possessed significant protection against retinal damage induced by intravitreal injection of NMDA. SEA0400 significantly reduced NMDA or high intraocular pressure-induced retinal cell damage in mice. It also reduced the expression of phosphorylated mitogen-activated protein kinases (ERK1/2, JNK, p38) induced by NMDA injection [81] and failed to produce its protective effect against Ca^{2+} overload-mediated factors other than NCX1. This finding may suggest that the Ca^{2+} overload produced by NCX1 working in its reverse mode may contribute to NMDA-mediated excitotoxicity.

Of the NCX subtypes, NCX1 is the most highly expressed in microglia [82]. Increased NCX1 expression and activity, working in reverse mode, has been found in microglial cells inside and surrounding brain infarction core. It has been suggested that these changes carry protective role in the postinfarction period [83].

NCX2

In contrast to NCX1$^{+/-}$, transient cerebral ischemia in vivo produced a larger infarction and more cell death in the NCX2$^{-/-}$ mouse brain [84] while in wild-type brain, NCX2-expressing neurons were largely spared from cell death after ischemia. NCX2-deficient hippocampal cells showed slower recovery in population spike amplitudes, a sustained elevation of Ca$^{2+}_i$, and an increased membrane depolarization after in vitro ischemia. This suggests that NCX2 exports Ca^{2+} (working in forward mode) in ischemia/reperfusion and thus protects neuronal cells from death by reducing Ca$^{2+}_i$ [85]. In addition, overexpression of NCX2 (which is not susceptible to cleavage by calpain) protected neurons against glutamate excitotoxicity, a process that is, at least partially, dependent upon Ca^{2+}-induced injury [74].

In the previously discussed model of Pignataro et al., ODN downregulation of NCX2 protein levels had no effect on infarct size. This suggests that NCX2 does not play a major role in cell death following permanent ischemia [79]. Jeon et al. [85] have demonstrated larger focal injury in rats deficient of the NCX2 protein following transient MCAO. The overexpression of NCX2 in cerebellar granule neurons confers neuroprotection against excitotoxicity in vitro [74]. Taken together, these studies show that NCX2 has a protective effect under the ischemia/reperfusion state, whereas its role in permanent ischemic models may be less important.

NCX3

Similar to NCX2, NCX3 knockout mice display increased brain injury following transient global and transient focal cerebral ischemia [84, 86, 87]. In vitro studies using cortical neuronal cultures subjected to transient oxygen glucose deprivation also revealed increased neuronal injury in NCX3 knockout mouse-derived cultures [87]. Furthermore, the reduced NCX3 activity limits Ca^{2+}-induced neurotoxicity during severe transient but not during severely sustained ischemic insults [78]. Although NCX3 was not shown to be cleaved during ischemia/reperfusion injury (see before) [76], Bano et al. have demonstrated in a rat model of focal ischemia that the NCX3 isoform is cleaved by calpain following permanent ischemia and glutamate-induced excitotoxicity. NCX3 is crucial in extruding the Ca^{2+} load resulting from glutamate exposure, and the cleavage of the NCX protein contributes to Ca^{2+} overload [74, 88]. In the permanent ischemia model, ODN downregulation of the NCX3 protein level showed a larger infarction size, rendering NCX3 the only subtype that is protective against reperfusion and permanent injury.

Pre- and Postconditioning

Preconditioning is a phenomenon whereby a subliminal injurious stimulus is applied before a longer, harmful ischemia, whereas postconditioning refers to a short sublethal brain ischemia subsequent to a prolonged harmful ischemic episode, which may confer ischemic neuroprotection [89]. NCX,

NCX3 in particular, seems to also play a role in such protective phenomena. Interestingly, NCX3 was upregulated when the brain was exposed to a transient ischemic episode for 30 min (but not 120 min, see before) [77]. Although 120 min of transient MCAO reduced the expression of NCX1 (see before), the 30-min episode did not change its expression. More surprising, the matter was changed when tMCAO for 120 min was preceded by a transient ischemic episode (for 30 min) 72 h previously; both NCX1 and NCX3 were then upregulated. Moreover, small interfering RNA (siRNA) against NCX1 and NCX3 before preconditioning significantly reduced the protective effect of preconditioning [77]. Of the NCX subtypes, NCX3 protein and ncx3 mRNA were the ones that were upregulated in those brain regions protected by postconditioning. NCX3 silencing, induced by intracerebroventricular infusion of siRNA, partially reverted the postconditioning-induced neuroprotection [90]. Further studies are needed to clarify the mode of action of NCX3 under these conditions.

Mitochondrial NCX

The mitochondrial Na^+/Ca^{2+} exchanger (NCX_{mito}), located in the inner mitochondrial membrane, extrudes Ca^{2+} ions from its matrix in exchange with cytosolic Na^+ ions. The stoichiometry of NCX_{mito}-operated Na^+/Ca^{2+} exchange has long been the subject of controversy, but evidence of an electrogenic 3:1 Na^+/Ca^{2+} exchange is increasing. NCX_{mito} has a role in controlling neuronal Ca^{2+} homeostasis and neuronal bioenergetics. The three isoforms of plasma membrane NCX can contribute to NCX_{mito} in neurons and astrocytes [91]. Based on its role in regulating both mitochondrial and potentially cytoplasmic Ca^{2+}, NCX_{mito} seems to be an interesting candidate to study under ischemic conditions. Experimental evidence suggests that supraphysiological activation of NCX_{mito} contributes to neuronal cell death in the ischemic brain, and its activity is related to reduction in the ability to reestablish normal ionic homeostasis. These data suggest that NCX_{mito} could represent an important target for the development of new neurological drugs [92].

NCKX

Despite its importance in maintaining Na^+, Ca^{2+}, and K^+ homeostases in the CNS, the role of NCKX under ischemic conditions, at least relative to NCX, has not generated enough interest. The role of this protein in the development of ischemic damage was assessed by knocking down its expression with ODNs intracerebroventricularly, infused by an osmotic minipump for 48 h, starting from 24 h before permanent MCAO. NCKX2 knockdown increased the extent of the ischemic lesion [93]. Further studies conducted by the same group showed that after MCAO, NCKX2 mRNA and protein expression were downregulated in the ischemic core and peri-infarctional areas. $NCKX2^{-/-}$ primary cortical neurons displayed a higher vulnerability and a greater Ca^{2+}_i increase under hypoxic conditions, compared with $NCKX2^{+/+}$ neurons. These results indicate that NCKX2 may be involved in brain ischemia, and it may represent a new potential target to be investigated in the study of the molecular mechanisms involved in cerebral ischemia [94]. However, other reports have shown that under conditions in which NCX is inhibited, NCKX can provide a way by which Ca^{2+} can get access and contribute to ischemic damage [95], suggesting that the protective role of NCKX is evident only when NCX is functioning.

Conclusions and Unanswered Questions

It is clear that different NCX subtypes exert variable roles in neuronal ischemic injury. The lack of pharmacological tools or agents that specifically modify the function of particular NCX subtypes has greatly limited our understanding of the differential roles of each subtype in neuronal ischemic injury. Genetic models and knockdown of the NCX subtypes have helped to some extent in exploring their roles. The availability of Cre/loxp system to generate conditional $NCX1^{-/-}$ ($NCX1^{-/-}$ mice are embryonic lethal at 11 days postcoitum due to absence of spontaneous heart beats [96]) may help in further exploring the function of this particular subtype [97]. The potential lack of temporal and spatial selectivity of some antisense knockdown strategies limits their usefulness as experimental tools in studying NCX.

NCX subtypes carry variable roles in ischemic injury; furthermore, the mode of action of each subtype varies in ischemia and reperfusion states, which necessitates time-sensitive and subtype-specific therapeutic targeting. The role of other Na^+/Ca^{2+} exchanger systems, NCKX and NCX_{mito} in neuronal injury is still unclear and further investigations are highly required to understand their role.

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