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The role of intracellular sodium (Na^+) in the regulation of calcium (Ca^{2+})-mediated signaling and toxicity

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ABSTRACT

It is known that activated N-methyl-D-aspartate receptors (NMDARs) are a major route of excessive calcium ion (Ca^{2+}) entry in central neurons, which may activate degradative processes and thereby cause cell death. Therefore, NMDARs are now recognized to play a key role in the development of many diseases associated with injuries to the central nervous system (CNS). However, it remains a mystery how NMDAR activity is recruited in the cellular processes leading to excitotoxicity and how NMDAR activity can be controlled at a physiological level. The sodium ion (Na^+) is the major cation in extracellular space. With its entry into the cell, Na^+ can act as a critical intracellular second messenger that regulates many cellular functions. Recent data have shown that intracellular Na^+ can be an important signaling factor underlying the up-regulation of NMDARs. While Ca^{2+} influx during the activation of NMDARs down-regulates NMDAR activity, Na^+ influx provides an essential positive feedback mechanism to overcome Ca^{2+}-induced inhibition and thereby potentiate both NMDAR activity and inward Ca^{2+} flow. Extensive investigations have been conducted to clarify mechanisms underlying Ca^{2+}-mediated signaling. This review focuses on the roles of Na^+ in the regulation of Ca^{2+}-mediated NMDAR signaling and toxicity.

Keywords: NMDA Receptors; Sodium and Calcium Influx; Sodium and Calcium Signaling; Excitability; Toxicity

1. INTRODUCTION

Cytoplasmic Ca^{2+} is the most common signaling factor in all types of cells. Normal intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) is approximately 40,000-fold lower than extracellular $[Ca^{2+}]$, which ranges from 1 to 2 mM [1,2]. Ca^{2+} ions enter neurons via various pathways including voltage-gated Ca^{2+} channels, ligand-gated Ca^{2+} channels and the Ca^{2+} exchangers [2,3]. It is known that activated NMDAR channels are a major route of excessive Ca^{2+} entry in neurons [4-10]. While excessive intracellular Ca^{2+} may activate degradative processes and thereby cause toxic effects [2,10-12], NMDAR channel activity may be inhibited by intracellular Ca^{2+} through: 1) α-actinin/cytoskeleton dissociation from the NR1 subunit of NMDARs [13]; 2) calmodulin activation [13-16], and 3) activation of phosphatases, such as calcineurin which dephosphorylates NMDARs [17-19]. The Ca^{2+}-induced down- regulation of NMDARs is considered an important negative feedback mechanism to control NMDAR activity [20-23]. Based on these findings we questioned: How do excessive amounts of Ca^{2+} get into neurons through NMDARs if NMDARs are inhibited by Ca^{2+} influx?

Na^+ is the major cation in the extracellular space, and it can enter cells through a variety of routes including permeation through ligand- (e.g., glutamate) and voltage-gated cation channels, uptake via membrane exchangers and gradient-driven co-transporters [24]. NMDAR channels are highly permeable to both Na^+ and Ca^{2+}. Short burst or tetanic stimulation of afferents that induces synaptic LTP increases $[Na^+]$ up to 40 or 100 mM in spines and adjacent dendrites [25,26]. These increases can essentially be prevented by the blockade of NMDARs, indicating that they are mainly mediated by Na^+ entry through NMDARs [25,26].

Our initial studies demonstrated that intracellular Na^+ is an up-regulator of NMDARs, such that raising $[Na^+]_i$ or activating Na^+ permeable channels may increase NMDAR-mediated currents [27-29]. We then identified that in hippocampal neurons an increase of 5 ± 1 mM in $[Na^+]_i$ represents a threshold required to mask the down- regulation of NMDARs induced by Ca^{2+} influx. Further increases in Na^+ influx not only significantly enhance Ca^{2+} influx induced by the activation of NMDARs, but also overcome the Ca^{2+}- dependent inhibition of NMDARs [27,30]. This review focuses on the roles of Na^+ in the development of tissue injury and in the regulation of Ca^{2+}-mediated NMDAR signaling and toxicity.

2. Na^+ IN THE PROCESS OF TISSUE INJURY

A significant increase in $[Na^+]_i$ is a characteristic event associated with tissue injury [31-37]. Application of voltage-gated Na^+ channel blockers reduce both Na^+ entry and apoptotic neuronal death [32] whereas increases of Na^+ entry by application of the voltage-gated Na^+ channel activator, veratridine, induce neuronal apoptosis and caspase-3 activation [32,33]. There is a report showing that during anoxia Na^+ entry can occur through either Gd^{3+}-sensitive channels or via $Na^+/K^+/2Cl^-$ co-transporters in cultured hippocampal neurons [38], implying that multiple pathways for Na^+ entry may be activated during tissue injury.

It is known that Na^+ influx into the cell is accompanied by chloride ions (Cl^-) and water, which can lead to acute neuronal swelling and damage [4,39]. Previous studies have shown that Na^+ entry may cause an increase in cytosolic Ca^{2+} through either Na^+/Ca^{2+} exchangers or activation of voltage-gated Ca^{2+} channels [40,41], thereby activating Ca^{2+}-dependent signaling mechanisms. Moreover, Na^+ entry via Na^+/H^+ exchange may cause changes in intracellular pH, and thereby regulate many cellular functions including enzyme activity, neuronal growth and death [38,42-46]. A recent study showed that Na^+ influx plays an important role in the onset of anti-Fas-induced apoptosis and that blocking Na^+ influx may rescue programmed cell death in Jurkat cells [47]. Cox and colleagues reported that the binding of agonists to opioid receptors on guinea pig cortical neuron membranes is significantly reduced by increases in $[Na^+]_i$ of 10-30 mM [48]. Maximal inhibition of μ-, δ- and κ-opioid receptor binding by Na^+ is approximately 60%, 70% and 20%, respectively [48]. Co-occurrence of Na^+,K^+-ATPase dysfunction and Na^+ influx causes α-amino-3-hydroxyl-5-methyl-4-isoxa-zole-propionate receptor (AMPAR) proteolysis and a rapid reduction of AMPAR cell-surface expression [49]. Na^+-mediated K^+ channels such as Slo gene-encoded K^+ channels [50-54], are widely distributed throughout the nervous system and are involved in both the regulation of the after-potential following action potentials [51,55], and the protection of neurons from hypoxic stimulation [51,52,54].

While the details of the mechanisms remain to be clarified, significant pharmacological data have demonstrated the protective effects of blocking Na^+ influx during injuries to the nervous tissue. The blockade of voltage-gated Na^+ channels can prevent neurons from traumatic spinal cord injury [33,56-60] and the loss of white matter [30,56,60,61], concurrently reducing the sensitization associated with pain [62-65] and preventing seizures during kindling development [66]. The inhibition of Na^+/H^+ exchange attenuates ischemia-induced cell death [67,68]. As a result, a major focus of pharmaceutical research has been on the search for effective therapeutic approaches that target voltage-gated Na^+ channels [33,36].

3. ROLES OF Na^+ IN THE REGULATION OF Ca^{2+}-MEDIATED NMDAR SIGNALING AND TOXICITY

3.1. Calcium Influx through Activated NMDARs is Regulated by Na^+ Influx

Activated NMDARs are highly permeable to both Na^+ and Ca^{2+} [20,22,23]. Prolonged increases of intracellular Ca^{2+} during NMDAR activation may act as a negative feedback mechanism controlling NMDAR activity [20,22,23]. In light of our findings demonstrating that: 1) intracellular Na^+ up-regulates NMDAR channel gating and 2) multiple types of receptor/channels such as AMPARs, voltage-gated Na^+ channels, non-selective cation channels and remote NMDARs may regulate NMDAR activity through a Na^+-dependent mechanism [27,28], we investigated how NMDARs are regulated when both Ca^{2+} and Na^+ flow into neurons during the same time period through activated NMDARs [27,30]. Recordings were conducted in the cell-attached single-channel configuration. In this recording model, recorded surface NMDARs are isolated by a recording electrode from the bath environment and therefore cannot be directly stimulated by bath-applied agents. We recorded the activity of surface NMDARs before and after activation of remote NMDARs (outside the patch) induced by bath application of NMDA or L-aspartate [30]. To prevent toxic effects which may be induced by application of NMDA or L-aspartate, a standard extracellular solution in which NaCl and KCl were replaced by Na_2SO_4 and Cs_2SO_4, was utilized [30]. Consistent with previous findings [17,28,39, 69,70], no damage of neurons bathed with this standard solution was observed following NMDA or aspartate application. NMDAR single-channel activity was evoked with 10 μM NMDA and 3 μM glycine included in the standard extracellular solution filling the recording electrodes.

We found that bath application of NMDAR agonists may change NMDAR channel activity recorded in cell-attached patches in a concentration-dependent manner. While a significant increase in NMDAR channel gating occurred during L-aspartate (>100 μM) application to neurons bathed with the standard extracellular solution, the activity of NMDARs was inhibited in neurons when Na^+ influx was blocked by replacing extracellular Na^+ with Cs^+ or N-methyl-D-glutamine (NMDG) [28,30].

We measured the ratio of fluorescence at 346 nm versus 380nm for the Na^+-sensitive dye, sodium-binding benzofuran isophthalate (SBFI), and the Ca^{2+}-sensitive

dye, Fura-2, in the soma region of neurons. When the Na^+ gradient across the cell membrane was decreased by reducing extracellular Na^+ concentration ($[Na^+]_e$) to 20 mM and the Na^+ ionophore, monensin (10 μM) was included in the extracellular solution, basal $[Ca^{2+}]_i$ and $[Na^+]_i$ of neurons were approximately 84 nM and 16 mM, respectively. Under this condition bath application of L-aspartate increased $[Ca^{2+}]_i$ by 66 nM, decreased $[Na^+]_i$ by 5.8 mM and inhibited NMDAR activity [30]. On average, the overall channel open probability and mean open time were reduced to 64% and 77% of controls. The burst and cluster lengths were also significantly reduced. These inhibitory effects produced by the bath application of L-aspartate were prevented by either application of APV or removal of Ca^{2+} from extracellular solution, indicating that the activation of remote NMDARs may also down-regulate recorded NMDAR activity through Ca^{2+} influx [30]. Thus, it is demonstrated that NMDARs can be up- and down-regulated by influxes of Na^+ and Ca^{2+}, respectively.

We then measured changes of $[Na^+]_i$ and $[Ca^{2+}]_i$ in neurons bathed with extracellular solution containing a $[Na^+]$ of 10, 20 or 145 mM before and during the activation of NMDARs induced by bath application of L-aspartate. We found that with an increase in $[Na^+]_e$, the activation NMDARs produced increases in $[Na^+]_i$ as expected, but also increased $[Ca^{2+}]_i$. Excluding the effect of Ca^{2+} influx-induced Ca^{2+} release (CICR) from intracellular stores, the increase in $[Ca^{2+}]_i$ of neurons bathed with extracellular solution containing 145 mM Na^+ was still significantly higher than that found in neurons bathed with extracellular solution containing 10 mM Na^+ [28,30]. When $[Na^+]_e$ was reduced to 10 mM, the activation of NMDARs produced increases in $[Na]_i$ and $[Ca^{2+}]_i$ by around 0.8 mM and 35 nM, respectively. Under this condition, the activation of remote NMDARs inhibited NMDAR activity recorded in cell-attached patches [30]. When $[Na^+]_e$ was increased to 20 mM, NMDAR activation produced a 5 mM increase in $[Na^+]_i$ and a 50 nM increase in $[Ca^{2+}]_i$, but no change in the activity of recorded NMDARs [30]. Similarly, increasing $[K^+]_e$ by 30 mM in an extracellular solution containing 170 mM Na^+ and 1 μM TTX produced increases in $[Na^+]_i$ and $[Ca^{2+}]_i$ by around 7 mM and 48 nM, respectively, but again showed no change in the activity of NMDARs recorded in cell-attached patches either [28,30]. Thus, an increase in $[Na^+]_i$ of approximately 5 mM appeared to be a critical concentration for masking the inhibitory effects induced by Ca^{2+} influx on NMDARs in cultured hippocampal neurons [30]. Since a modest increase of $[Ca^{2+}]_i$ by approximately 35 nM inhibited NMDAR activity when $[Na^+]_e$ was reduced to 10 mM [30], it was possible that Na^+ influx not only enhanced Ca^{2+} influx but also masked the inhibitory effects of Ca^{2+}.

To confirm this hypothesis, we recorded NMDAR single-channel activity before and during the activation of remote NMDARs in cell-attached patches with pipettes filled with a Ca^{2+}-free extracellular solution containing 200 mM Na^+ from neurons that had been pre-treated with BAPTA-AM (10 μM for 4 hrs) and bathed with the same Ca^{2+}-free extracellular solution, or with pipettes filled with extracellular solution containing 0.3 or 1.2 mM Ca^{2+} from neurons bathed with the extracellular solution containing the same amount of Ca^{2+}, respectively. We found that the activation of remote NMDARs produced a similar up-regulation of NMDAR channel activity when local and bath $[Ca^{2+}]$ was set at 0, 0.3 and 1.2 mM, implying again that the effects of Ca^{2+} influx in the regulation of NMDARs by remote NMDARs are overcome by Na^+ under normal condition [30]. Furthermore, removal of extracellular Ca^{2+} did not produce any effect on the up-regulation of NMDARs by remote NMDARs in neurons bathed with the standard extracellular solution containing 200 mM Na^+ [28,30]. Thus, we conclude that Ca^{2+} influx through activated NMDARs is regulated by Na^+ influx, and that the effect of Na^+, which overcomes Ca^{2+}-induced inhibition, provides an essential positive feedback mechanism enhancing both the NMDAR activity and the inward flow of Ca^{2+}.

3.2. Depletion of Extracellular Ca^{2+} Enhances Na^+ Influx and Thereby Causes NMDAR-Mediated Toxicity

Based on the findings that glutamate concentration may increase in both humans [71,72] and animals after nervous system injury [73], and that application of NMDAR antagonists may protect neurons from excitotoxic injuries in both humans [74,75] and animal [39,74,76], it has been believed that NMDAR-mediated excitoxicity plays a ke- y role in the development of neuronal death associated with stroke/traumatic CNS injury. However, it remained unclear how NMDARs were recruited to cause neurotoxicity.

We examined the effects of extracellular Ca^{2+} depletion and reperfusion, which may occur in stroke patients, on cultured hippocampal neurons [27,77]. Neurons were bathed initially with an extracellular solution containing: 140 mM NaCl, 5 mM CsCl, 1.8 mM $CaCl_2$, 33 mM glucose, 25 mM HEPES; pH: 7.35; osmolarity: 310-320 mOsm. The reduction of $[Ca^{2+}]_e$ from 1.8 mM to 0.5 or 0 mM caused a significant increase in Caspase-3 activity and morphological changes in neurons such as swelling, beading, and/or process disintegration. Significantly less formazan was observed in 3-(4,5-dimethylthiazol-2-yl) 2,5-diphenyl-tetrazolium bromide (MTT) assays in which neurons were treated with the extracellular solution containing 0.5 or 0 mM Ca^{2+}, indicating a change in mitochondrial function associated with neuronal injury [78-81]. Unexpectedly, application of NMDAR antagonists APV (100 μM) and MK801 (2 μM) significantly prevented the above mentioned changes in neurons only

when the drugs were applied concomitant to the reduction of $[Ca^{2+}]_e$ from 1.8 to 0 mM. No protective effects of the drugs could be found when they were applied during Ca^{2+} reperfusion or when $[Ca^{2+}]_e$ was reduced from 1.8 to 0.5 mM [77]. These findings suggest that the depletion of extracellular Ca^{2+} may evoke NMDAR-mediated neurotoxicity [77], and also raised the questions of how and when NMDAR activity is recruited to induce neuronal injury following the removal of extracellular Ca^{2+}.

To address this question, we recorded NMDAR single-channel activity before and during a depletion of extracellular Ca^{2+} from 1.8 to 1.3, 0.5 or 0 mM in cell-attached patches from cultured hippocampal neurons. To prevent cell damage during the reduction of extracellular Ca^{2+}, the Cl^- in the standard extracellular solution was replaced by SO_4^{2-} [28,30,39,77]. Bath application of a low $[Ca^{2+}]$ solution to neurons caused a parallel shift of the current-voltage (I/V) relationship in NMDAR single-channels recorded in cell-attached patches, which indicates that there is a cell-depolarization, but no change in single-channel conductance [30,77]. In order to account for this, the holding potential was re-adjusted to maintain a 70 mV patch-potential from the reversal potential of recorded channels. We found that a depletion of extracellular Ca^{2+} from 1.8 to 1.3 or 0.5 mM did not induce any significant change in the activity of recorded channels until $[Ca^{2+}]_e$ was reduced from 1.8 to 0 mM [28,30,77]. The channel activity could be subsequently abolished with application of the NMDAR antagonist, MK801, confirming that a $[Ca^{2+}]_e$ reduction from 1.8 to 0 mM produces increases in NMDAR activity [28,30,77]. Since the concomitant blockade of NMDARs to the reduction of $[Ca^{2+}]_e$ from 1.8 to 0.5 mM may actually increase the number of injured neurons [77], the up-regulation of NMDARs appears to be essential in triggering of toxicity mediated by NMDARs and the application of NMDAR antagonists in the Ca^{2+} reperfusion model may be protective only when NMDARs are recruited.

To identify the mechanisms by which the removal of extracellular Ca^{2+} results in the up-regulation of NMDARs, we measured $[Ca^{2+}]_i$ and $[Na^+]_i$ in cultured hippocampal neurons before and during reductions of extracellular Ca^{2+}. A $[Ca^{2+}]_e$ reduction-dependent decrease in $[Ca^{2+}]_i$ and increase in $[Na^+]_i$ were observed [77]. A depletion of extracellular Ca^{2+} from 1.8 to 0 mM produced sufficient increases in $[Na^+]_i$ capable of enhancing NMDAR activity [28,30,77]. Furthermore, we found that the up-regulation of NMDAR activity induced by extracellular Ca^{2+} depletion was prevented by the blockade of Na^+ influx [77].

Previous studies showed that the removal of extracellular Ca^{2+} to 0 mM may increase NMDAR single-channel conductance [20,23,82], and that reducing intracellular Ca^{2+} may reduce the Ca^{2+}-dependent inhibition of NMDARs and thereby enhance NMDAR channel activity [13-20,23]. Therefore, it is possible that NMDAR gating may be enhanced by the removal of extracellular Ca^{2+} through Na^+ and/or Ca^{2+}-dependent mechanisms.

The ensemble currents produced by the summation of consecutive super-clusters were compared before (1.8 mM) and after reducing $[Ca^{2+}]_e$ to 0 mM. We found that the removal of extracellular Ca^{2+} may significantly increase the decay time of ensemble currents and that this effect can be abolished by blocking Na^+ influx. This suggests that the removal of extracellular Ca^{2+} may affect NMDAR-mediated whole-cell responses through the action of Na^+ [77].

Large reductions in $[Ca^{2+}]_e$ have been found during instances of high neuronal activity [83-85], the development of seizures [86], hypoglycemic coma [87], and periods of hypoxia and ischemia [88,89]. Ca^{2+}-depletion has also been reported to induce cell injury and death [90]. Thus, the Na^+-dependent enhancement of NMDAR activity induced by depletion of extracellular Ca^{2+} may be an important mechanism underlying the development of neurotoxicity in the CNS.

3.3. Na^+ Regulation of Ca^{2+} Homeostasis

Under resting conditions $[Ca^{2+}]_i$ in neurons is normally maintained at 10–100 nM, and is tightly regulated by both Ca^{2+} influx and efflux across the membrane. $[Ca^{2+}]_i$ can be increased by Ca^{2+} entry through Ca^{2+} channels (including ligand- and voltage-gated Ca^{2+} channels and non-selective cation channels) located on the plasma membrane and by CICR from the endoplasmic reticulum (ER) upon binding of inositol trisphosphate (IP3) to the inositol trisphosphate receptor (IP3R). Ca^{2+}-mediated injury is usually acute and rapid [91]. Disturbances of Ca^{2+} homeostasis in the cytoplasm, ER, or mitochondria can be harmful to cells [3]. Since Ca^{2+} stores are closely connected within the cells and interact with each other, dysregulation of one compartment is usually followed by responses from the others. Together, they may overwhelm the cell's capacity to maintain overall homeostasis and kill the cell [3].

In the plasma membrane Ca^{2+}-ATPase and the Na^+/Ca^{2+} exchanger act to transport cytosolic Ca^{2+} to the extracellular space. The Na^+/Ca^{2+} exchanger has a low affinity for Ca^{2+} but a high velocity; as such, it removes Ca^{2+} only when cytosolic concentrations are high. The Ca^{2+}-ATPase, has a high affinity for Ca^{2+} and pumps out Ca^{2+} even at low cytosolic concentrations [3,92,93]. In resting cells, $[Ca^{2+}]$ in the mitochondrial matrix is around 100 nM. When cytosolic $[Ca^{2+}]$ rises, Ca^{2+} can enter the mitochondria through a uniporter and thereby regulate Ca^{2+} signals [94]. In mitochondria the Na^+/Ca^{2+} exchanger extrudes Ca^{2+} [3,94,95]. However, the activity of the Na^+/Ca^{2+} exchanger may be reversed on the influx of Na^+ [96]. This reversal in Na^+/Ca^2 exchange is observed under pathological conditions [3]. If the mitochondrial

Na^+/Ca^{2+} exchanger is overwhelmed by Ca^{2+} entry, the Ca^{2+} levels in the mitochondrial matrix may increase enough to trigger a mitochondrial permeability transition. The sustained transitions may cause mitochondrial depolarization, inhibition of ATP production, and cell death [97-99]. In the nucleus Ca^{2+} is involved in the gene transcription and DNA metabolism [100-102]. Unlike in the mitochondria, nuclear Ca^{2+} is found to be rapidly equilibrated with cytosolic Ca^{2+}. This may occur by diffusion across nuclear pores [103] and/or Ca^{2+} channels in the nuclear envelope [104].

CICR during NMDAR activation has been reported [20,105,106]. We observed that when extracellular solution contained more Na^+, NMDAR activation produced greater increases in both $[Na^+]_i$ and $[Ca^{2+}]_i$ [30]. Furthermore, in neurons bathed with extracellular solution containing 145 mM Na^+, NMDAR activation-induced increases in $[Ca^{2+}]_i$ were significantly reduced from 100 ± 30 nM (n = 5) to 62 ± 8 nM (n = 8) with thapsigargin (0.1 μM) treatment [30], which depletes intracellular stores of Ca^{2+} by blocking Ca^{2+} re-uptake. In the absence of thapsigargin, NMDAR activation only produced a 35 ± 8 nM (n=8) increase in $[Ca^{2+}]_i$ in neurons bathed with extracellular solution containing 10 mM Na^+ [30]. The blockade of Ca^{2+} influx by removal of extracellular Ca^{2+} abolished the NMDAR activation-induced increase in $[Ca^{2+}]_i$, (data not shown) [2,107]. The increase in $[Ca^{2+}]_i$ induced by Ca^{2+} release from intracellular stores during NMDAR activation in neurons bathed with extracellular solution containing 10 mM Na^+ was significantly reduced when compared with that in neurons bathed with extracellular solution containing 145 mM Na^+ [30]. These data suggest that CICR from intracellular stores during NMDAR activation may be regulated by intracellular Na^+. Stys and colleagues provided direct evidence showing that intra-axonal Ca^{2+} release during ischemia in rat optic nerves is mainly dependent on Na^+ influx. This Na^+ accumulation stimulates three distinct intra-axonal sources of Ca^{2+}: 1) the mitochondrial Na^+/Ca^{2+} exchanger driven in the Na^+ import/Ca^{2+} export mode; 2) positive modulation of ryanodine receptors; and 3) promotion of IP3 generation by phospholipase C [108].

4. QUESTIONS AND FUTURE STUDIES

Na^+ entry is a key factor that initiates fast action potentials and shapes sub-threshold electrical properties to thereby regulate neuronal excitability and neuronal discharge activity [109-113]. Present data have shown that: 1) intracellular Na^+ up-regulates NMDARs; 2) via increasing intracellular Na^+, multiple types of receptor/channels such as AMPA receptors, voltage-gated Na^+ channels and non-selective cation channels, may regulate NMDAR activity; 3) Na^+ influx may enhance Ca^{2+} influx, mask the Ca^{2+}-dependent inhibition of NMDARs and significantly alter Ca^{2+} homeostasis.

Based on combined investigations of protein crystal structures in-vitro and functions in cells, Na^+ binding motifs have been characterized in a number of proteins such as thrombin, Na^+/K^+-ATPase and various neurotransmitter transporters. Thrombin is a serine protease, the activity of which is regulated by Na^+ binding. The sequence, CDRDGKYG, in the Na^+ binding loop is highly conserved in thrombin from 11 different species [114]. Investigations into the crystal structure of a bacterial homologue of the Na^+/Cl^- dependent transporters from Aquifex aeolicus revealed that there are two Na^+ binding sites, named Na1 and Na2 [115]. Na^+/K^+-ATPase is found to have three Na^+ biding sites. Na1 is formed entirely by the side chain oxygen atoms of residues on three helices in the transmembrane regions (TM) 5, 6 and 8. Na2 is formed almost "on" the TM4 helix with three main chain carbonyls plus four side chain oxygen atoms (Asp 811 and Asp 815 on TM6 and Glu 334 on TM4). The Na3 binding site is contiguous to Na1. The carbonyls of Gly 813 and Thr 814 (TM6), the hydroxyl of Tyr 778 (TM5), and the carboxyl of Glu 961 (TM9) contribute to the Na3 binding site [116,117].

To date there is no evidence of a similar amino acid sequence corresponding to a Na^+ binding site, as seen in these Na^+ binding proteins, present in NMDAR subunit proteins (Yu, unpublished data). Molecular mechanisms underlying the regulation of NMDARs and Ca^{2+} signaling by intracellular Na^+ remain unclear. Investigations aiming to identify critical Na^+ targeting site(s) in the regulation of NMDARs and Ca^{2+} homeostasis are essentially needed for understanding activity-dependent neuroplasticity in the CNS.

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REFERENCES

[1] Berridge, M.J., Lipp, P. and Bootman, M.D. (2000) The versatility and universality of calcium signaling. *Nat Rev Mol Cell Biol*, **1,** 11-21.

[2] Clapham, D.E. (1995) Calcium signaling. *Cell*, **80,** 259-268.

[3] Dong, Z., Saikumar, P., Weinberg, J.M. and Venkatachalam, M.A. (2006) Calcium in cell injury and death. *Ann. Rev Pathol.*, **1,** 405-434.

[4] Choi, D.W. (1995) Calcium: Still center-stage in hypoxic-ischemic neuronal death. *Trends. Neurosci.*, **18,** 58-60.

[5] Forder, J.P. and Tymianski, M. (2009) Postsynaptic mechanisms of excitotoxicity: Involvement of postsynaptic density proteins, radicals, and oxidant molecules. *Neuroscience*, **158,** 293-300.

[6] Lipton, S.A. (2006) Paradigm shift in neuroprotection by

NMDA receptor blockade: Memantine and beyond. *Nat. Rev. Drug Discov.*, **5,** 160-170.

[7] Mody, I. and MacDonald, J.F. (1995) NMDA receptor-dependent excitotoxicity: The role of intracellular Ca2+ release. *Trends. Pharmacol. Sci.*, **16,** 356-359.

[8] Soriano, F.X. and Hardingham, G.E. (2007) Compartmentalized NMDA receptor signaling to survival and death. *J. Physiol.*, **584,** 381-387.

[9] Tymianski, M. and Tator, C.H. (1996) Normal and abnormal calcium homeostasis in neurons: A basis for the pathophysiology of traumatic and ischemic central nervous system injury. *Neurosurgery*, **38,** 1176-1195.

[10] Zipfel, G.J., Babcock, D.J., Lee, J.M. and Choi, D.W. (2000) Neuronal apoptosis after CNS injury: The roles of glutamate and calcium. *J. Neurotrauma.*, **17,** 857-869.

[11] Bredesen, D.E. (2000) Apoptosis: Overview and signal transduction pathways [In Process Citation]. *J. Neurotrauma*, **17,** 801-810.

[12] Fiskum, G. (2000) Mitochondrial participation in ischemic and traumatic neural cell death [In Process Citation]. *J. Neurotrauma.*, **17,** 843-855.

[13] Krupp, J.J., Vissel, B., Thomas, C.G., Heinemann, S.F. and Westbrook, G.L. (1999) Interactions of calmodulin and alpha-actinin with the NR1 subunit modulate Ca2+-dependent inactivation of NMDA receptors. *J. Neurosci.*, **19,** 1165-1178.

[14] Ehlers, M.D., Zhang, S., Bernhardt, J.P. and Huganir, R.L. (1996) Inactivation of NMDA Receptors by direct interaction of calmodulin with the NR1 subunit. *Cell*, **84,** 745-755.

[15] Wechsler, A. and Teichberg, V.I. (1998) Brain spectrin binding to the NMDA receptor is regulated by phosphorylation, calcium and calmodulin. *EMBO J.*, **17,** 3931-3939.

[16] Zhang, S., Ehlers, M.D., Bernhardt, J.P., Su, C.T. and Huganir, R.L. (1998) Calmodulin mediates calcium dependent inactivation of N-methyl-D-aspartate receptors. *Neuron*, **21,** 443-453.

[17] Lieberman, D.N. and Mody, I. (1994) Regulation of NMDA channel function by endogenous Ca2+- dependent phosphatase. *Nature*, **369,** 235-239.

[18] Mulkey, R.M., Endo, S., Shenolikar, S. and Malenka, R.C. (1994) Involvement of a calcineurin/inhibitor-1 phosphatase cascade in hippocampal long-term depression. *Nature*, **369,** 486-488.

[19] Tong, G., Shepherd, D. and Jahr, C.E. (1995) Synaptic desensitization of NMDA receptors by calcineurin. *Science*, **267,** 1510-1512.

[20] Dingledine, R., Borges, K., Bowie, D. and Traynelis, S.F. (1999) The glutamate receptor ion channels. *Pharmacol. Rev.*, **51,** 7-61.

[21] Kyrozis, A., Albuquerque, C., Gu, J. and MacDermott, A.B. (1996) Ca(2+)-dependent inactivation of NMDA receptors: Fast kinetics and high Ca2+ sensitivity in rat dorsal horn neurons. *J. Physiol. (Lond)*, **495,** 449-463.

[22] Mayer, M.L. and Westbrook, G.L. (1987) The physiology of excitatory amino acids in the vertebrate central nervous system. *Prog. Neurobiol.*, **28,** 197-276.

[23] McBain, C.J. and Mayer, M.L. (1994) N-Methyl-D-aspartic acid receptor structure and function. *Physiol. Rev.*, **74,** 723-760.

[24] Nicholls, D. and Attwell, D. (1990) The release and uptake of excitatory amino acids. *Trends in Pharmacological Sciences*, **11,** 462-468.

[25] Rose, C.R. (2002) Na+ signals at central synapses. *Neuroscientist.*, **8,** 532-539.

[26] Rose, C.R. and Konnerth, A. (2001) NMDA receptor-mediated Na+ signals in spines and dendrites. *J. Neurosci.*, **21,** 4207-4214.

[27] Yu, X.M. (2006) The role of intracellular sodium (Na+) in the regulation of NMDA receptor-mediated channel activity and toxicity. *Mol Neurobiol.*, **3,** 63-79.

[28] Yu, X.M. and Salter, M.W. (1998) Gain control of NMDA-receptor currents by intracellular sodium. *Nature*, **396,** 469-474.

[29] Yu, X.M. and Salter, M.W. (1999) Src, a molecular switch governing gain control of synaptic transmission mediated by N-methyl-D-aspartate receptors. *Proc. Natl. Acad. Sci. USA*, **96,** 7697-7704.

[30] Xin, W.K. *et al.* (2005) A functional interaction of sodium and calcium in the regulation of NMDA receptor activity by remote NMDA receptors. *J. Neurosci.*, **25,** 139-148.

[31] Ballard-Croft, C., Carlson, D., Maass, D.L. and Horton, J.W. (2004) Burn trauma alters calcium transporter protein expression in the heart. *J. Appl. Physiol.*, **97,** 1470-1476.

[32] Banasiak, K.J., Burenkova, O. and Haddad, G.G. (2004) Activation of voltage-sensitive sodium channels during oxygen deprivation leads to apoptotic neuronal death. *Neuroscience,* **126,** 31-44.

[33] Baptiste, D.C. and Fehlings, M.G. (2007) Update on the treatment of spinal cord injury. *Prog. Brain Res.*, **161,** 217-233.

[34] Bauer, R., Walter, B., Fritz, H. and Zwiener, U. (1999) Ontogenetic aspects of traumatic brain edema: Facts and suggestions. *Exp. Toxicol Pathol.*, **51,** 143-150.

[35] Friedman, J.E. and Haddad, G.G. (1994) Anoxia induces an increase in intracellular sodium in rat central neurons in vitro. *Brain Res.*, **663,** 329-334.

[36] Schwartz, G. and Fehlings, M.G. (2002) Secondary injury mechanisms of spinal cord trauma: A novel therapeutic approach for the management of secondary pathophysiology with the sodium channel blocker riluzole. *Prog. Brain Res.*, **137,** 177-190.

[37] Strichartz, G., Rando, T. and Wang, G.K. (1987) An integrated view of the molecular toxinology of sodium channel gating in excitable cells. *Ann. Rev. Neurosci.*, **10,** 237-67.

[38] Sheldon, C., Diarra, A., Cheng, Y.M. and Church, J. (2004) Sodium influx pathways during and after anoxia in rat hippocampal neurons. *J. Neurosci.*, **24,** 11057-11069.

[39] Choi, D.W. (1993) NMDA receptors and AMPA/kainate receptors mediate parallel injury in cerebral cortical cultures subjected to oxygen-glucose deprivation. *Prog. Brain Res.*, **96,** 137-43.

[40] Blaustein, M.P., Fontana, G. and Rogowski, R.S. (1996) The Na(+)-Ca2+ exchanger in rat brain synaptosomes: Kinetics and regulation. *Ann. N. Y. Acad. Sci.*, **779,** 300-17.

[41] Koch, R.A. and Barish, M.E. (1994) Perturbation of intracellular calcium and hydrogen ion regulation in cultured mouse hippocampal neurons by reduction of the sodium ion concentration gradient. *J. Neurosci.*, **14,** 2585-2593.

[42] Baxter, K.A. and Church, J. (1996) Characterization of acid extrusion mechanisms in cultured fetal rat hippocampal neurones. *J. Physiol. (Lond)*, **493,** 457-470.

[43] Boonstra, J. *et al.* (1983) Ionic responses and growth stimulation induced by nerve growth factor and epidermal growth factor in rat pheochromocytoma (PC12) cells. *J. Cell Biol.*, **97,** 92-98.
[44] Moolenaar, W.H., Defize, L.H. and de Laat, S.W. (1986) Ionic signaling by growth factor receptors. *J. Exp. Biol.*, **124,** 359-73.
[45] Moolenaar, W.H., Tsien, R.Y., van der Saag, P.T. and de Laat, S.W. (1983) Na+/H+ exchange and cytoplasmic pH in the action of growth factors in human fibroblasts. *Nature*, **304,** 645-648.
[46] Sin, W.C. *et al.* (2009) Regulation of early neurite morphogenesis by the Na+/H+ exchanger NHE1. *J. Neurosci.*, **29,** 8946-8959.
[47] Bortner, C.D. and Cidlowski, J.A. (2003) Uncoupling cell shrinkage from apoptosis reveals that Na+ influx is required for volume loss during programmed cell death. *J. Biol Chem*, **278,** 39176-39184.
[48] Werling, L.L., Brown, S.R., Puttfarcken, P. and Cox, B.M. (1986) Sodium regulation of agonist binding at opioid receptors. II. Effects of sodium replacement on opioid binding in guinea pig cortical membranes. *Mol Pharmacol*, **30,** 90-95.
[49] Zhang, D. *et al.* (2009) Na, K-ATPase activity regulates AMPA receptor turnover through proteasome-mediated proteolysis. *J. Neurosci.*, **29,** 4498-4511.
[50] Bhattacharjee, A. *et al.* (2003) Slick (Slo2.1), a rapidly-gating sodium-activated potassium channel inhibited by ATP. *J. Neurosci*, **23,** 11681-11691.
[51] Bhattacharjee, A. and Kaczmarek, L.K. (2005) For K(+) channels, Na(+) is the new Ca(2+). *Trends Neurosci*, **28,** 422-428.
[52] Dryer, S.E. (2003) Molecular identification of the Na+-activated K+ channel. *Neuron*, **37,** 727-728.
[53] Niu, X.W. and Meech, R.W. (2000) Potassium inhibition of sodium-activated potassium (K(Na)) channels in guinea-pig ventricular myocytes. *J. Physiol*, **526(Pt 1),** 81-90.
[54] Yuan, A. *et al.* (2003) The sodium-activated potassium channel is encoded by a member of the Slogene family. *Neuron*, **37,** 765-773.
[55] Liu, X. and Stan, L.L. (2004) Sodium-activated potassium conductance participates in the depolarizing afterpotential following a single action potential in rat hippocampal CA1 pyramidal cells. *Brain Res.*, **1023,** 185-192.
[56] Agrawal, S.K. and Fehlings, M.G. (1996) Mechanisms of secondary injury to spinal cord axons in vitro: Role of Na+, Na(+)-K(+)-ATPase, the Na(+)-H+exchanger, and the Na(+)-Ca2+ exchanger. *J. Neurosci.*, **16,** 545-552.
[57] Agrawal, S.K. and Fehlings, M.G. (1997) The effect of the sodium channel blocker QX-314 on recovery after acute spinal cord injury. *J. Neurotrauma.*, **14,** 81-88.
[58] Fehlings, M.G. and Agrawal, S. (1995) Role of sodium in the pathophysiology of secondary spinal cord injury. *Spine*, **20,** 2187-2191.
[59] Hains, B.C., Saab, C.Y., Lo, A.C. and Waxman, S.G. (2004) Sodium channel blockade with phenytoin protects spinal cord axons, enhances axonal conduction, and improves functional motor recovery after contusion SCI. *Exp. Neurol.*, **188,** 365-377.
[60] Teng, Y.D. and Wrathall, J.R. (1997) Local blockade of sodium channels by tetrodotoxin ameliorates tissue loss and long-term functional deficits resulting from experimental spinal cord injury. *J. Neurosci.*, **17,** 4359-4366.
[61] Cummins, T.R. and Waxman, S.G. (1997) Downregulation of tetrodotoxin-resistant sodium currents and upregulation of a rapidly repriming tetrodotoxin-sensitive sodium current in small spinal sensory neurons after nerve injury. *J. Neurosci.*, **17,** 3503-3514.
[62] Appelgren, L., Janson, M., Nitescu, P. and Curelaru, I. (1996) Continuous intracisternal and high cervical intrathecal bupivacaine analgesia in refractory head and neck pain [see comments]. *Anesthesiology*, **84,** 256-272.
[63] Cox, J.J. *et al.* (2006) An SCN9A channelopathy causes congenital inability to experience pain. *Nature*, **444,** 894-898.
[64] Dib-Hajj, S., Black, J.A., Cummins, T.R. and Waxman, S.G. (2002) NaN/Nav1.9: A sodium channel with unique properties. *Trends Neurosci.*, **25,** 253-259.
[65] Waxman, S.G. and Hains, B.C. (2006) Fire and phantoms after spinal cord injury: Na+ channels and central pain. *Trends Neurosci.*, **29,** 207-215.
[66] Rogawski, M.A. and Loscher, W. (2004) The neurobiology of antiepileptic drugs. *Nat. Rev. Neurosci.*, **5,** 553-564.
[67] Mentzer, R.M., Lasley, R.D., Jessel, A. and Karmazyn, M. (2003) Intracellular sodium hydrogen exchange inhibition and clinical myocardial protection. *Ann. Thorac. Surg.*, **75,** S700-S708.
[68] Vornov, J.J., Thomas, A.G. and Jo, D. (1996) Protective effects of extracellular acidosis and blockade of sodium/hydrogen ion exchange during recovery from metabolic inhibition in neuronal tissue culture. *J. Neurochem.*, **67,** 2379-2389.
[69] Kohr, G., De Koninck, Y. and Mody, I. (1993) Properties of NMDA receptor channels in neurons acutely isolated from epileptic (kindled) rats. *J. Neurosci.*, **13,** 3612-3627.
[70] Yu, X.M., Askalan, R., Keil, G.J.I. and Salter, M.W. (1997) NMDA channel regulation by channel-associated protein tyrosine kinase src. *Science*, **275,** 674-678.
[71] Baker, A.J., Moulton, R.J., MacMillan, V.H. and Shedden, P. M. (1993) Excitatory amino acids in cerebrospinal fluid following traumatic brain injury in humans. *J. Neurosurg.*, **79,** 369-372.
[72] Persson, L. and Hillered, L. (1992) Chemical monitoring of neurosurgical intensive care patients using intracerebral microdialysis. *J. Neurosurg.*, **76,** 72-80.
[73] Benveniste, H., Drejer, J., Schousboe, A. and Diemer, N.H. (1984) Elevation of the extracellular concentrations of glutamate and aspartate in rat hippocampus during transient cerebral ischemia monitored by intracerebral microdialysis. *J. Neurochem.*, **43,** 1369-1374.
[74] Chen, H.-S.V. and Lipton, S.A. (2005) Pharmacological implications of two distinct mechanisms of interaction of memantine with NMDA-gated channels. *J. Pharmacol. Exp. Ther.*, **314,** 961-971.
[75] Lipton, S.A. (2004) Paradigm shifts in NMDA receptor antagonist drug development: Molecular mechanism of uncompetitive inhibition by memantine in the treatment of Alzheimer's disease and other neurologic disorders. *J. Alzheimers. Dis.*, **6,** S61-S74.
[76] Lipton, P. (1999) Ischemic cell death in brain neurons. *Physiol Rev.*, **79,** 1431-1568.
[77] Xin, W.K. *et al.* (2005) The removal of extracellular calcium: A novel mechanism underlying the recruitment of N-methyl-d-aspartate (NMDA) receptors in neurotoxicity. *Eur. J. Neurosci.*, **21,** 622-636.

[78] Giardina, S.F. and Beart, P.M. (2002) Kainate receptor-mediated apoptosis in primary cultures of cerebellar granule cells is attenuated by mitogen-activated protein and cyclin-dependent kinase inhibitors. *Br. J. Pharmacol.*, **135,** 1733-1742.

[79] Ohyashiki, T., Satoh, E., Okada, M., Takadera, T. and Sahara, M. (2002) Nerve growth factor protects against aluminum-mediated cell death. *Toxicology*, **176,** 195-207.

[80] Sawyer, T.W. (1995) Practical applications of neuronal tissue culture in in vitro toxicology. *Clin. Exp. Pharmacol. Physiol.*, **22,** 295-296.

[81] Wang, X., Mori, T., Sumii, T. and Lo, E.H. (2002) Hemoglobin-induced cytotoxicity in rat cerebral cortical neurons: Caspase activation and oxidative stress. *Stroke*, **33,** 1882-1888.

[82] Westbrook, G.L., Krupp, J.J. and Vissel, B. (1997) Cytoskeletal interactions with glutamate receptors at central synapses. *Soc. Gen. Physiol Ser.*, **52,** 163-175.

[83] Hardingham, N.R. *et al.* (2006) Extracellular calcium regulates postsynaptic efficacy through group 1 metabotropic glutamate receptors. *J. Neurosci.*, **26,** 6337-6345.

[84] Rusakov, D.A. and Fine, A. (2003) Extracellular Ca2+ depletion contributes to fast activity-dependent modulation of synaptic transmission in the brain. *Neuron*, **37,** 287-297.

[85] Vassilev, P.M., Mitchel, J., Vassilev, M., Kanazirska, M. and Brown, E.M. (1997) Assessment of frequency-dependent alterations in the level of extracellular Ca2+ in the synaptic cleft. *Biophys. J.*, **72,** 2103-2116.

[86] Heinemann, U. and Hamon, B. (1986) Calcium and epileptogenesis. *Exp. Brain Res.*, **65,** 1-10.

[87] Ekholm, A., Kristian, T. and Siesjo, B.K. (1995) Influence of hyperglycemia and of hypercapnia on cellular calcium transients during reversible brain ischemia. *Exp. Brain Res.*, **104,** 462-466.

[88] Harris, R.J., Symon, L., Branston, N.M. and Bayhan, M. (1981) Changes in extracellular calcium activity in cerebral ischaemia. *J. Cereb. Blood Flow Metab.*, **1,** 203-209.

[89] Nicholson, C., Bruggencate, G.T., Steinberg, R. and Stockle, H. (1977) Calcium modulation in brain extracellular microenvironment demonstrated with ion-selective micropipette. *Proc. Natl. Acad. Sci. USA*, **74,** 1287-1290.

[90] Smith, M.T., Thor, H. and Orrenius, S. (1981) Toxic injury to isolated hepatocytes is not dependent on extracellular calcium. *Science*, **213,** 1257-1259.

[91] Trump, B.F. and Berezesky, I.K. (1995) Calcium-mediated cell injury and cell death. *FASEB J.*, **9,** 219-228.

[92] Carafoli, E., Santella, L., Branca, D. and Brini, M. (2001) Generation, control, and processing of cellular calcium signals. *Crit Rev Biochem. Mol Biol.*, **36,** 107-260.

[93] Berridge, M.J., Bootman, M.D. and Roderick, H.L. (2003) Calcium signaling: Dynamics, homeostasis and remodeling. *Nat Rev Mol Cell Biol*, **4,** 517-529.

[94] Duchen, M.R. (2000) Mitochondria and calcium: From cell signaling to cell death. *J. Physiol.*, **529 (Pt 1),** 57-68.

[95] Gunter, T.E., Yule, D.I., Gunter, K.K., Eliseev, R.A. and Salter, J.D. (2004) Calcium and mitochondria. *FEBS Lett.*, **567,** 96-102.

[96] Hilgemann, D.W., Collins, A. and Matsuoka, S. (1992) Steady-state and dynamic properties of cardiac sodium-calcium exchange: Secondary modulation by cytoplasmic calcium and ATP. *J. Gen. Physiol.*, **100,** 933-961.

[97] Bernardi, P. and Rasola, A. (2007) Calcium and cell death: The mitochondrial connection. *Subcell. Biochem.*, **45,** 481-506.

[98] Crompton, M. (1999) The mitochondrial permeability transition pore and its role in cell death. *J. Biochem.*, **341 (Pt 2),** 233-249.

[99] Lemasters, J.J., Theruvath, T.P., Zhong, Z. and Nieminen, A.L. (2009) Mitochondrial calcium and the permeability transition in cell death. *Biochim. Biophys. Acta*, **1787,** 1395-1401.

[100] Hardingham, G.E., Cruzalegui, F.H., Chawla, S. and Bading, H. (1998) Mechanisms controlling gene expression by nuclear calcium signals. *Cell Calcium*, **23,** 131-134.

[101] McKenzie, G.J. *et al.* (2005) Nuclear Ca2+ and CaM kinase IV specify hormonal- and Notch-responsiveness. *J. Neurochem.*, **93,** 171-185.

[102] Papadia, S., Stevenson, P., Hardingham, N.R., Bading, H. and Hardingham, G.E. (2005) Nuclear Ca2+ and the camp response element-binding protein family mediate a late phase of activity-dependent neuroprotection. *J. Neurosci.*, **25,** 4279-4287.

[103] Allbritton, N.L., Oancea, E., Kuhn, M.A. and Meyer, T. (1994) Source of nuclear calcium signals. *Proc. Natl. Acad. Sci. USA*, **91,** 12458-12462.

[104] Mazzanti, M., DeFelice, L.J., Cohen, J. and Malter, H. (1990) Ion channels in the nuclear envelope. *Nature*, **343,** 764-767.

[105] Sattler, R. and Tymianski, M. (2000) Molecular mechanisms of calcium-dependent excitotoxicity. *J. Mol. Med.*, **78,** 3-13.

[106] Verkhratsky, A. (2002) The endoplasmic reticulum and neuronal calcium signaling. *Cell Calcium*, **32,** 393-404.

[107] Verkhratsky, A.J. and Petersen, O.H. (1998) Neuronal calcium stores. *Cell Calcium*, **24,** 333-343.

[108] Nikolaeva, M.A., Mukherjee, B. and Stys, P.K. (2005) Na^{+}-dependent sources of intra-axonal Ca^{2+} release in rat optic nerve during in vitro chemical ischemia. *J Neurosci*, **25,** 9960-9967.

[109] Cantrell, A.R. and Catterall, W.A. (2001) Neuromodulation of Na+ channels: An unexpected form of cellular plasticity. *Nat. Rev Neurosci.*, **2,** 397-407.

[110] Catterall, W.A., Goldin, A.L. and Waxman, S.G. (2003) International union of pharmacology. XXXIX. compendium of voltage-gated ion channels: Sodium channels. *Pharmacol Rev.*, **55,** 575-578.

[111] Goldin, A.L. (2001) Resurgence of sodium channel research. *Ann. Rev. Physiol.*, **63,** 871-894.

[112] Hille, B. (1992) Ionic channels of excitable membranes. Sinauer, Sunderland.

[113] Waxman, S.G., Dib-Hajj, S., Cummins, T.R. and Black, J.A. (2000) Sodium channels and their genes: Dynamic expression in the normal nervous system, dysregulation in disease states (1). *Brain Res.*, **886,** 5-14.

[114] Di Cera, E. *et al.* (1995) The Na [IMAGE] binding site of thrombin. *J. Biol. Chem.*, **270,** 22089-22092.

[115] Yamashita, A., Singh, S.K., Kawate, T., Jin, Y. and Gouaux, E. (2005) Crystal structure of a bacterial homologue of Na+/Cl: Dependent neurotransmitter transporters. *Nature*, **437,** 215-223.

[116] Li, C., Capendeguy, O., Geering, K. and Horisberger, J.D. (2005) A third Na+-binding site in the sodium pump. *Proc. Natl. Acad. Sci. USA*, **102,** 12706-12711.

[117] Ogawa, H. and Toyoshima, C. (2002) Homology modeling of the cation binding sites of Na+K+-ATPase. *Proc. Natl. Acad. Sci. USA*, **99,** 15977-15982.