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Relationship Between Na^+, K^+-ATPase and NMDA Receptor at Central Synapses

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Abstract: Specific receptors for classical neurotransmitters receptors and neuropeptides, as well as the Na^+, K^+-ATPase, are all molecular entities inserted into synaptic region membranes and localized contiguously. Herein, available experimental evidence showing close interactions between the activity of the Na^+, K^+-ATPase and the N-methyl-D-aspartate (NMDA) ionotropic glutamate receptor was reviewed, supporting a functional link between these macromolecules. The Na^+, K^+-ATPase and NMDA receptor are involved in ion movements through membranes. The former acts as an ion transporter, whereas the latter acts as an ion channel. The modulation of their activity plays a critical role in controlling neuronal function. Examples were taken from studies performed with specific agonists or antagonists of the NMDA receptor. Regarding the Na^+, K^+-ATPase, its involvement was postulated after observing its inhibition by ouabain or related cardiac glycosides. Additionally, experimental conditions known to prevent normal Na^+, K^+-ATPase (i. e., sodium pump functioning) led to similar valuable information. These findings indicate potential cross-talk between this enzyme and the NMDA receptor. The Na^+, K^+-ATPase and NMDA play very important roles in the regulation of learning and memory in the hippocampus. The fact that important changes here described were recorded in the hippocampus indicate a different vulnerability of this area to toxicity induced by the Na^+, K^+-ATPase inhibitor ouabain. Some interesting relationships include calcineurin actions, the participation of ERK or Src family kinases, and signaling cascades initiated by calcium. At present, many other examples of signaling related to the NMDA receptor cannot be correlated with Na^+, K^+-ATPase activity. It is desirable that the development of future research offer new clues for the relationship between Na^+, K^+-ATPase and NMDA receptor activation.

Keywords: Cross-talk, interaction, Na^+, K^+-ATPase, NMDA receptor, relationship.

1. INTRODUCTION

Specific receptors for classical neurotransmitters receptors and neuropeptides, as well as the Na^+ and K^+-dependent ATPase (Na^+, K^+-ATPase), are all molecular entities inserted into synaptic region membranes and localized contiguously. Therefore, it is tempting to speculate that when a given classical neurotransmitter and / or a neuropeptide are / is released, a molecular interaction is favored between the corresponding specific receptor(s) and this enzyme. Taking into account that the Na^+, K^+-ATPase is a ubiquitous membrane-bound enzyme, the possibility of its regulation by released active substances at diverse neuron sites seems tenable.

The effect of most active substances is subsequent to their binding at specific sites of specific macromolecules, the receptors, inserted into synaptic region membranes. The involvement of a particular receptor in a process is often inferred from the use of specific antagonists, able to nullify its effects.

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In synaptic region membranes, neurotransmitter receptors [1] and the Na^+, K^+-ATPase [2] are highly concentrated. Therefore, these macromolecules may well interact provided that they are contiguously located. Results recorded in several experimental models suggested a close relationship between the activity of the Na^+, K^+-ATPase and N-methyl-D-aspartate (NMDA) ionotropic glutamate receptor in intact cells [3].

In the present article available experimental evidence was reviewed, which shows close interactions between the activity of the Na^+, K^+-ATPase and the NMDA receptor, supporting a functional link between these macromolecules.

In some cases, results disclose cell functioning as a unit, as in assays performed in a whole animal, in an isolated organ, in slices, or else in culture systems. On the other hand, assays carried out in cell-free systems such as those employing whole tissue homogenates or subcellular fractions; disclose a direct effect on the macromolecules, obviously independent of signaling processes. Interestingly, an exception is the isolated nerve ending (synaptosome) which behaves as a whole miniature cell in many aspects [see 4].

Protein phosphorylation is a key process in biological regulation which involves a protein kinase, a protein phosphatase and a substrate protein. The kinases catalyze the

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transfer of the terminal γ phosphate of ATP to the hydroxyl moiety in the correponding amino acid residue, in a reaction requiring Mg^{2+}. In turn, the protein phosphatases catalyze the cleavage of this phosphoester bond through hydrolysis. Phosphorylation of diverse protein types is involved in regulating or in carrying out nervous system processes. At nervous system level, protein phosphorylation is the major molecular mechanism through which the function of neural proteins is regulated in response to extracellular signals, including the response to neurotransmitter stimuli. Indeed, it is the major mechanism of neural plasticity, including memory processing. Regarding the subject of the present review, it should be recalled that the Na^+, K^+-ATPase and subunits of the NMDA receptor [5] are phosphorylation substrates.

2. Na^+, K^+-ATPase

2.1. Function at Central Nervous System

In specialized cells, maintenance of the Na^+ and K^+ gradients between the intracellular and extracellular compartments is a prerequisite for basic cellular homeostasis and for diverse functions. The Na^+, K^+-ATPase (sodium- and potassium-activated adenosine 5'-triphosphatase), discovered by Skou [6] also termed Na^+ pump or Na^+, K^+-pump, is an ubiquitous membrane transport protein in mammalian cells, which is essential to establish and maintain high K^+ and low Na^+ concentration in the cytoplasm. The sodium pump regulates K^+ entry with Na^+ exit from the neuron, and therefore is responsible for Na^+ / K^+ equilibrium maintenance through neuronal membranes. Impairment of such equilibria leads to nerve ending depolarization with Ca^{2+} entry to the cell, followed by neurotransmitter release and neuronal swelling, obviously detrimental to cell function [7].

As occurs with all ATPases, it hydrolyzes ATP and occludes ions within the membrane inserted segment of the protein during the translocation process. Therefore, the ionophore is accessible from one membrane side at any given time. This transport system couples the hydrolysis of one molecule of ATP to exchange three sodium ions for two potassium ions, thus maintaining the normal gradient of these cations in animal cells. It acts as an electrogenic ion transporter, which is autophosphorylated on an aspartic acid residue by the γ phosphate group of the ATP molecule that it hydrolyzes [8, 9].

It is known that oxidative metabolism is very active in brain, where large amounts of chemical energy as ATP molecules are consumed, mostly required for the maintenance of the ionic gradients that underlie resting and action potentials involved in nerve impulse propagation, neurotransmitter release and cation homeostasis [9].

Likewise, the sodium pump plays a crucial role in a wide variety of physiological processes, including cell volume regulation, osmotic balance and nervous system differentiation [10].

The Na^+, K^+-ATPase concentrates in surrounding membranes of nerve endings [2], a crucial site in neurotransmission. The involvement of the Na^+, K^+-ATPase in diverse biological processes is often studied employing ouabain and related cardiac glycosides, which proved to behave as selective and powerful inhibitors [9].

In comparison with its environment, every living cell is negatively charged, thus cell environment constitutes a battery able to perform work. The cell employs this electrochemical gradient to provide itself with nutrients, either ionic or non-ionic, from the surrounding medium, and to extrude metabolites and ions from its interior. In this way, the composition of the intracellular compartment remains constant while allowing adaptation to permanent environment changes [8].

Since the sodium pump is responsible for the establishment and maintenance of this electrochemical gradient in animal cells, it is obviously essential for neuronal communication. Besides, electrochemical Na^+ gradient resulting from its activity is the driving force behind secondary transport systems. The Na^+, K^+-ATPase is an ion transporter crucial not only for neural but also for glial physiology by direct electrogenic activity and regulation of ion gradients.

In order to maintain neuronal cytoplasmic Ca^{2+} concentration one-ten thousand times lower that in the extracellular millieu, two mechanisms are operative: a calcium pump and a Na^+ / Ca^{2+} exchanger. The latter depends on functional Na^+, K^+-ATPase, in a process inhibited by omitting Na^+ and including ouabain. Therefore, failure of the Na^+, K^+-pump produces depletion of intracellular K^+, accumulation of intracellular free Ca^{2+} by activation of voltage-gated Ca^{2+} channels and reversion of the Na^+ / Ca^{2+} exchanger [11, 12].

The various mechanisms that consume energy at synapses are summarized in Fig. (**1**).

2.2. Brain Enzyme Structure

The Na^+, K^+-ATPase is an olygomeric protein consisting of α and β subunits, both required for enzyme function. In mammals, four genes (α1- α4) encode the α subunit and three genes (β1-β3) encode the β subunit. The α subunit has the catalytic and the ouabain sites; it has a relative molecular mass of about 100-113 kDa, depending on the presence of different isoforms. This subunit is expressed under several isoforms. Subunits α1, α2 and α3 bind ouabain with low, intermediate and high affinity, respectively. α1 isoform is ubiquitously distributed with highest expression levels in kidney; α2 isoform predominates in skeletal muscle, brain and heart, and α3 isoform seems to be the most abundant form in brain, though it is also present in heart. In neurons the last two isoforms are present whereas in glial cells α1 and α2 isoforms are localized [13-16]. There is another isoform, termed α4, which was identified only in testis [17].

According to temporal and spatial expression in the brain, the various α and β isoforms may be expressed in the same cell type [15, 18] α subunit crosses ten times the membrane, forming transmembrane domains M1 to M10; both the N- and C-termini are localized on the cytosolic side. The binding sites for substrate ATP and the inhibitor ouabain, as well as ion occlusion occur in this subunit [19].

A crystal structure of the Na^+, K^+-ATPase with bound ouabain, at 2.8 A resolution in a state analogous to E2.2 K^+.Pi was described. In this structure, ouabain is deeply

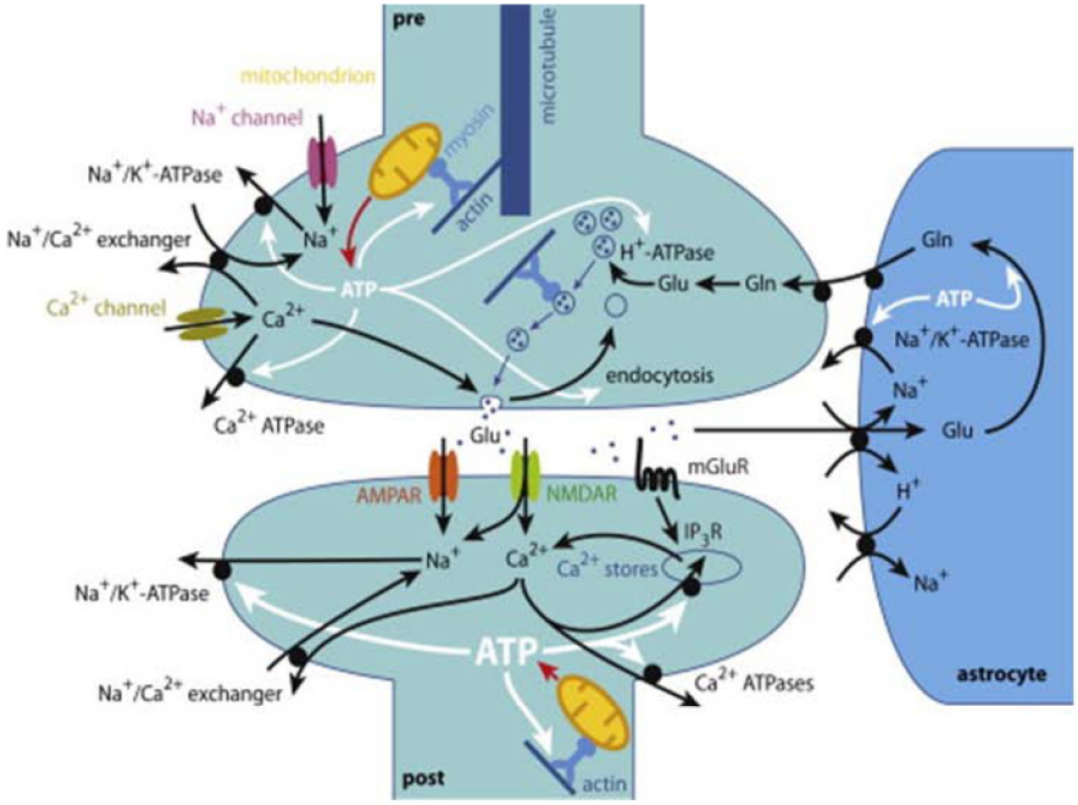

Fig. (1). Mechanisms that consume energy at synapses. Presynaptically, ATP is used for the sodium pump, the Ca^{2+}-ATPase, vacuolar H^+-ATPase, which energizes vesicle transmitter uptake, and motor proteins that move mitochondria and vesicles. In addition, vesicle retrieval by dynamin consumes GTP. Postsynaptically, ATP consume is larger and is mainly on the pumping out ions mediating synaptic currents. In astrocytes, ATP is used to extrude Na^+, to maintain the resting potential and to remove the ions driving glutamate uptake, and on the conversion of glutamate into glutamine. From Reference [203], with permission.

inserted into the transmembrane domain with the lactone ring situated very close to the bound K^+, in contrast to previous models. The structure represents a low-affinity ouabain-bound state because of the antagonism between ouabain and K^+. This crystal structure gives an explanation for mutagenesis data obtained with the high-affinity state, indicating that the binding site for ouabain is essentially the same. According to a homology model for the high affinity state, it is a closure of the binding cavity that confers a high affinity [19']. It has been hypothesized that cardiotonic steroids bind primarily to the E2-P ground state through a channel accessible extracellularly and that the binding of extracellular sodium ions to potassium binding sites relieves inhibition by cardiotonics. This reactivation is dependent on the presence or absence of the sugar moiety on the cardiotonic, and a single sugar is enough to impair reactivation [19''].

In brain, the three isoforms present cell-type and development-specific expression patterns. The α2 isoform is widely expressed in neurons in late gestation but it is primarily expressed in astrocytes in adult brain; most interesting, mice lacking the α2 isoform do not survive after birth [20].

The β subunit regulates the α subunit regarding the activity and conformational stability [21, 22]. It is highly glycosylated, has a relative molecular mass of roughly 60 kDa and the mass of the protein moiety is 36-38 kDa. The β subunit presents a single-transmembrane span with the N-terminus localized on the intracellular membrane side [23]. This subunit seems to participate in the modulation of enzyme affinity for K^+ and Na^+ [24, 25]. It is important for ATP hydrolysis, ion transport, and binding of inhibitors such as ouabain. In order to accomplish ion transport, an interaction between β and α subunit must take place [8].

With respect to β subunit isoforms, β2 type is related to glial cells as an adhesion cell molecule; it is expressed where β1 isoform is absent, including astrocytes and tissues in the central nervous system (glia, choroid plexus, arachnoid membrane), exerting specialized ion-translocating characteristics [8, 26].

The assay of binding affinities for several digitalis glycosides to human Na^+, K^+-ATPase α1β1, α2 β1 and α3 β1 subunits indicates that the sugar determines isoform selectivity [26'].

In association with the α-β dimmer there is a third subunit with a mass of 7-11 kDa, which belongs to the family of small membrane proteins with sequence domain beginning with PFXYD (FXYD proteins). This subunit modulates transport function of the enzyme [27], exerts a regulatory role in a tissue-specific manner but seems not essential for functional Na^+, K^+-ATPase [8, 28]. Regarding subunit assembly, it is known that the diversity of α-β heterodimers *in vivo* is determined by cell-specific co-expression of particular isoforms and by selective association of the α and β isoforms [29].

FXYD proteins are small proteins which behave as channels or regulators of ion channels [30]. The FXYD proteins act as regulators of the Na^+, K^+-ATPase in a tissue-specific manner; they are hydrophobic type I proteins with a single-transmembrane span containing an extracellular invariant sequence. These proteins are not an integral part of the Na^+, K^+-ATPase but modulate catalytic enzyme properties by molecular interactions with specific enzyme domains [30, 31]. The mammalian FXYD proteins from FXYD1 to FXYD7 exhibit tissue-specific distribution [32]. FXYD7 is expressed exclusively in brain; it bears N-terminal, post-translationally added modifications on threonine residues, such as O-glycosylations which are important for protein stabilization. It is associated with α1-β isozymes and is most likely involved in neuronal excitability [33, 34].

Phospholemman (FXYD1) is highly expressed in selected structures at central nervous system (CNS) level. Phospholemman antibodies precipitate the Na^+, K^+-ATPase α1- α3 subunit isoforms from cerebellum, indicating that the interaction is not specific to a particular α isoform and that phospholemman is present in both neurons and glia [35]. During brain ventricle development, the Na^+, K^+-ATPase is essential for normal function, including the formation of a cohesive neuroepithelium, the restriction of neuroepithelial permeability and the production of cerebrospinal fluid. The α1 Na^+, K^+-ATPase isoform is required for all these processes whereas FXYD1 isoform is required only for the formation of a cohesive neuroepithelium [36]. Structural, functional and pharmacological comparisons among the α1, α2 and α3 Na^+, K^+-ATPase isoforms were carried out. The three isoforms differ in its sensitivity to diverse agents and FXYD1 protects the isoforms in different ways [36'].

2.3. Regulation of Enzyme Activity and Expression

Due to changing physiological needs, diverse regulatory mechanisms are operative to ensure appropriate expression of the Na^+, K^+-ATPase and required enzyme activity. Na^+, K^+-ATPase activity can be regulated by multiple mechanisms. This is coherent with the diverse functional roles in different conditions, leading this protein vulnerable to pathogenic insults and a target for therapeutics [28, 37]. Besides its dependence for ATP, Na^+, K^+-ATPase activity is regulated by phosphorylation state [28], neurotransmitters [38, 39], including diverse peptides [40], as well as by endogenous ouabain-like substances [41-44].

It is known that chronic infusion of exogenous ouabain to normotensive rats leads to hypertension involving central mechanisms [45, 46]. Ouabain administration increases Na^+, K^+-ATPase α3 subunit whereas it fails to modify enzyme activity in hypothalamus. The inability to detect a decrease in enzyme activity in the hypothalamus in response to ouabain was attributed, at least in part, to an increase in enzyme expression and the dissociation of ouabain during sample processing [47]. On the other hand, acute ouabain administration increases the expression of Na^+, K^+-ATPase α3 subunit in cerebral cortex whereas it fails to modify that in the hippocampus and decreases enzyme activity in cortical synaptosomal membranes [48].

Members of cardiac glycoside family have been employed for many years in therapeutic for the treatment of heart failure and atrial arrhythmia. Diverse evidences indicate signalling modes of action of Na^+, K^+-ATPase, involving cardiac glycosides in the regulation of important cellular processes. This issue highlights potential therapeutic roles in various diseases, including cancer processes [48'].

To conclude, the Na^+, K^+-ATPase inhibitors differentially modify the expression of Na^+, K^+-ATPase α3 subunit and enzyme activity, most likely involving compensatory mechanisms.

2.4. Regulation by Phosphorylation / Dephosphorylation

Phosphorylation of Na^+, K^+-ATPase catalytic subunit inhibits enzyme activity [49] and inhibition of protein kinase C (PKC) restores enzyme activity [50, 51]. Dephosphorylation of the Na^+, K^+-ATPase is mediated by calcineurin, a serine / threonine phosphatase involved in a wide range of cellular responses to Ca^{2+} mobilizing signals, in the regulation of neuronal excitability by controlling the activity of ion channels, in the release of neurotransmitters and hormones, in synaptic plasticity, as well as in gene transcription [52, 53]. Likewise, changes in dopamine- and cAMP-regulated phosphoprotein of 32 kDa (DARPP-32) activity would lead to changes in protein phosphatase 1 activity and hence altered Na^+, K^+-ATPase dephosphorylation [54, 55]. It should be mentioned that Ca^{2+} influx through the NMDA receptor activates calcineurin and protein phosphatase 1 [56]. A crystal structure of the pig kidney Na^+, K^+-ATPase in its phosphorylated form stabilized by high affinity binding of ouabain was described. The steroid binds to a site at transmembrane segments αM1-αM6, plugging the ion from the extracellular side. A rotation occurs in response to phosphorylation and αM1-2 move towards the ouabain molecule, leading to high affinity interactions and closing the ion pathway from the extracellular side [56'].

2.5. Na^+, K^+-ATPase Modulators

The knowledge of Na^+, K^+-ATPase modulation by diverse molecules may contribute to understanding regulatory mechanisms involved in normal and pathological conditions. Taking into account that the Na^+, K^+-ATPase is an ubiquitous membrane-bound enzyme, it may be regulated by released active substances at diverse neuron sites. The search for possible modulation of sodium pump by neuroactive substances (including neurotransmitters) has been the aim of diverse studies and results recorded have been summarized [38, 39, 43, 57, 58].

Taking into account their chemical nature, endogenous Na^+, K^+-ATPase modulators may be grouped into steroids (identical or closely resembling ouabain), and non-steroids (of diverse chemical structure). Digitalis-like factors are endogenous mammalian cardenolides with chemical features similar to plant-derived digitalis compounds, which may act as effectors of ion-transport due to interaction with Na^+, K^+-ATPase. The term *endobain* has been introduced for *endo*genous oua*bain*-like substances [59] and gathered information reviewed [43].

Regarding non-steroid modulators, low MW substances, either non-peptidic [60-62] have been described. Another ouabain-like factor unrelated to ouabain has been identified in human urine and proved to be chemically a vanadium discorbate adduct [63]. Among non-steroid factors, an

endogenous brain Na^+, K^+-ATPase inhibitor, termed endobain E, which shares several biological properties with ouabain has been described. Endobain E is highly hydrophilic, non-lipidic, non-peptidic and anionic in nature, acid stable but alkali labile [64]; it differs from ouabain in HPLC retention time, chromatographic behavior and UV spectra [65], and contains ascorbic acid and another unidentified compound [66]. Some endobain E properties include the inhibition of Na^+, K^+-ATPase activity but not of other membrane bound enzymes [67], the blockade of high affinity [^{3}H]ouabain binding [68], norepinephrine release enhancement [69] and uptake decrease [70] from rat hypothalamus slices, phosphoinositide turnover enhancencement in neonatal brain [71, 72], as well as excitatory amino acid release increase from synaptosomes [73]. However, on assaying *in vitro* ligand binding to the NMDA receptor, the effect of endobain E markedly differs from that of ouabain [74, 75].

Intracerebroventricular administration of endogenous Na^+, K^+-ATPase inhibitor endobain E enhances the expression of α3 subunit in both the cerebral cortex and the hippocampus. Na^+, K^+-ATPase activity of synaptosomal membranes from cerebral cortex enhances after treatment with endobain E [48].

When added *in vitro* during the enzyme assay some neurotransmitters are able to modify Na^+, K^+-ATPase activity [see 10, 76]. Catecholamines norepinephrine and dopamine modify synaptosomal membrane Na^+, K^+-ATPase activity. They behave as enzyme inhibitors or stimulators, according to the absence or presence of a brain soluble fraction during enzyme assay [see 38, 77].

Studies with Na^+, K^+-ATPase modifiers like rottlerin (inhibitor) and monensin (stimulator) disclosed an interesting link between this enzyme, L-glutamate transporters and the cytoskeleton in astrocytes. Results indicate that astrocytic morphology and excitatory amino acid transporter activity are co-regulated by a tightly coupled, homeostatic relationship among L-glutamate uptake, the electrochemical gradient and Na^+, K^+-ATPase activity [77'].

Insulin stimulates the activity of membrane-bound ATPase isolated from rat brain. It was suggested that the sodium pump is intimately involved with the physiological action of insulin and that it may act as a transducer between the binding of insulin to its receptor on the plasma membrane and the cellular actions of insulin [78]. Besides the enhancement of Na^+, K^+-ATPase activity, insulin induces the translocation of Na^+, K^+-ATPase molecules to skeletal muscle plasma membrane, *via* phosphorylation of the α-subunits by ERK1/2 MAP kinase [79].

Diverse examples provided by the literature support the notion that neuropeptide receptors play major roles in diverse peptide effects on ion transport at synaptic level [see 40]. Synaptosomal membrane Na^+, K^+-ATPase activity is inhibited *in vitro* by neurotensin, suggesting a regulatory action of the peptide on this enzyme activity [80, 81].

2.6. The Na^+, K^+-ATPase as a Signal Transducer

Besides the pumping of ions, the Na^+, K^+-ATPase seems to act as a signal transducer [82]. Ouabain binding to Na^+, K^+-ATPase changes enzyme interaction with neighboring membrane proteins inducing the formation of multiple signaling modules. This leads to Src kinase activation, transactivation of the epidermal growth factor receptor and enhanced formation of reactive oxygen species. Interaction of such signals activates several other cascades, including the enhancement of phospholipase C [83, 84]. Multiple protein kinase cascades, including mitogen-activated protein kinases and protein kinase C isozymes became activated in a cell-specific manner. Mitochondrial production of reactive oxygen species is activated and intracellular calcium concentration regulated. Cross-talk among the activated pathways may result in changes in the expression of diverse genes [85].

Inhibition of rat neuronal Na^+, K^+-ATPase α3 isoform at low (100 nM) ouabain concentration produces the activation of MAP kinase cascade *via* PKC and PIP_3 kinase. At variance, the ouabain-resistant α1 isoform (inhibition with 1 mM ouabain) of the Na^+, K^+-ATPase regulates MAP kinase *via* Src kinase-dependent reactions. Assays with Annexin V-FITC apoptotic test indicated that α3 isoform stimulates whereas α1 isoform suppresses apoptotic process in cerebellum neurons. Therefore, Na^+, K^+-ATPase α1 and α3 isoforms participate in different signaling pathways in neuronal cells [86].

The inhibition of Src blocks many of the ouabain-activated signaling pathways. It is known that Src binds directly to the Na^+, K^+-ATPase and that ouabain modulates the interaction between the enzyme and Src, leading to Src activation. The possibility that signaling Na^+, K^+-ATPase is concentrated in a separate pool on the plasma membrane has been advanced and potential interaction between the Na^+, K^+-ATPase and caveolins studied, due to enzyme concentration in caveloae/rafts [84, 87].

In rat cardiac myocytes and renal cells, ouabain interaction with a minor fraction of the Na^+, K^+-ATPase leads to a cascade of events. After sodium pump inhibition by ouabain, intracellular Na^+ concentration increases with a subsequent gradual enhancement or oscillations in intracellular Ca^{2+} concentration. It is not clear whether such increase in intracellular Ca^{2+} concentration is part of or a result of the cascade, or alternatively, a totally independent phenomenon [88]. This process seems to involve the stimulation of a clathrin-dependent endocytosis pathway that translocate the Na^+, K^+-ATPase to intracellular compartments, suggesting a role of endocytosis in ouabain-induced signal transduction [89].

Inhibition of the Na^+, K^+-ATPase by ouabain results in phosphatidylinositol turnover enhancement in rat cerebral cortex, an effect markedly higher in neonatal than in adult brain [90, 91]. Similarly, phosphatidylinositol turnover increase occurs with the endogenous ouabain-like substance endobain E, an effect recorded in neonatal but not in adult rat cerebral cortex [91]. Taking into account the above mentioned findings, endobain E could well act as a physiological inducer of this signaling system [72].

Na^+, K^+-ATPase signal transduction triggers dendritic growth, and transcriptional programs dependent on cAMP response element binding protein (CREB) and CRE-

mediated gene expression, primarily regulated via Ca^{2+}/calmodulin-dependent kinases [92]. On the other hand, inhibition of calcineurin, which leads to Na^+, K^+-ATPase activity decrease, induces depressive-like behavior via mTOR signaling pathway [93].

2.7. The Na^+, K^+-ATPase and the Nitrergic System

Administration of L-arginine reduces Na^+, K^+-ATPase activity in midbrain of adult rats. Nitric oxide synthase inhibition by N (ω)-nitro-L-arginine methyl esther (L-NAME) administration prevents such enzyme decrease, an effect most likely due to free radical generation induced by nitric oxide (NO) formation [94].

Peptide neurotensin inhibits synaptosomal membrane Na^+, K^+-ATPase activity, an effect which involves high affinity neurotensin receptor (NTS1) [80, 81]. Nitric oxide (NO) acts as a neurotransmitter or as a neuromodulator when it is synthesized by neuronal nitric oxide synthase (nNOS) [95]. Administration to rats at early postnatal stage of L-NAME, a nitric oxide synthase inhibitor, leads to NO dysfunction. This treatment entirely prevents later Na^+, K^+-ATPase response to neurotensin [96].

3. THE NMDA RECEPTOR

3.1. Glutamatergic Receptors. Classes

Neurotransmitter glutamate is released at most excitatory synapses in CNS, depolarizing neurons by interaction with specific receptors, classified as either ionotropic, with a ligand-gated ion channel, or metabotropic, operated *via* G proteins. Functional classes for ionotropic glutamate receptors include the NMDA, α-amino-3 hydroxy-5 methyl-4-isoxazole propionic acid (AMPA) and kainate receptors, represented by distinct molecular families of receptor genes. Regarding metabotropic glutamate receptors (mGluRs), three functional classes (I, II, and III) are operative. They comprise eight subtypes (mGluR1 through mGluR8) all of which have been cloned [97, 98].

The NMDA receptors are highly permeable to Ca^{2+} and exhibit voltage-dependent inhibition by Mg^{2+}. They are activated by glutamate and by glycine acting at a co-agonist binding site. D-serine is an endogenous ligand for the NMDA glycine site [99, 100].

The NMDA receptors are critical to fast signaling at CNS which are involved in a wide broad of physiological processes and in excitotoxicity. Modulation of its activity plays a critical role in controlling neuronal function. Evidences indicate its involvement in synaptogenesis, synaptic plasticity, neurogenesis, as well as in learning and memory [101-104].

The NMDA receptors do not constitute a homogeneous population of receptors but exist as multiple subpopulations differing in functional properties. The extracellular N-terminal region exerts a central role. The identity of this region, distal to the membrane, which precedes the agonist-binding domain, is crucial for biophysical and pharmacological properties of the different NMDA receptor subtypes [105]. The family of the NMDA receptors exert diverse roles in CNS function and in several neuropathological and psychiatric conditions.

3.2. NMDA Receptor Structure

The NMDA receptor consists of four or five subunits. Functional NMDA receptor is constituted by heteromeric complexes of GluN1 subunit (8 splice variants) in combination with GluN2 (GluN2A-GluN2D) or GluN3 (GluN3A-GluN3B) subunits. Its functionality requires the presence of at least one GluN1 subunit, which is essential for the NMDA-receptor-channel complex [106-111]. GluN2 or GluN3 family subunits are related to modulatory properties of the receptor [98, 103].

The NMDA receptors occur with a molecular composition of the variants of GluN1 subunit in association with GluN2 subunit dimer, the main form of this receptor. They are obligate tetramers composed of two GluN1 and typically two GluN2 subunits.

The subunit composition of the NMDA receptors is tightly regulated during brain development. Whereas GluN2B initially dominate the NMDA receptors, GluN2A incorporation increases after birth. The specificity of GluN2B-mediated signaling is due to its particular interaction with the protein effector α Ca^{2+}-calmodulin kinase II and the regulation of the mTOR pathway. These processes can not be rescued by replacement with GluN2A in genetically modified mouse [112].

GluN2 subunits are diferentially expressed during development and among brain regions which contribute to functional diversity of the NMDA receptors. GluN2 subunit diversity offers an opportunity to pharmacologically modify the function of selected groups of neurons for therapeutic purposes [113].

The induction of long term depression (LTD) in pyramidal neurons of young rat visual cortex is NMDA receptor-dependent and requires the presence of GluN2B subunit but not GluN2A subunit [114].

The four GluN2 subunits (GluN2A-D) may form diheteromeric NMDA receptor subtypes which exert diverse physiological and pathological roles. NMDA receptor subtype specificity of Mg^{2+} blockage, single-channel conductance and selective permeability to Ca^{2+} are controlled by the amino acid residue at a single GluN2 site in the M3 transmembrane region. GluN2-GluN1 subunit interaction mediates site effects. A single GluN2 subunit residue couples with the pore-forming loop of the GluN1 subunit leading to variations in the properties of the NMDA receptor which are critical for synaptic plasticity [115].

Two key residues in the lower lobe of the GluN2 agonist binding domain control the selectivity of NMDA receptor antagonist DQP-1105 for this subunit. These results lead to the suggestion that ligands can access in a subunit-selective manner to a site located in the lower, membrane-proximal portion of the agonist-binding domain [116].

Evidence indicates that GluN2B subunit exerts a critical role in post ischemic synaptic plasticity [117]. Antagonists at GluN2B sites have shown an improved side effect profile in animal models of pain [118] and neuropathic pain [119] and proved effective in the treatment of ischemia brain injury [120].

GluN3B shares high sequence homology with GluN3A and is expressed mainly in motor neurons, whereas GluN3A is more widely distributed. GluN3A or GluN3B co-assemble with GluN1 in *Xenopus oocytes* to form excitatory glycine receptors which are not affected by glutamate or NMDA but inhibited by D-serine (a co-activator of conventional NMDA receptors) [121].

Similar to GluN1 (but unlike GluN2), GluN3 binds glycine and D-serine with high affinity. For native GluN3A, glycine is the endogenous ligand with unique pharmacological characteristics. [122, 123]. Most interesting, GluN3 subunits in the absence of GluN2 assemble with GluN1 into excitatory glycine receptors [124].

3.3 NMDA Receptor Modifiers

The NMDA receptor is modulated by several endogenous substances, evidencing the importance of fine-tuning for this receptor function [99, 101, 125]. NMDA receptor complex subpopulations respond with different sensitivity to modulation by several neuroactive substances.

At present there are many classes of drugs which are able to modulate NMDA receptor activity. They include competitive antagonists at the glutamate and glycine binding sites, channel blockers, antagonists for the GluN2B-selective N-terminal domain binding site, as well as positive or negative allosteric modulators. Some of them are channel blocking agents like [^{3}H]dizocilpine (MK 801) and phenylcyclidine [126, 127], non-competitive agents ifenprodil, polyamines [128-130] and haloperidol [131, 132].

The group of allosteric modulators include the pan potentiator UBP646, the NR2A -selective potentiator/GluN2C and GluN2D inhibitor UBP512-, the GluN2D-selective potentiator UBP551, the GluN2C/GluN2D-selective potentiator CIQ, a negative allosteric modulator UBP618, the GluN2C/GluN2D-selective inhibitor QZN46 and the GluN2A inhibitors UBP608 and TCN201. These drugs do not bind within the glutamate or glycine binding sites, the N-terminal regulatory domain or the ion channel pore [see 133].

DPQ-1105 is a representative compound of a new class of NMDA receptor antagonists. It inhibits non-competitively GluN2C- and GluN2D-containing receptors.

Its chemical structure is 4-(5-(4-bromophenyl)-3-(6-methyl-2-oxo-4-phenyl-1,2-dihydroquinolin-3-yl)-4,5-dihidro-1H-pyrazol-1-yl)-4-oxobutanoic acid. The evaluation of antagonist DQP-1105 inhibition of chimeric NMDA receptors allowed the identification of two key residues in the lower lobe of the GluN2 subunit agonist binding domain that control the selectivity of DQP-1105. Ligands can access, in a subunit-selective way, to a new site located in the lower, membrane-proximal portion of the agonist-binding domain [116].

Neurosteroids are a family of compounds that affect the activity of the NMDA receptors [see 134]. To illustrate, neurosteroids 17β-estradiol and allopregnanolone modulate the function and trafficking of the NMDA receptor. Besides, 17β-estradiol induces synaptogenesis and synaptic plasticity by increasing NMDA expression [135, 136]. Expression of GluN1/GluN2B receptors are differentially modified by 17β-estradiol according to the neurosteroid concentration employed [137].

Neurosteroids promote learning and memory by the modulation of synaptic functions in the hippocampus [138, 139]. It is known that cytochrome P450scc is the enzyme that converts cholesterol to pregnenolone, which is required for the biosynthesis of all other neurosteroids [138, 140]. Aminoglutethimide (AG), the selective inhibitor of cytochrome P450scc reduces NMDA receptor activity, and suppresses long term potentiation (LTP). These findings indicate that endogenous neurosteroids locally synthesized in brain are required to maintain the normal excitatory synaptic transmission and plasticity in dentate gyrus of the rat hippocampus [141]. Endogenous neurosteroids pregnenolone sulfate and 3α-hydroxy-5β-pregnan-20-one sulfate bind to the NMDA receptor and differentialy modify its activity. The resulting effect is dependent on subunit receptor composition [142].

D(-)-2-Amino-5-phosphonovaleric acid (D-AP5), a selective NMDA receptor antagonist, blocks the induction of LTD. The selective GluN2B-containing NMDA receptor antagonists Ro 25-6981 and ifenprodil, also prevented the induction of LTD. Zn^{2+}, a voltage-independent GluN2A-containing NMDA receptor antagonist, fails to influence the induction of LTD [114].

The dopaminergic antagonists haloperidol and trifluperidol are subtype-selective NMDA receptor antagonists on assaying [^{3}H]-1-[1-(2-thienyl) cyclohexyl] piperidine (TCP) and [^{3}H]ifenprodil binding to rat brain membranes [143]. Haloperidol binds to the NMDA receptors containing GluN2B subunit, with the same subunit specificity as ifenprodil, polyamines and magnesium. Although spermidine, haloperidol and ifenprodil share subunit selectivity and overlapping pharmacology response, they have specific structural determinants likewise, as shown in studies carried out in mutant models [132].

Alteration in levels of mRNA for NMDA receptor subunits were recorded by several authors after administration of antipsychotic drugs [144, 145], though other authors reported no changes by this treatment [146].

On the other hand, dieldrin, an antagonist for GABA(A) receptor, produces NMDA receptor internalization, an effect which occurs on GluN1 and GluN2B subunits but not on GluN2A subunit [137].

Ethanol, a noncompetitive NMDA antagonist [147, 148], up regulates NMDA receptor number and function both *in vitro* [149-151] and *in vivo* [152-154]. The effect of chronic ethanol treatment on NMDA receptor subunit regulation seems to be area-specific. Findings indicate that chronic ethanol up regulates the NMDA receptor function and binding in cortical neurons [152], an effect involving the enhancement of GluN2B mRNA level [153]. However, immunoblotting procedures indicate up regulation of GluN1 subunit in the hippocampus but not in the nucleus accumbens,the cerebral cortex or the striatum [151].

NMDA receptor antagonists are capable of increasing NMDA receptor levels *in vitro* and *in vivo*. NMDA receptor density increases in cultured cortical neurons after exposure

to the NMDA antagonists D-AP5, CGS 19755, dizocilpine and phenylcyclohexylpiperidine, phenylcyclidine (PCP), but not after exposure to the AMPA/kainate receptor antagonist 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX) [155, 156]. The presence of ketamine in forebrain cultures, a noncompetitive NMDA receptor ion channel blocker, results in an up-regulation of NMDA receptor GluN1 subunit protein which is accompanied by enhanced apoptosis [157].

3.4. NMDA Receptor Changes in Cell Free Systems

Potential action of the Na^+, K^+-ATPase inhibitor endobain E on the NMDA receptor was studied in a cell-free system to exclude ionic gradient participation, membrane depolarization and neurotransmitter release. It was observed that endobain E exerts a direct effect on the NMDA receptor, as assayed on rat cerebral cortex membranes in the binding of ligand [^{3}H]dizocilpine [74]. Endobain E allosterically decreases [^{3}H]dizocilpine binding to the NMDA receptor, an effect dependent on receptor activation by glutamate and glycine [75]. As endobain E effect is interfered by ketamine and Mg^{2+}, this factor most likely modulates the NMDA receptor by its binding to the inner face of the associated channel [158].

The assay of [^{3}H]dizocilpine binding indicated that both substances endobain E and ouabain are able to modify the NMDA receptor: the former decreases whereas the later enhances the binding of ligand [^{3}H]dizocilpine to CNS membranes. Therefore, NMDA receptor modulation takes place by a direct mechanism unrelated to the ability of endobain E and ouabain to alter Na^+ / K^+ equilibrium due to Na^+, K^+-ATPase inhibition [74].

3.5. Synaptic Plasticity

Experiments carried out in mice lacking the NMDA receptor indicated that glutamatergic synaptic transmission and its modulation by the NMDA receptors play key roles in the control of AgRP (Agouti-related peptide) neurons and determine the cellular and behavioral response to fasting [159].

The NMDA receptors are regulated and subjected to the activity which depends on long-term plasticity. These receptors may participate in the transfer of information at synapses mainly during periods of repetitive activity. They are also involved in dendritic synaptic integration and are important for the generation of persistent activity of neural assemblies. The mechanism and functional consequences of NMDA receptor plasticity have been advanced. NMDA receptor transmission seems to be a particular mechanism for the fine tuning of information encoding and storage throughout the brain [see 160].

3.6. Cell Signaling Processes

The NMDA receptor plays a vital and unique role in subcellular signaling. Data from the literature indicate a relationship between NMDA receptor activation and signaling processes, mainly for extracellular signal-regulated kinase (ERK) pathway [161-163].

GluN2B is coupled to inhibition rather than activation of Ras-ERK pathway, which drives surface delivery of GluN1. In brain, the synaptic Ras GTPase activating protein (GAP) SynGAP is associated with GluN2B and is required for inhibition of NMDA receptor-dependent ERK activation. It has been suggested that the coupling of GluN2B to SynGAP may well explain the subtype-specific function of GluN2B-NMDA in inhibition of Ras-ERK, removal of synaptic AMPA receptors and weakening of synaptic transmission [162].

The NMDA receptors bidirectionally modulate ERK through the coupling of synaptic NMDA receptors to an ERK activation pathway which is coupled to receptors containing both GluN2A and GluN2B NMDA subunits. Synaptic NMDA receptor activation of ERK in rat cortical cultures is partially inhibited by the highly selective GluN2B antagonist Ro25-6981, as well as the less selective GluN2A antagonist NVP-AAM0077 [164].

The NMDA receptors are known to mediate affective pain by activating a signaling pathway that involves cAMP/protein kinase A (PKA) and /or ERK/CREB. In such a process receptor glycine site and GluN2B subunit are involved. NMDA application up regulates pERK and pCREB, an effect inhibited by NMDA receptor antagonist 2-amino-5-phosphonovaleric acid [165].

Calcium influx initiates signaling cascades which are important for synaptic plasticity and cell survival. However, NMDA receptor overstimulation leads to toxicity and neuronal death which may be explained by the subcellular localization of these receptors: The NMDA receptors located at synaptic sites stimulate cell survival pathways whereas those at extra synaptic sites signal cell death [see 166].

Calcium influx *via* NMDA receptors regulates the intracellular trafficking of the NMDA receptors, leading to long-lasting change of NMDA-receptor mediated synaptic transmission, involved in development, learning and synaptic plasticity.

Phosphorylation of GluN2B at tyrosine 14172 in spinal dorsal horn contributes to NMDA-induced pain hypersensitivity in mice, an effect attenuated by ifenprodil. Administration of NMDA elicits pronounced allodynia, concurrent with an increase in GluN1 and GluN2B subunits at the postsynaptic density (PSD)-enriched fraction. This effect decreases by ifenprodil [114].

Activation of spinal NMDA receptors is able to accumulate GluN2B receptors at synapses *via* SKF signaling. It was postulated that this effect might exaggerate NMDA receptor-dependent nociceptive transmission and contribute to NMDA-induced nociceptive behavioral hyper responsiveness [114].

It is known that Src family kinases (SFKs) play critical roles in the regulation of many cellular functions, including those which involve ligand gated ion channels. SFKs is a convergent point of multiple signaling pathways which regulate NMDA receptors at CNS level. Among SFK molecules, Src and Fyn are closely associated through direct or indirect binding with the NMDA receptors. The NMDA receptor is associated with a signaling complex which consist of the SFK-activating phosphatase, protein tyrosine phosphatase α and the SFK-inactivating kinase, C-terminal Src kinase. Intramolecular interactions with SH2 or SH3 domain lock SFKs in a closed conformation and their

disruption may lead to the activation of SFKs. Enzyme activity of neuronal Src protein is related to its stability and the interactions with the SH2 adn SH3 domains may constrain neuronal Src activation and regulate its catalytic activity [see 167].

The phosphorylation and trafficking of the NMDA receptors are tightly regulated by the Src family tyrosine kinase Fyn, through dynamic interactions with several scaffolding proteins in the NMDA receptor complex [168-170]. GluN2B-containing NMDA receptors are up regulated by Fyn, which acts as a convergence point for diverse signaling pathways. Fyn seems to mediate ethanol effects by regulating the phosphorylation of GluN2B NMDA receptor subunits. Fyn regulates alcohol withdrawal and acute tolerance to ethanol through a GluN2B-dependent mechanism [171].

4. CROSS-TALK BETWEEN THE Na^+, K^+-ATPase AND THE NMDA RECEPTOR

The administration of the endogenous Na^+, K^+-ATPase inhibitor endobain E and ouabain led to differential regulation of Na^+, K^+-ATPase $\alpha3$ and NMDA subunit expression [48, 172, 173]. Results recorded after administration of Na^+, K^+-ATPase inhibitors on NMDA receptor subunits and Na^+, K^+-ATPase expression point to an interplay between the regulation of NMDA receptor and Na^+, K^+-ATPase activities.

The Na^+, K^+-ATPase inhibitor ouabain evokes neurotransmitter release in several experimental models, including that of excitatory amino acids from cerebellar granule cells in culture [174] and cerebral cortex synaptosomes [73, 175]. Ouabain is also responsible for an increase in intracellular sodium concentration and plasma membrane depolarization, and for calcium entry to the cell through several calcium channels, such as those related to the NMDA receptor. In support, ouabain-induced glutamate release is inhibited by calcium channel blockers and, though partially, by NMDA and AMPA / kainate receptor antagonists [174].

Ouabain administration produces an acute and significant decrease of the apparent diffusion coefficient of water, which results in infarct-like histological brain lesions. The comparison of ouabain injection *versus* NMDA injection to neonatal rats allowed the conclusion that ouabain induces cellular membrane depolarization which leads to excitotoxicity. NMDA blocker dizocilpine attenuates the volume of tissue exhibiting diminished apparent diffusion coefficient of water. Therefore, ouabain-induced excitotoxicity is suited to detect relevant neuroprotection, being more sensitive to attenuation of synaptic glutamate levels [176].

Expression of brain NMDA receptor subunits is altered in different experimental conditions, as occurred after intracerebral administration of Na^+, K^+-ATPase inhibitors to rats. Changes recorded are dependent on the brain area and ouabain dose employed. The treatment leads to up-regulation of cerebral cortex GluN1 and GluN2D NMDA receptor subunits. In the hippocampus, the expression of GluN1 subunit enhances, remains unaltered or decreases, according to the ouabain dose administered but ouabain invariably leads to down-regulation of GluN2A, GluN2B and GluN2C NMDA receptor subunits [173]. These changes were attributed to an indirect effect [173], considering that ouabain is a Na^+, K^+-ATPase inhibitor which enhances the release of glutamate (or other glutamate receptor agonist), and might be correlated with the binding increase to CNS membranes of NMDA antagonist [^{3}H]dizocilpine, which suggested NMDA receptor activation by ouabain [74].

Ouabain administration most likely leads to membrane depolarization, producing excitotoxicity with overstimulation of the NMDA receptor; this effect is followed by receptor down-regulation as a compensatory response. However, this explanation may be valid for changes recorded in the hippocampus, but not for those observed in the cerebral cortex, where expression of GluN1 and GluN2D subunits results significantly enhanced [173].

It is tempting to speculate that changes in the NMDA and Na^+, K^+-ATPase expression and activity are interrelated. For instance, it may occur that the administration of Na^+, K^+-ATPase inhibitors modify first the enzyme, which in turn, enhances the expression of certain NMDA subunits as a compensatory mechanism. Alternatively, a direct effect on NMDA receptor subunit expression could induce changes in the Na^+, K^+-ATPase.

Na^+, K^+-ATPase activity in rat cerebral cortex, striatal and hippocampal synaptosome (miniature cells) preparations is diminished by incubation with glutamate [177] whereas in isolated synaptosomal membranes, glutamate fails to modify Na^+, K^+-ATPase activity [38]. The latter is attributable to NMDA receptor functionality impairment in this cell-free system.

There are several examples indicating the regulation of sodium pump isoform activities.

Glutamate enhances sodium pump activity *via* an increase in the highly digitalis-sensitive type isoform, most likely in response to an enhancement of $[Na^+]i$ in neurons, subsequent to passive Na^+ influx through glutamate receptor-mediated cation channels. The suggestion that Na^+ pump isoforms in neurons differ in their physiological function and that the brain type isoform plays a crucial role in restoring Na^+ and K^+ concentration gradients after neuronal excitation has been advanced [178]. Regarding this point, inhibition of $\alpha2$ / $\alpha3$ Na^+, K^+-ATPase isoforms leads to potentiation of glutamate neurotoxicity [179]. Accordingly, glutamate receptor modulation stimulates Na^+, K^+-ATPase activity [180]. Taken jointly, the mentioned findings support the notion that the activity of Na^+, K^+-ATPase in intact neurons exerts a critical impact on NMDA receptor function.

Electrophysiological responses indicate that electrogenic sodium pump inhibition underlies delayed depolarization of cortical neurons after mechanical injury or exposure to glutamate, proving dependent on NMDA receptor activation [181]. This finding provides another example of a functional relationship between the sodium pump and the NMDA receptor. Most interesting, glial and neuronal clearance of glutamate from the synaptic cleft depends of Na^+, K^+-ATPase activity, since the sodium gradient drives high affinity glutamate uptake. Therefore, Na^+, K^+-ATPase inhibition

modifies excitotoxicity through a decreased glutamate transport activity [182].

It is known that adenosine A2A receptors control synaptic plasticity. The activation of these receptors decreases Na^+, K^+-ATPase activity in astrocytes, leading to glutamate uptake inhibition. Coimmunoprecipitation and in situ proximity ligation assays indicated a physical association of astrocytic adenosine A2A receptors with Na^+, K^+-ATPase $\alpha 2$ subunit. This coupling of adenosine A2A receptors to the glutamate transport regulation through Na^+, K^+-ATPase $\alpha 2$ subunit modulation suggested a link between neuronal activity with ion homeostasis which control glutamatergic activity [182'].

Incubation of primary cultured neurons with glutamate results in Na^+, K^+-ATPase activation. This effect is prevented by dizocilpine, indicating that it is mediated by activation of the NMDA receptor. Enzyme enhancement seems due to decreased phosphorylation since it is reversed by phorbol esters, which behave as PKC activators. Accordingly, N-(6-aminohexyl)-5-chloro-1-naphthalenesulfonamide (W-7) and cyclosporine, both calcineurin inhibitors prevent Na^+, K^+-ATPase activation by glutamate. Therefore, NMDA receptor activation by glutamate most likely leads to calcineurin activation; in turn, the latter dephosphorylates a Na^+, K^+-ATPase amino acid residue previously phosphorylated by PKC, and thus the Na^+, K^+-ATPase becomes activated [183].

Calcineurin is a Ca^{2+}/calmodulin-dependent phosphatase 2B which modulates NMDA receptor activity [184]. Calcineurin acts via the C-terminus of GluN2A subunit, increasing desensitization of the NMDA receptors [185]. Calcineurin plus calmodulin increases single channel shut time. In addition to its direct effects on single channel activity, calcineurin regulates the actions of calmodulin on NMDA receptor activity [186]. The protein scaffold AKAP is required for NMDA receptor-dependent long-term depression because of its interaction with calcineurin [187].

Intraventricular administration of NMDA into mice produces acute seizures followed by impairments in locomotor and behavioral activities. Subsequent neurochemical changes, including decreases in neuronal membrane Na^+, K^+- and Mg^{2+}-ATPase activities were recorded. Behavioral and neurochemical changes were ameliorated by mice pretreatment with phytopolyphenols [188].

It is known that Na^+ and Ca^{2+} influx occur *via* the Na^+, K^+-ATPase and the Na^+ / Ca^{2+} exchanger. Changes in protein expression of the Na^+, K^+-ATPase and Na^+ / Ca^{2+} exchanger, as well as those of GluN2A and GluN2B NMDA subunits occur in the penumbra after focal cerebral ischemia. The Na^+, K^+-ATPase decreases whereas the exchanger enhances in the penumbra. Concomitantly, GluN2A subunit increases whereas GluN2B subunit decreases [189].

Studies in CNS membrane fractions indicate that ouabain and endogenous ouabain-like factor endobain E, both inhibitors of the Na^+, K^+-ATPase, exert opposite effects on dizocilpine binding to the NMDA receptor, since the former enhances whereas the latter depresses ligand binding to isolated brain membranes. Therefore, in a cell free system, NMDA receptor modulation most likely takes place by a direct effect independent of Na^+/ K^+ equilibrium regulated by the sodium pump [74].

Another example of potential relationship between the NMDA receptor and the activity of Na^+, K^+-ATPase is the following. It is known that malonate, the reversible succinate dehydrogenase inhibitor, induces neurotoxicity. This effect is greatly exacerbated by depolarization produced by co-injection of Na^+, K^+-ATPase inhibitor ouabain or by high K^+ concentration. This combined toxicity is blocked by non-competitive NMDA receptor antagonist dizocilpine, thus indicating NMDA receptor participation [190].

In the developing brain, susceptibility to hypoxia depends on developmental stage and modulation of excitatory neurotransmitter receptors such as the NMDA receptor, as well as lipid membrane composition and peroxidation rate, among other factors. In fetal and newborn animal models, brain tissue hypoxia results in damage to cell membrane as disclosed by increased membrane lipid peroxidation and decreased Na^+, K^+-ATPase activity; concomitantly, hypoxia alters the NMDA receptor-ion channel, as well as recognition and modulatory sites [191].

Dihidroouabain, a Na^+, K^+-ATPase inhibitor, potentiates NMDA current in rat hippocampal CA1 pyramidal neurons, an effect blocked by PP2 (selective Src tyrosine kinase inhibitor) and PD-98059 (selective inhibitor of the MAPKs cascade). These findings indicate that Src mediates the cross-talk between the Na^+, K^+-ATPase and the NMDA receptor to transduce the signals from the Na^+, K^+-ATPase to MAPK cascade. These findings provide insights into therapeutic target for a deeper understanding of the nature of cognitive disorder [192].

Intrahipppocampal administration of NMDA induces NF-kB activation and increases NOS and $\alpha(2/3)$-Na^+, K^+-ATPase activities. Besides, NMDA treatment further enhances ouabain-induced NF-kB activation, an effect partially blocked by NMDA receptor antagonist dizocilpine. The suggestion that ouabain-induced NF-kB activation is at least partially dependent on Na^+, K^+-ATPase modulatory effect of the NMDA receptor has been advanced [193].

It should be also considered a potential indirect effect of ouabain which modifies NMDA receptor environment, i.e. binding of the drug to a membrane component other than the Na^+, K^+-ATPase. In support, a 31.5-kD ouabain receptor protein independent of the Na^+, K^+-ATPase has been isolated from transverse tubule membrane-junctional sarcoplasmic reticulum complex of cat cardiac muscle [194]. This suggest that the Na^+, K^+-ATPase may not be the only macromolecule with a receptor site for ouabain.

Creatine increases hippocampal Na^+, K^+-ATPase activity. This effect is antagonized by dizocilpine and ifenprodil but not by DNQX or L-NAME. Furthermore, calcineurine inhibition by cyclosporine A and PKC and PKA activators attenuated Na^+, K^+-ATPase enhancement by creatine, suggesting that creatine effect on the Na^+, K^+-ATPase occurs via NMDA receptor calcineurin pathway [195].

The administration of dizocilpine produces a decrease in zebrafish brain Na^+, K^+-ATPase activity. This change is concomitant with the enhancement of thiobarbituric acid reac-

tive substances, suggesting that oxidative damage might be involved in the effect [196].

Another relationship between the Na^+, K^+-ATPase and the NMDA receptor is through the transcription factor Sp4. This factor governs the developmental pattering of dendrites and contributes to diverse processes including learning and memory [197]. It has been associated to psychiatric disorders such as schizophrenia and bipolar disorder [198]. The neuron-specific Sp4 factor regulates the expression of key glutamatergic receptor subunits [199]. The phosphorylation of Sp4 factor is decrease in response to membrane depolarization. Inhibition of the voltage-dependent NMDA receptor increases Sp4 phosphorylation. Accordingly, stimulation with NMDA reduces the levels of Sp4 phosphorylation. This process is dependent on the protein phosphatase 1/2A signaling pathway [200].

On the other hand, Sp4 factor regulates the expression of all 13 subunits of mitochondrial cytochrome c oxidase in neurons [201], essential for energy generation. As mentioned above, the sodium pump is a major source of demand in neurons. The employ of multiple approaches indicate that Sp4 factor functionally regulates the Atp1a1, Atp1a3, and Atp1b1 subunit genes of the Na^+, K^+-ATPase in neurons. Most interesting, transcripts of these three genes are up-regulated by depolarizing KCl stimulation and down-regulated by tetrodotoxin, an impulse blocker, indicating that their expression is activity-dependent. Taken jointly, results indicate that Sp4 factor plays an important role in the transcriptional coupling between energy generation and consumption in neurons [202].

One example to illustrate the cross-talk between the Na^+, K^+-ATPase and the NMDA receptor is presented in Fig. (**2**).

CONCLUDING REMARKS

The Na^+, K^+- ATPase, neurotransmitter receptors and neuropeptide receptors are all molecular entities inserted into synaptic region membranes and probably contiguous; therefore, it is tempting to speculate that when a given classical neurotransmitter and / or a neuropeptide are / is released, a molecular interaction is favored between the corresponding specific receptor(s) and this enzyme.

Interplay between specific proteins in the synaptic region membrane directly involved in neurotransmission may conceivably indicate a potential relationship between the activity of Na^+, K^+-ATPase and the NMDA receptor.

Herein, available evidence arising from a variety of experimental designs is reviewed, showing close relationship between the activity of the Na^+, K^+-ATPase and the glutamate NMDA receptor. Examples were taken from studies performed with specific agonists or antagonists for the NMDA receptor, which may include electrophysiological assays. Regarding the Na^+, K^+-ATPase, its involvement is postulated after observing its inhibition by ouabain or related cardiac glycosides. Additionally, experimental conditions known to prevent normal Na^+, K^+-ATPase activity (i. e., sodium pump functioning) led to similar valuable information. They indicate potential cross-talk between this enzyme and the NMDA receptor. Therefore, the modulation of its activity plays a critical role in controlling neuronal function.

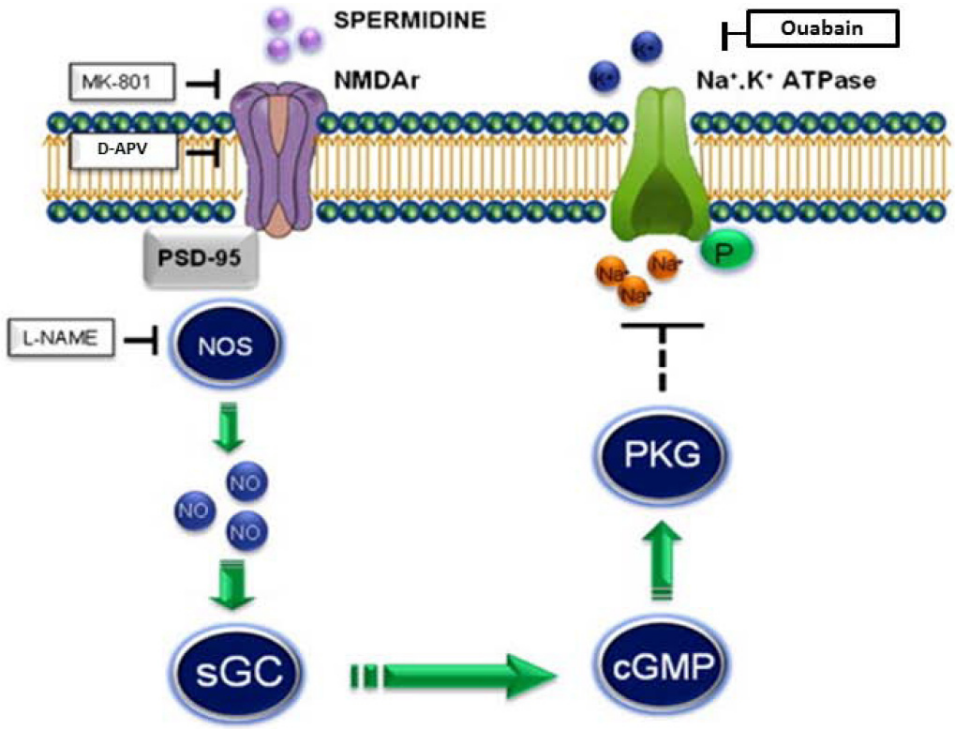

Fig. (2). Na^+, K^+-ATPase decrease by spermidine through NMDA receptor/NOS/ cyclicGMP/PKG pathway. Activation of the NMDA receptor increases NOS activity and NO synthesis. Blockade of the NMDA receptor, inhibition of NOS, cyclic guanilate cyclase, and PKG prevent inhibitory effect of spermidine on the Na^+, K^+-ATPase. Modified from Reference [204].

Diverse examples provided by the literature support the notion that neuropeptide receptors play major roles in diverse peptide effects on ion transport at synaptic level [see 40]. Taking into account that the Na^+, K^+-ATPase is a ubiquitous membrane-bound enzyme, the possibility of its regulation by released active substances at diverse neuron sites seems tenable.

Examination of polypeptide levels of NMDA receptor GluN1, GluN2A, GluN2B, GluN2C and GluN2D in rat CNS following acute treatment with Na^+, K^+-ATPase inhibitors ouabain and endobain E, indicates that the expression of NMDA receptor subunits is modified differentially by Na^+, K^+-ATPase inhibitors.

The Na^+, K^+-ATPase and the NMDA receptor are involved in ion movements through the membranes. Results recorded in several experimental models suggested a close relationship between the activity of Na^+, K^+-ATPase and neurotransmitter receptors, including the NMDA receptor [3].

Phosphorylation of diverse protein types is involved in regulating or in carrying out nervous system processes. Interestingly, the Na^+, K^+-ATPase and the NMDA receptor act as phosphorylation substrates. Nonetheless, whether Na^+, K^+-ATPase regulation modulates receptor activity or *vice versa* is an open question. In either case, it is tenable that vicinal rather than distant regulation takes place at the synaptic membrane. Although in most instances an interplay between the Na^+, K^+-ATPase and the NMDA receptor may take place, it should be stressed that changes in receptors are not invariably accompanied by Na^+, K^+-ATPase modulation.

Potential interplay between the NMDA receptor and the Na^+, K^+-ATPase in the whole CNS system is sustained by the observation of both qualitative and quantitative alterations of NMDA receptor subunit expression after administration of Na^+, K^+-ATPase inhibitors. Differential effect of Na^+, K^+-ATPase inhibitors on the expression of some NMDA receptor subunits may be of interest for signaling pathways involving the activity of both, the Na^+, K^+-ATPase and the NMDA receptor. Several reports showed alteration in gene expression of mRNAs encoding the NMDA receptor subunits.

It should not be expected that every change in Na^+, K^+-ATPase will lead to NMDA modification. It seems necessary to evaluate each change according to the brain area, receptor subunit, as well as the doses of the drug employed. Generally speaking, inhibition of the Na^+, K^+-ATPase not necessarily is accompanied by a NMDA receptor change and *viceversa*.

The enhancement of the GluN1 and GluN2A NMDA receptor subunits in some cases seems to be a compensatory adaptation of the system, an action attributable to a direct effect on the NMDA receptor, or alternatively, through its inhibitory action on the Na^+, K^+-ATPase.

If a given effect on the NMDA receptor is recorded in a cell free system, it should hardly attributated to Na^+/ K^+ equilibrium alteration through membranes because the participation of ionic gradients is not operative.

The Na^+, K^+-ATPase and the NMDA receptor play very important roles in the regulation of learning and memory in the hippocampus. The fact that the greatest changes here described were recorded in the hippocampus may indicate a different vulnerability of this area to the toxicity induced by ouabain.

Some interesting relationships include calcineurin actions, the participation of ERK or Src family kinases, as well as the signaling cascades initiated by calcium. At present, many other examples of signaling related to the NMDA receptor cannot be correlated with Na^+, K^+-ATPase activity. It is desirable that the development of future research offer new clues for the relationship between the Na^+, K^+-ATPase and NMDA receptor activation.

CONFLICT OF INTEREST

The authors confirm that this article content has no conflict of interest.

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