Pharmacological Research 101 (2015) 86–93

Contents lists available at ScienceDirect

Pharmacological Research

journal homepage: www.elsevier.com/locate/yphrs

Review

Interactions of pannexin 1 with NMDA and P2X7 receptors in central nervous system pathologies: Possible role on chronic pain

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ARTICLE INFO

Article history:
Received 16 June 2015
Received in revised form 17 July 2015
Accepted 17 July 2015
Available online 23 July 2015

Keywords:
Pannexin 1
NMDA receptor
P2X7 receptor
Src kinase
Neuropharmacology of chronic pain
Neuropathic pain

ABSTRACT

Pannexin 1 (Panx1) is a glycoprotein that acts as a membrane channel in a wide variety of tissues in mammals. In the central nervous system (CNS) Panx1 is expressed in neurons, astrocytes and microglia, participating in the pathophysiology of some CNS diseases, such as epilepsy, anoxic depolarization after stroke and neuroinflammation. In these conditions Panx1 acts as an important modulator of the neuroinflammatory response, by secreting ATP, by interacting with the P2X7 receptor (P2X7R), and as an amplifier of NMDA receptor (NMDAR) currents, particularly in conditions of pathological neuronal hyperexcitability. Here, we briefly reviewed the current evidences that support the interaction of Panx1 with NMDAR and P2X7R in pathological contexts of the CNS, with special focus in recent data supporting that Panx1 is involved in chronic pain signaling by interacting with NMDAR in neurons and with P2X7R in glia. The participation of Panx1 in chronic pain constitutes a novel topic for research in the field of clinical neurosciences and a potential target for pharmacological interventions in chronic pain.

Contents

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http://dx.doi.org/10.1016/j.phrs.2015.07.016
1043-6618/

1. Introduction

Pannexin 1 (Panx1) is a membrane channel, abundantly expressed in the central nervous system (CNS) of mammals in all cell types (microglia, astrocytes, oligodendrocytes and neurons) [1]. Specifically, Panx1 transcript has been found in cerebellum, cortex, retina and cerebral cortex; in hippocampus, amygdala, substantia nigra, olfactory bulb and spinal cord, among other neural structures [2]. This protein functions as a *bona fide* membrane channel, with high non selective conductance to small molecules (1.5 kDa) [3], high permeability to ATP, Ca^{+2}, glutamate and some inflammatory mediators, and can be activated by several mechanisms, such as mechanical stimulation, increases of extracellular K^+, proteolytic cleavage of its C-terminus, raising of the intracellular Ca^{+2}, and several intracellular signaling [4].

Understanding the physiological and pathological role of Panx1 is fundamental, because this high conductance channel has the particular property of potentiating the activity of some ligand-gated receptors of the CNS in pathological conditions, such as neuroinflammation, anoxic depolarization, stroke, neuronal death, seizure, among others [5]. Thus, in this review we highlighted diverse aspects of Panx1 in CNS, and its interactions with *N*-methyl-*D*-aspartate receptor (NMDAR) and the ionotropic purinergic P2X7 receptor (P2X7R), two of the main components involved in a wide variety of CNS diseases. Furthermore, we review the available evidence of the participation of Panx1 in chronic pain, focusing the discussion toward the potential role of Panx1 in chronic pain signaling via interactions with NMDAR and/or P2X7R.

1.1. Panx1 characteristics

Panx1 is a glycoprotein that belongs to the family of the pannexin membrane channels expressed in diverse cell types in chordates [2]. This protein has three isoforms, Panx1, Panx2 and Panx3, with different structure and function [6]. In the year 2000, Panchin and colleagues identified in the mammalian genome a homolog sequence of innexins, the gap junction protein in invertebrates, by performing BLASTP and PSI-BLAST searches against GenBank using Inx sequences [7], being initially classified as part of the family of the gap junctions [8]. Due to this phylogenic argument, many authors currently keep referring to pannexins as "hemichannels", arguing the possibility of this protein to bond to another pannexin and form gap junctions. However, although several studies have shown structural similarity of the innexins with pannexins (reviewed by Dahl and Muller [9]), confirming that both proteins belong to the same family [8,11], there is consistent evidence that demonstrate that these proteins are functional membranes channels and do not act as an intercellular channel in appositional membranes like connexins does [11,12]. Several arguments support this idea: (a) Panx1 channels expressed in individual cells, such as erythrocytes, do not form gap junctions [12]; (b) Panx1 are located exclusively at the apical membrane of polarized cells, such as epithelial cells of the airways [13]; (c) in neurons, Panx1 are distributed asymmetrically in synapses only at the postsynaptic density, in co-localization and co-expression with the postsynaptic membrane protein PSD95 [14]; (d) pannexins, including Panx1, are glycoproteins and they have a N-glycosylation site which would prevent the coupling of these channels by steric hindrance [15,16].

1.2. Structure

The three-dimensional structure of Panx1 remains unclear. However, due to the gross structural similarities with connexin and innexins, it is predicted that Panx1 has a hexameric conformation, with four transmembrane segments, two extracellular loops and one intracellular, with its N and C terminal at the intracellular space [7,10,15,17]. Particularly interesting is the N-glycosylation site at cysteine 254 of the second extracellular loop, which has a role in the trafficking of the protein to the membrane, and would prevent the binding of a Panx1 to another to form a gap junction [11]. Using substituted-cysteine accessibility method and electron microscopy of Panx1 pore structure, it has been found that residues from the N-terminus, from the first transmembrane domain, and from the extreme of the C-terminus, constitute the hydrophilic pore lining [18], with a pore diameter estimated at a size of ~7–21 Å [19].

1.3. Expression

In humans, Panx1 is expressed in a wide variety of tissues: virtually at the entire digestive system, in skeletal muscle, heart, endothelium, skin, among others, initially identified by Northern blotting [8]. In central nervous system, Panx1 has been localized in microglia, astrocytes, oligodendrocytes and neurons [20,21] in regions such as cerebellum, cortex, retina, cerebral cortex; in hippocampus, amygdala, substantia nigra, olfactory bulb, and spinal cord [6,21–24].

1.4. Biophysics

Using patch clamp single channel recording in hippocampal neurons, the conductance of Panx1 was 527pS in a protocol of oxygen-glucose deprivation [25,26]. In isolated hippocampal neurons, current–voltage (IV) relationship is linear in ramps of ±80 mV [27]. During ischemia Panx1 looses its rectification leading to an increase of the time-dependent currents [25], thus supporting that Panx1 is in fact a membrane channel with a variable voltage-dependent conductance.

1.5. Mechanisms of Panx1 channel activation in the CNS

Under resting conditions, Panx1 remains closed to prevent loss of the electrochemical gradients across the plasma membrane and responds to changes in voltage, as previously mentioned. At resting potentials, the channel open probability is very low. However, at positive potential, Panx1 shows high conductance in neurons [28]. Besides the voltage dependence to open the channel, there are at least five physiological Panx1 opening mechanisms in the CNS.

1.5.1. Mechanical stimulation

While there are descriptions in the literature about activation of Panx1 in the CNS by the action of mechanical forces [26], this feature has been described in different cell types (erythrocytes, capillary endothelium, etc.) as a channel opening mechanism on hypotonic stress conditions or during stretching the cell. Although it has not been demonstrated in the CNS, it could potentially be a mechanism of response in traumatic injury of the CNS [29].

1.5.2. Increases in the extracellular concentration of K^+

This opening mechanism has been poorly researched despite that it is an interesting approach to investigate the role of

pannexin channels in neurons [30]. This mechanism is an effective way to increase the opening probability of channels under physiological, and in some cases, pathological conditions. At concentrations near 100 mM of extracellular K^+, Panx1 channels can be activated at resting potential and even in hyperpolarizing conditions, up to −100 mV [31]. It had previously been reported activation of the channel at higher concentrations of K^+ [26], but it should be remembered that Panx1 is activated by membrane depolarization and thus is difficult to distinguish whether the effect is caused by depolarization or high concentration of K^+. Silverman et al. [31] proposed that activation of Panx1 by high extracellular K^+ is not due to depolarization resulting from the elimination of the transmembrane concentration gradient, because of the fact that activation of Panx1 occurred even with voltage clamped at rest membrane potentials.

1.5.3. Rupture (cleavage) of the C-terminal

The mechanism involved in the opening of Panx1 mediated by rupture of the C-terminal is a "ball-chain" mechanism, as in many voltage-gated channels [32]. When breaking the C-terminus by proteolytic action of caspases, opening of the channel pore occurs. Thus, when caspases 5–7 interact with the C terminal of the channel, between residues 376 and 379, this segment cleaves and this leads to a powerful and sustained opening of Panx1. Secondary to this opening, the cell activates apoptotic signaling [33,34].

1.5.4. Intracellular Ca^+

High concentrations of intracellular Ca^{+2} have been shown to be one of the most effective mechanisms for Panx1 opening in various tissues such as endothelium or the heart muscle [35,36]. In the CNS, the opening of Panx1 by Ca^{+2} has been described in microglia and astrocytes, subsequent to activation of purinergic receptors [9].

1.5.5. Src family kinase

Post-translational modifications, such as phosphorylation [37] or nitrosylation [38], can modulate the activity of Panx1. The activation of Panx1 by P2X7 receptors may be mediated by Src family kinases (SFKs), because disruption of P2X7 binding to SFKs with an interfering peptide prevented Panx1 opening by 3′(2′)-O-(4-Benzoyl)benzoyl-ATP in J774 macrophages [39]. SFK activity increases in hippocampal neurons during ischemia [40], and Panx1 is activated in ischemic hippocampal neurons [25]. Therefore, Panx1 may be activated by SFK. Indeed, interfering Src activation or a putative SFK phosphorylation site on Panx1 prevented anoxia-induced Panx1 opening [41].

2. Function of Panx1 channel in the CNS

There is abundant literature about the physiological and pathophysiological functions of Panx1. One main function is the release of ATP by astrocytes and microglia into the extracellular medium, along its concentration gradient. Panx1 also participates in the regulation of inflammasome and has been linked to neuronal death associated to coupling of P2X7 receptor to Panx1 [43]. Additionally, diverse studies suggest that Panx1 is an important component in various pathological conditions in the brain, such as the enhancement of glutamatergic signaling in hippocampal synapses during epilepsy, in the pathophysiology of neuronal death in ischemia, as well as in neuroinflammatory processes and dysfunctions of neuronal excitability in general [5,43]. In these reviews, the authors suggest that Panx1 functions coupled to various types of ionotropic and metabotropic receptors, modulating the activity of such channels. When coupled, the channel would work as an enhancer of the activity of these receptors, thereby contributing to various and numerous physiological and pathological processes in the CNS. In this section, we focus the discussion toward the interaction of Panx1 with NMDAR and P2X7R in the pathophysiology of CNS diseases.

2.1. Panx1 and NMDAR

The NMDAR is a ligand-dependent receptor widely expressed in the CNS, with various physiological functions, such as participation in long-term potentiation-dependent learning and memory processes in various brain regions, and in other ionotropic channel-dependent pathologies such as neuronal death or central sensitization during chronic pain. In all these cases, NMDAR causes an increase of the synaptic strength [44]. NMDAR activation is dependent on synaptic activity and depolarization of the membrane is required to remove one molecule of Mg^{2+} present in the pore following activation of the receptor by its ligands, glutamate and D-serine. Structurally, NMDARs are tetramers comprised of the combination of three subunits, NR1, NR2 (4 isoforms, A-D) and NR3 (2 isoforms, A-B). Its assembly can be formed by two different subunits dimensional structure (thus a heterodimer, 2 NR1/2 NR2A), or three subunits of different three-dimensional structure (therefore a heterotrimer, 2 NR1/NR2A/NR2B) [45]. The NR1 subunit is essential for the functionality of the receptor, whereas the NR2 subunits determine the biophysical properties of the channel conductance and the mean open time or sensitivity to Mg^{+2} block [46].

The NMDAR has been functionally linked to the Panx1 channel in diverse investigations. In 2006, Thompson and colleagues showed that Panx1 is activated in response to oxygen and glucose deprivation in isolated hippocampal neurons, generating an intense secondary current that depolarize the membrane (anoxic depolarization), which finally led to neuronal death [25]. They recorded inward currents in these neurons under blockade of several important components in the process (ASIC1 channel, NMDA, AMPA and P2X7 receptors), but the current was still observed. However, this current decreased drastically in the presence of a carbenoxolone (CBX), an unspecific hemichannel blocker. Despite the apparent independence of channel activation of other receptors, subsequent data from Thompson et al. [47] and Weilinger et al. [41,42] showed that this anoxic depolarization induced activation of the NMDAR in hippocampal slices. This activation initiated an intracellular signaling cascade including increased Src kinase, and this molecule interacts with Panx1 by phosphorylating its C-terminus at Y308, generating channel opening. Indeed, when applying PP2, a Src blocker, there was significantly attenuation of the Panx1 channel activity during anoxic depolarization [41]. Thus, preventing the activation of Panx1 by NMDARs and Src kinases may become a powerful tool to reduce anoxic depolarization and neuronal death in ischemia [49].

Another pathological condition where NMDAR interacts functionally with Panx1 is in states of epilepsy, where epileptic seizures are caused by dysregulation of cortical rhythmic activity. Furthermore, Panx1 been observed in the postsynaptic density of pyramidal cells of the hippocampus in co-expression with PSD95, a major protein of the postsynaptic density, thereby suggesting a modulatory role in the excitability of the postsynaptic neurons [14]. Since Panx1 may facilitate neuronal depolarization and generate hyperexcitability, it was proposed that the channel could also participate, exacerbate, or even cause a seizure. To induce the experimental status epilepticus, hippocampal slices were stimulated with NMDA [47,48]. After blocking the activity of Panx1 with RNAi or via knock-out, it was observed a significant reduction of the neuronal activity following stimulation of the slices with NMDA or kainic acid [49], thus demonstrating that activation of Panx1 potentiates NMDAR activity. Additionally, Panx1 antagonists reduced the occurrence of epileptic events in hippocampal slices treated with glutamate and without Mg^{+2}, that is on high, intense and sustained NMDAR activity [25,50].

On the other hand, an amino acid sequence similar to a consensus site for Src has been identified in the C-terminal of Panx1 [51]. In 2012, Weilinger and colleagues developed a peptidic sequence that binds to C-terminal between the 305–318 amino acids. At very low concentrations (1 μM), this peptide was able to block the opening of Panx1 during anoxic depolarization [41].

As observed, the literature suggests that there is a functional link between NMDAR activation and Panx1 in neurons, mediated by intracellular signaling via SFK, and this relationship could be occurring in the whole CNS, in pathological processes. In this direction, potential research approaches emerging from the functional relationship between NMDAR and Panx1 are interesting. Apparently, the common link between these receptors is the neuronal hyperexcitability mediated by hyperactivity of NMDAR. Apart of the previously described conditions (ischemia and epilepsy), NMDAR participates in a wide variety of pathological conditions in the CNS [52], and could potentially be linked to co-activation with Panx1 via Src family kinases [53].

2.2. Panx1 and P2X7 receptors

P2X receptors are ionotropic membrane channels activated by the binding of extracellular ATP. When opened, they allow the selective passage of cations of small size (Na^+, K^+ and Ca^{+2}) [54,55]. ATP required to activate P2X receptors is released from presynaptic neurons, but may also be locally supplied by Panx1 [56,57]. P2X receptors comprise a structurally distinct family of seven isoforms of ATP ligand-gated ion channels [58]. They possess cytosolic amino and carboxyl ends, and two transmembrane regions of which the first is related with the opening of the channel and the second with the channel formation. The interaction between the two transmembrane segments of the P2X subunits may also modulate the opening and closing of the channel [59]. These ionotropic receptors are expressed in mammals, throughout all systems, participating in diverse physiological and pathological processes [56].

Among all purinergic receptors, P2X7R has been functionally linked to Panx1 in many investigations, in which P2X7R is intensely stimulated, leading to activation of Panx1 by intracellular mechanisms [60–62]. The synergistic participation of P2X7R and Panx1 has been demonstrated in several physiological mechanisms such as calcium waves, inflammation and apoptosis [63]. When activated, the P2X7R are permeable to various cations, leading to membrane depolarization. Furthermore, they are highly permeable to Ca^{+2} thereby increasing its intracellular concentration, thus activating Panx1 by this mechanism [64]. Panx1, in turn, releases ATP to the extracellular space, which re-activates the P2X7R positively, modulating the receptor activity. Once activated, the intracellular Ca^{+2} is able to open Panx1, even at resting membrane potential [12,65,66]. When steadily stimulated by ATP (mostly provided by Panx1), the P2X7R generates a high conductance pore in the cell membrane which allows the entry of Ca^{+2} into the cytosol and the exit of molecules of up to 900 Da, including IL1β [66].

In CNS, the P2X7R–Panx1complex has been linked to many neuroinflammatory processes, such as neuronal death, inflammasome activation, ischemia, epilepsy and chronic pain [67]. When vesiculated ATP is released, it activates P2X7R which in turn opens Panx1. This channel releases more ATP that causes a rapid and reversible opening of the P2X7R, which triggers a series of cellular responses such as activation of caspase-1 and the release of interleukin-1β (IL-1β), and cell proliferation or apoptosis can occur depending on the cell type in which the receptor is expressed [54,55,68]. Activation of P2X7R and the subsequent influx of calcium into the cell has also been associated with other signaling molecules, such as glycogen synthase kinase-4 (GSK-3) [69], mitogen-activated protein kinases (MAPK) [70], NF-kB [71], Ca^{+2}-calmodulin kinase II [72], as well as to the release of neurotransmitters such as acetylcholine [73], glutamate [74] and gamma-amino butyric acid (GABA) [75].

3. Panx1 and chronic pain

Chronic pain leads to plastic changes in the spinal cord, generating hypersensitivity and central sensitization [76], caused by the release of glutamate, ATP and several peptides (such as substance P, brain-derived neurotrophic factor, calcitonin gene-related peptide) from peripherally injured nerves, which activate NMDA, purinergic, neurokinin, calcitonin receptor-like receptor and TrkB receptors in neurons, but also in glia surrounding the synapses, thereby enhancing this condition by the release of glial proinflammatory gliotransmitters, such as glutamate, ATP and cytokines [77–80,82]. Due to this central changes, in neuropathic pain, one of the most common modalities of this condition, there is no transduction and the treatment tends to be more refractory [81]. This process can be prevented, or eventually reverted, by administrating antagonists of the neuronal and glial mediators and receptors [83].

As described before, Panx1 activation has been associated with NMDAR and P2X7R. Interestingly, NMDAR is an essential component of chronic pain mainly in neurons [84], while P2X7R, mostly in glia, has been implicated in the maintenance of chronic pain states [82,83,85]. The NMDAR and P2X7R open Panx1, and this channel potentiates the activity of both receptor in pathologies of the CNS. Since both receptors have an important role in chronic pain states, we hypothesized that Panx1 participate in this pathological state at a CNS level.

Recently, we tested this hypothesis in rats with a section of the sural nerve (a neuropathic pain model) treated with different Panx1 blockers (10panx peptide; carbenoxolone, CBX; probenecid, PRB). 10 days after the induction of neuropathy, the mechanical nociceptive threshold was tested using paw pressure testing. Intrathecal administration of either 10panx, CBX or PRB significantly decreased the mechanical hyperalgesia in neuropathic rats, without affecting the mechanical threshold in sham animals, thus suggesting that Panx1 is involved in the mechanical hyperalgesia observed in neuropathy [86]. Further, we tested whether Panx1 may affect spinal cord transmission properties during chronic pain. Using the same animal model, we studied the variations in spinal wind up activity, in vivo, after intrathecal injection of Panx1 blockers. Spinal wind up is a frequency-dependent increase in the excitability of spinal cord neurons, evoked by electrical stimulation of afferent C-fibers, strongly related with NMDA receptor activation [87]. It was shown that Panx1 blockers reduced by about 75% the wind up activity in the rat spinal cord, strongly suggesting an interaction between NMDAR activation and Panx1 [86]. Since pharmacological blockage of Panx1 reduced both the mechanical hyperalgesia and the spinal nociceptive transmission in neuropathic rats, we concluded that Panx1 participates in spinal transmission of neuropathic pain.

Months later, based on previous evidence showing that Panx1 can release many signaling molecules and that blocking Panx1 in spinal cord reduces chronic pain [86], a group of researchers from the Anderson Cancer Center studied the distribution of Panx1 in dorsal root ganglion (DRG) neurons and how nerve injury could affect Panx1 expression in DRG, using the spinal nerve ligation model of rat neuropathy [88]. They demonstrated that both the Panx1 protein and its mRNA significantly increased in the DRG, after the peripheral lesion. These data suggest that the nerve injury increased the number of Panx1-immunoreactive DRG neurons, as measured by immunocytochemical labeling. In addition, after the lesion they found increased levels of two activating histone marks (H3K4me2 and H3K9ac) together with decreased occupancy of two repressive histone marks (H3K9me2 andH3K27me3) near the promoter region of Panx1 in the DRG, with no effect on the DNA

methylation level, thus demonstrating upregulation of Panx1 in the DRG and epigenetic changes via histones modifications [88]. Furthermore, to demonstrate that the increased of Panx1 expression in the DRG has a pathological correlation with pain hypersensitivity induced by nerve injury, they injected Panx1 blockers and Panx1 specific siRNA. They found that both, blockers and siRNA, significantly reduced mechanical hyperalgesia in the von Frey test. Finally, they showed that Panx1 siRNA significantly reduced caspase-1 release induced by neuronal depolarization in a DRG cell line. Therefore, they concluded that nerve injury increases Panx1 expression levels in the DRG through altered histone modifications, and that this upregulation contributes to the development of neuropathic pain and stimulation of inflammasome signaling [88].

Additionally, two other recent reports demonstrated that CBX attenuated mechanical hypersensitivity in models of pathological pain induced in rat either by partial transection of the infraorbital nerve [3] or by peripheral nerve injury [89]. Considering that CBX is a non selective inhibitor of gap junctions and that it also blocks Panx1 at low concentration, this evidence constitutes only an indirect link regarding the participation of Panx1 on chronic pain.

These promising researches constitute the very first evidence of the participation of Panx1 in chronic pain. They have begun to open discussion about the role of Panx1 in this pathological condition and the potential relevance for the treatment of chronic pain, as a new pharmacological target. Although the methods used by these authors do not explain the physiological role of this channel, it is possible to postulate diverse hypothesizes about how does Panx1 participates in chronic pain. An intriguing question is: where Panx1 is mainly acting? This simple inquiry could be crucial for the understanding of its role in chronic pain. Since it is known that Panx1 is expressed in neurons, astrocytes and microglia (as mentioned in Section 1.3), it is possible to hypothesize the participation of Panx1 in the spinal cord, both in neurons and glia. These hypothesis are summarized in Fig. 1.

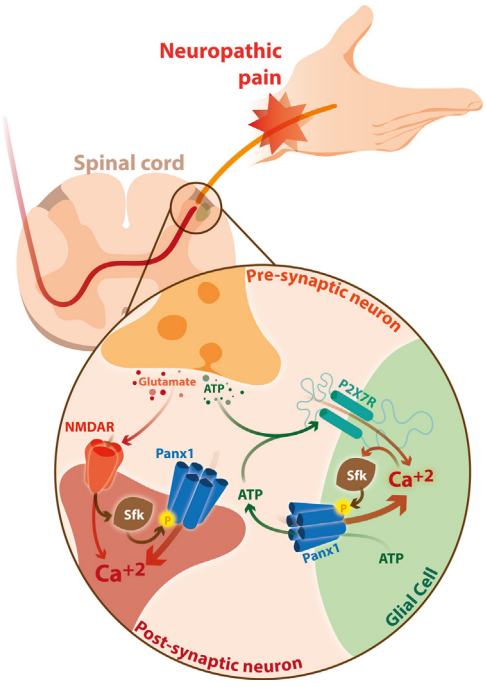

Fig. 1. Possible mechanisms of the participation of Panx1 in chronic pain: The Neuronal and Glial Hypothesis. Damaged primary afferent fibers projects to the lamina I and II at the dorsal horn of the spinal cord. These pre-synaptic neurons release diverse neurotransmitter that produces pathological neuroplastic changes of the post-synaptic neurons and activates surrounding glial cells, both expressing Panx1. In the neuronal hypothesis, we postulate that NMDAR interacts with Panx1 in neuropathic pain, by activating Src-family kinases (sfk) that may phosphorylate Panx1. This high conductance channel may increase calcium inward currents as reported in hippocampus, thus potentiating the NMDAR activity. In the glial hypothesis, we postulate that glial P2X7R is activated by ATP, released from pre-synaptic neurons, which in turn activates Panx1, probably by sfk. This channel releases ATP, amplifying this purinergic signal, which induces the production of pro-inflammatory cytokines.

3.1. The neuronal hypothesis: Panx1 and NMDAR interaction

As stated before, NMDAR is a key component of the machinery of chronic pain I the spinal cord. Particularly interesting is the activation of a family of tyrosine kinases derived from sarcomas, or Src kinases, as a mechanism of NMDAR activation in chronic pain conditions. In vitro studies demonstrate that Src is crucial in the establishment and maintenance of hippocampal LTP, and the Src antagonist PP2 has proven to be highly efficient to inhibit the generation of LTP [90–92]. In 2008, Liu and colleagues [93] published an interesting finding about the way to treat chronic pain by blocking NMDAR, but without producing cardiovascular, locomotive or memory effects, which are the main side effects observed following the administration of NMDAR antagonists. By synthesizing a peptide that interacts with the amino acids 40–49 of Src (Src40-49), the researchers disrupted the interaction between Src and the NMDA receptor complex by inhibiting the binding of the kinase to ND2, a region of NMDAR that modulates its activity, without affecting the catalytic activity of Src. In neurons in culture, the peptide reduced NMDAR mediated currents [93]. Later, the authors investigated the in vivo effects of the peptide Src40-49Tat. In a rat model of inflammatory pain, pre-treatment with Src40-49Tat suppressed NMDAR-mediated pain behavior and also inhibited thermal and mechanical hypersensitivity [93]. When the peptide was tested in models of neuropathic pain, the peptide reversed the mechanical hypersensitivity and hypersensitivity to cold, and when administered intrathecally, the antinociceptive effect persisted for 5 h. Interestingly, these effects were not observed in $Src^{-/-}$ mice, indicating that the effect was dependent on Src [93]. In the spinal cord, Src40-49Tat disrupted the interaction Src-ND2 and reduced the increase in tyrosine phosphorylation of the NR2B subunit of NMDAR, which occurred in response to inflammatory and neuropathic pain. Thus, disruption of the interaction of Src with ND2 was able to produce long-lasting analgesia, without inducing side effects [93].

Given this relationship between NMDAR and Src in nociception, and the interaction of Src with Panx1 after NMDAR activation in anoxia (Section 2.1), it is possible to hypothesize that Panx1 could be an important element in the nociceptive signaling of dorsal horn neurons in chronic pain conditions. More specifically, Panx1 channels could be acting as a potentiator or amplificator of NMDAR, as proposed by Isakson and Thompson for the hippocampus [5]. Considering the reduction of the spinal wind up by Panx1 inhibitors [86], it can be inferred that NMDAR and Panx1 are, somehow, functionally coupled in spinal cord dorsal horn neurons. The 1 Hz electrical stimulation used to generate wind up increases the frequency of action potentials in primary sensory C fibers [94] and is related with activation of NMDAR [95–97]. Then, when wind up takes places, NMDAR are activated in lamina I and II [98,99]. If NMDAR are activated during spinal wind up, and wind up is reduced by Panx1 blockers [86], it is likely that activation of NMDAR signalizes to activate Panx1, and thus this channel could be the responsible for the wind up amplification of the spinal signal and, consequently, the generation of central sensitization in dorsal horn neurons. In this context Panx1 could be being activated by NMDAR receptors via Src, thus becoming an extra-source of inward calcium currents, as described in the hippocampus in vitro [49] and in vivo [41]. Finally, the fact that NMDAR forms a complex with PSD-95/GKAP [100] and Panx1 is co-localized with PSD95 [14] and also NMDAR [90], opens the attractive possibility that these protein

interacts and signalize via Src in a microdomain of dendritic spines, a hypothesis that should be tested.

As mentioned above, NMDAR has been demonstrated as crucial in the process of generating hyperalgesia during neuropathy by many authors, and there is the possibility that the inward calcium currents to the postsynaptic densities in the spinal cord could occur via Panx1 (Fig. 1). Due to the high conductance of this channel in neurons, Panx1 could be the main responsible for the calcium-mediated changes in postsynaptic dorsal horn neurons of nociceptive pathways and the associated pathological plastic changes in those neurons. Whether Panx1 plays a role in chronic pain conditions by amplifying the NMDAR signaling in spinal cord circuitries is a matter that deserves future investigation.

3.2. The glial hypothesis: Panx1 and P2X7R interaction

P2X7R–Panx1 interactions have been described in glia, suggesting that Panx1 participates in the maintenance of chronic pain by enhancing glial P2X7 receptor activity. In fact, Panx1 has been described in the microglia, releasing ATP in co-activation with P2X7 receptor in neuroinflammatory processes [57]. There are many investigations that implicate this molecule to activation of purinergic receptors and purinergic signaling, which are known to be involved in chronic pain and central sensitization [101]. P2X7R is a crucial element in the establishment and maintenance of chronic pain, as previously stated. After intense and continuous discharge of the damaged peripheral neuron there is ATP release [102], which in turn activates glial P2X7R among other purinergic receptors [103]. When activated, P2X7R raises intracellular calcium that activates MAPK and P38, precursors of pro-inflammatory cytokines [104], which sensitize neurons. In this context, P2X7R may be opening Panx1 via Src kinases, and this channel could enhance the nociceptive activity of P2X7R, by secreting ATP [39]. This evidence indirectly suggests that Panx1 could be involved in neuropathic pain following stimulation of P2X7R.

In 2012, Sorge and colleagues [105] suggested that the pore formation of P2X7R was crucial for chronic pain and allodynia, both in mice and humans. They showed that individuals that do not have the coding sequence of the gene encoding the formation of the pore of P2X7R, may present less allodynia than those with an intact P2X7R. Actually, in human chronic pain cohorts (osteoarthritis and mastectomy), genetic associations between lower pain intensity and hypofunctional His270 (rs7958311) allele of P2X7R was observed [105]. Several authors have suggested that the pore formed by intense activation of P2X7R is, in fact, the Panx1, and its activation causes strong dye flux in vitro, release of ATP and IL-1β, as previously mentioned [49]. Hence, if Panx1 is actually the forming pore of P2X7R, this could explain the participation of Panx1 in a powerful way. Therefore, glial P2X7R–Panx1 interaction has the potential to explain why the pharmacological blockage of Panx1 interferes in the nociceptive signaling (Fig. 1).

Finally, another interesting hypothesis of the participation of Panx1 interacting with P2X7R in chronic pain (although in the peripheral nervous system), could be that Panx1 supplies ATP from the DRG sensory neurons to the satellite glial cells, as stated by Zhang in 2015 [88]. DRG neurons have been described releasing somatic ATP triggering neuron-satellite glial cell communication [106]. Activation of P2X7 receptors of the satellite cells can lead to the release of tumor necrosis factor-alpha, IL-1β and other cytokines, which in turn can influence neuronal excitability and, consequently, the development and/or maintenance of pain after a nerve damage. Since Panx1 is upregulated in the DRG during neuropathic pain, and one of the most important roles of Panx1 is ATP release [57], the relation Panx1-P2X7R could help to understand how neuropathic pain begins, and how it is maintained, at least in the periphery.

4. Concluding remarks

Based on the fact that (i) Panx1 is vastly expressed in the CNS, in all cell types; (ii) functioning as a channel, with high conductance; (iii) permeable to divalent cations such as calcium, glutamate and ATP; (iv) these molecules and cations are crucial molecules in diverse CNS diseases; we can state that Panx1 is a relevant component in the pathophysiology of some CNS diseases via interaction with NMDAR and with P2X7R, by amplifying the intracellular signaling of the first, and supplying the second with its ligand, ATP.

There is also few but consistent evidence that Panx1 is important for chronic pain signaling in the dorsal horn of the spinal cord (and perhaps in DRG sensory neurons), as well as in the process of developing and maintaining central sensitization underlying chronic pain. In this direction, NMDAR-Panx1 signaling via Src kinase observed in different pathological contexts could support the contention that this interaction leads to chronic pain, possibly by amplification of NMDAR nociceptive signaling. Besides, due to the fact that Panx1 is highly permeable to ATP, the main activator of the P2X7R signaling, which is one of the pathways that mediates the pathological activation of the glial cells in the spinal cord and in the DRG, we can speculate whether Panx1 also participates in synergic activity with P2X7R during chronic pain.

At present, the interactions of Panx1 with NMDAR and P2X7R in CNS pathologies are known, but the existence of such interactions in chronic pain remains rather unknown. We hypothesized that Panx1 could participate in dorsal horn mechanisms of hyperalgesia/allodynia accompanying chronic neuropathic pain by interacting with the hyperexcitability of secondary neurons activated by NMDAR and with P2X7R in parallel pathways, with both interactions possibly occurring together. If so, this will open new perspectives in the development of new drugs targeting this component, on the basis that accurate inhibition of this channel may represent a pharmacological tool for diminishing neuroinflammation and hyperexcitability, at the same time. Further investigations in this field are required, to raise hope to those who endures chronic pain, daily.

Conflict of interest

The authors declare that they do not have any conflict of interest, financial interests that could create a potential conflict of interest or the appearance of a conflict of interest with regard to the work.

Acknowledgement

This work was supported by grant FB0807 from CEDENNA and grant from DICYT 021543CC.

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