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Review

Advances in the pharmacology of lGICs auxiliary subunits

Pablo Galaz[a], Rafael Barra[b], Hector Figueroa[c], Trinidad Mariqueo[d,*]

[a] Departamento de Bioquímica y Biología Molecular, Facultad de Ciencias Químicas y Farmacéuticas, Universidad de Chile, Santiago, Chile
[b] Laboratory of Neurochemistry, Medicine School, Faculty of Medical Sciences, University of Santiago of Chile, Chile
[c] Department of Basic and Medical Sciences, Faculty of Health Sciences, University of Talca, Chile
[d] Center For Medical Research, School of Medicine, University of Talca, Chile

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ABSTRACT

Ligand-gated ion channels (LGICs) are cell surface integral proteins that mediate the fast neurotransmission in the nervous system. LGICs require auxiliary subunits for their trafficking, assembly and pharmacological modulation. Auxiliary subunits do not form functional homomeric receptors, but are reported to assemble with the principal subunits in order to modulate their pharmacological profiles. For example, nACh receptors are built at least by co-assemble of α and β subunits, and the neuronal auxiliary subunits β3 and α5 and muscle type β, δ, γ, and ϵ determine the agonist affinity of these receptors. Serotonergic 5-HT_3B, 5-HT_3C, 5-HT_3D and 5-HT_3E are reported to assemble with the 5-HT_3A subunit to modulate its pharmacological profile. Functional studies evaluating the role of γ2 and δ auxiliary subunits of $GABA_A$ receptors have made important advances in the understanding of the action of benzodiazepines, ethanol and neurosteroids. Glycine receptors are composed principally by α_{1-3} subunits and the auxiliary subunit β determines their synaptic location and their pharmacological response to propofol and ethanol. NMDA receptors appear to be functional as heterotetrameric channels. So far, the existence of NMDA auxiliary subunits is controversial. On the other hand, Kainate receptors are modulated by NETO 1 and 2. AMPA receptors are modulated by TARPs, Shisa 9, CKAMP44, CNIH2-3 auxiliary proteins reported that controls their trafficking, conductance and gating of channels. P2X receptors are able to associate with auxiliary Pannexin-1 protein to modulate P2X7 receptors. Considering the pharmacological relevance of different LGICs auxiliary subunits in the present work we will highlight the therapeutic potential of these modulator proteins.

Contents

* Corresponding author.
E-mail address: tmariqueo@utalca.ca (T. Mariqueo).

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1. Introduction

Ligand-gated ion channels (LGICs) belong in a group of channel proteins which permeate ions (and molecules in some cases) in response to the binding of a chemical messenger. They are essential in chemical synapses as they mediate the chemical to electrical signal transformation. LGICs are classified into three superfamilies: Cys-loop, ionotropic glutamate receptor and P2X receptor (ATP-gated channels) families. Because of their role in neurotransmission, they are major pharmacological targets present in the nervous system.

These channels are built up from several subunits, which interact with different proteins during and after assembly. In the present review, we define a protein as being an auxiliary subunit when it meets the following criteria: (I) It must not be able to form a functional channel by itself. (II) It must establish a direct and stable interaction with pore-forming subunits. (III) It must be involved in channel modulation and/or trafficking, and (IV) necessary for normal functioning *in vivo*. These criteria are similar to those defined by Yan et al., for glutamate receptors [1].

2. The ligand-gated ion channels Cys-loop family

The LGIC Cys-loop family includes the nicotinic acetylcholine, serotonin 5-HT_3, $GABA_A$ and glycine receptors. These members are integral membrane proteins conformed by a pentameric arrangement and are classified as Cys-loop family due to the presence of an aminoacids loop in the large extracellular domain linked by a disulphide bond. Each subunit of Cys-loop receptors consists of a large N-terminal extracellular domain containing the ligand-binding site, four transmembrane segments (TM1-4), of which TM2 is involved in pore formation, and one large intracellular loop (IL) connecting TM3 and TM4, which participates in diverse intracellular modulations [2] (Fig. 1A).

3. The nicotinic receptor auxiliary subunits

The nicotinic acetylcholine receptor (nAChR) was the first neurotransmitter receptor chemically identified, isolated from *Torpedo* fish electric organ [3]. In vertebrates, there are many types of nAChR oligomers, which show differences in the composition of their subunits, depending on their functional roles and location. The "muscle type" nAChRs are structurally related to the *Torpedo* electric organ nAChR and they are located at the skeletal neuromuscular junction. In addition, nAChRs have been found in the peripheral and central nervous systems, which have been named "neuronal" nAChRs [4]. All nAChRs are cation-selective ionic channels and their subunits share the same topology. Their basic structure consists of a large N-terminal extracellular domain, a transmembrane (TM) domain comprising four segments (TM1–TM4), and a variable cytoplasmic or intracellular domain [5].

3.1. The muscle nicotinic receptor

The muscle-type nAChRs are made from $\alpha 1$, β, γ, δ and ϵ subunits, that are expressed from the genes *chrna1*, *chrb*, *chrng*, *chrnd* and *chrne*, respectively [5]. Two receptors types are built from the combination of these subunits: $(\alpha 1)2\beta 1\gamma\delta$ expressed in embryonic and $(\alpha 1)2\beta 1\epsilon\delta$ expressed in adult muscle tissue [6].

3.2. Role of $\beta 1$ subunit in metabolic stability of nAChRs

This subunit seems to play an important role in the regulation of turnover, expression, clustering and anchoring of nAChRs at the neuromuscular junction (NMJ). It has been reported that the metabolic stability of the nAChRs at the NMJ is related to the level of phosphorylation of the nAChR β1 subunit. Experiments show that the presence of neural agrin combined with pervanadate, an inhibitor of tyrosine phosphatase, slow down the degradation of surface receptors in cultured muscle cells. This effect is induced by the phosphorylation of β subunit [7]. This hypothesis is supported by the finding that the mutation of β at Y390 impairs agrin-induced clustering and anchoring of AChRs in cultured myotubes [8]. Furthermore, mice with mutations of the β subunit loop tyrosines show a reduction in the expression of AChRs [9].

3.3. Role of β and δ subunits in trafficking and AChR assembly

Recently it has been reported that β and δ subunits loops regulate the expression and trafficking of assembled AChRs to the cell membrane, by means of signals that control Golgi retention of these receptors. The authors used chimeric proteins made of CD4 fused to the muscle AChR subunit cytoplasmic loops. They found that CD4-β and δ subunits loops showed a very low expression level at the plasma membrane, while the other subunits loops became widely expressed. The low surface expression of CD4-β and δ subunits is explained by their rapid internalization from the cell membrane and by a significant retention performed by the Golgi apparatus. The authors suggest that these mechanisms regulate the surface trafficking of assembled AChRs and may avoid the surface presence of unassembled AChR subunits [7].

3.4. Role of ϵ subunit

The ϵ subunit has been reported to increase Ca^{2+} permeability of muscle nAChR when its own expression is enhanced [10], to confer selectivity of Waglerin-1 for the α–ϵ subunit interface site [11].

3.5. The neuronal nicotinic receptor

Different subtypes of neuronal nAChRs exist in the nervous system. The heteromeric channels are made of α (2–6) and β (2–4), while the homomeric channels are made of α (7 and 10) subunits [12]. Heteromeric neuronal AChRs contain two ACh binding sites

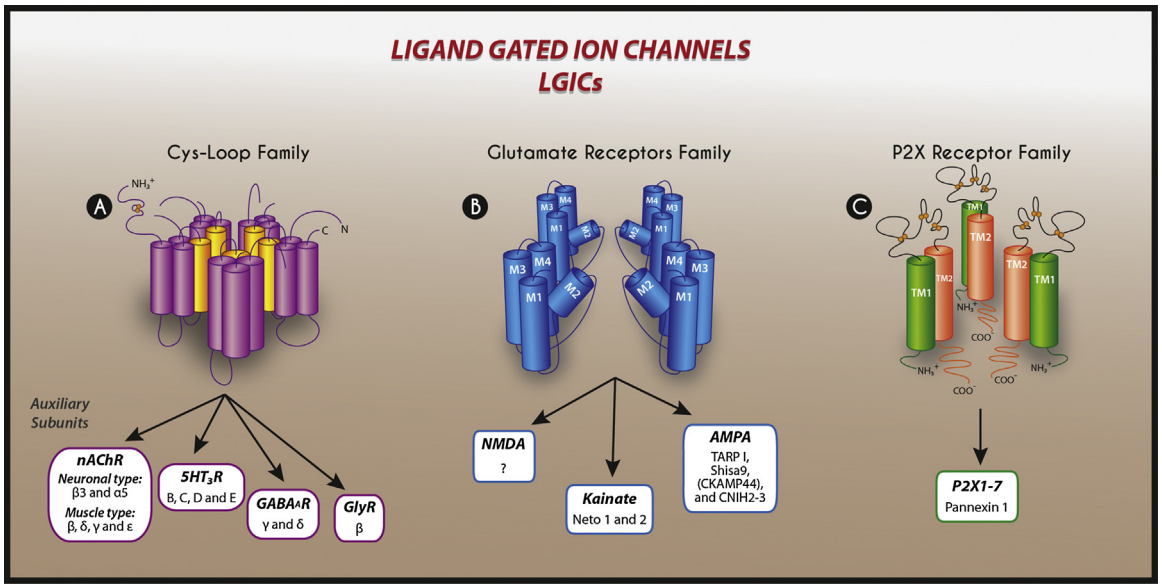

Fig.1. Ligand-gated ion channels auxiliary subunits. In A is represented the topology of Cys-Loop family and their members. In B, the Glutamate receptors and their family members. The question mark on the NMDA receptor box indicates that the existence of NMDA auxiliary subunits is under study. In C, the P2X receptors family and their auxiliary subunit.

located in the interfaces of α2, α3, α4 or α6 subunits with β2 or β4 adjacent subunits [5].

The additional auxiliary subunit contributes to the assembly, transport and function of the receptors, but does not form part of the ACh binding sites. This location can be filled by β2, β4 or α4 subunits [13]. On the other hand, β3 and α5 subunits can be placed only in this position.

3.6. Role of α5 subunit

Several cell-cased *in vitro* studies have shown that the α5 subunit regulates pharmacological properties, Ca^{2+} permeability and ACh sensitivity of α4β2 nAChR [13–16]. In addition, *in vivo* experiments have reported that the α5 subunit plays a relevant role in the regulation, expression and function control of α4-containing subunit neural nAChRs located in the ventral tegmental area of the brain [17].

3.7. Role of β3 subunit

It has been described that β3 subunits activate the expression and up-regulation of nicotinic receptors. Nicotinic acetylcholine receptors (AChRs) containing α6 subunits are found at monoaminergic nerve terminals and they seem to play relevant roles in the development of nicotine addiction and Parkinson's disease. Usually α6 AChRs contain β3 subunits are thought to work only as auxiliary subunits, since they do not form part of the acetylcholine binding sites [18].

3.8. Therapeutically relevance of nicotinic receptors

Several disorders of the nervous system as Alzheimer's disease, schizophrenia, depression, attention deficit hyperactivity disorder and tobacco addiction may improve if they are treated with drugs capable of modulating nAChRs [19,20]. As an example, some schizophrenic patients often show inability to focus attention, a problem thought to be caused by sensory-gating deficit affecting α7 nAChRs [21]. These patients respond to nicotine administration with improved sensory gating, presumably through α7 nAChR activation [22].

4. The 5-HT_3 receptor auxiliary subunits

The 5-HT_3 receptor is a cation-selective serotonin (5-hidroxytriptamine, 5HT) receptor that belongs to the superfamily of the Cys-loop ligand-gated ion channels (LGICs) permeable to Na^+, K^+ as well as other ions [23]. As other members of the Cys-loop LGIC family, the 5-HT_3 receptor has a pentameric structure [24]. In humans and other mammals, five different subunits, termed 5HTA to 5HTE have been described and thought to potentially combine to form distinct molecular versions of the 5-HT_3 receptor. The characterization of the homopentameric A [25,26] and heteropentameric AB receptor (5-HT_3AB) have been performed by means of heterologous expression [27]. The 5-HT_3 receptor is encoded by the genes *htr3a*, *ht3b*, *htr3c*, *htr3d* and *htr3e* [28].

Only 5-HT_3A subunits are able to form functional homomeric receptors, whereas the 5-HT_3B, C, D and E subunits are not able to build functional homopentameric receptors by themselves [29]. These 5-HT_3 receptor isoforms show explicit electrophysiological characteristics, such as single channel conductance and rectification. However, they exhibit few pharmacological differences in response to 5HT or to 5HT_3 antagonists [30,31]. In addition, the functional role of the subunits 5-HT_3C, 5-HT_3D and 5-HT_3E were investigated by means of patch clamp experiments using recombinant human subunits. All of them were non-functional when expressed as homomeric channels. However, co-transfection experiments aiming to assemble heteromeric receptors expressing 5-HT_3A with the other subunits, showed that the function or expression of the receptor was altered by 5-HT_3C and 5-HT_3E, but not by 5-HT_3D [32].

Serotonergic transmission is relevant in brain function, as it is involved in cognition, mood and emotions [33]. In addition, 5-HT is present in enterochromaffin cells of the mucosal epithelium and serotonergic enteric neurons of the bowel, where 5-HT participates in fast and slow synaptic transmission [34,35].

Although the basis for 5HT_3 receptor diversity is not well understood, and even the existence of functional 5HT_3C-E receptor subunits was doubted, some studies have demonstrated the presence of 5-HT_3C, 5-HT_3D and 5-HT_3E subunits *in vivo*, in gastrointestinal tissue, suggesting that these 5-HT_3 receptors subunits might have a specific role in the control of bowel functions [36,37].

Recently, in a study performed in human gut, it was shown the presence of a 5-HT_3 subunit transcript together with the expressed protein in myenteric and submucosal plexus in cellular bodies of neuronal cells [38]. These findings will promote future studies of the roles that distinct 5-HT_3 receptor subtypes may play in the function of enteric and nervous system.

5. The $GABA_A$ receptor auxiliary subunits

The γ-amino butyric acid (GABA) is the principal fast inhibitory neurotransmitter in the central nervous system. $GABA_A$ Receptors ($GABA_AR$) belong to LGICs Cys loop family and as glycine receptors, they are constituted by five subunits surrounding a central chloride ion channel. To date 19 subunits including α (α 1–6), β (β1–3), γ (γ1–3), δ, ϵ, π, ρ (ρ1–3), θ have been isolated, each of them have been expressed by a different *gabra* gene [39,40].

The majority of $GABA_AR$ are assembled in a stoichiometry 2α, 2β, 1γ or 1δ [41,42]. $GABA_AR$ containing γ subunit is expressed preferentially at synaptic level and interacts with the scaffolding synaptic protein gephyrin [43] regulating the phasic inhibitory currents, while δ subunit determines the extra-synaptic location and is responsible for the tonic GABAergic currents [44,45].

The pharmacological modulation of $GABA_AR$ is principally mediated by γ and δ auxiliary subunits. It was reported that benzodiazepines preferentially interact with $GABA_AR$ containing $\alpha(\alpha_{1,2,3,5})\beta\gamma_2$ subunits [46]. In this sense, benzodiazepine binding to the interface α/γ positively modulates phasic GABAergic currents [47,48].

Impairment in the inhibitory GABAergic synaptic transmission at spinal cord level is associated with chronic pain development [49]. Recent evidence suggests that compounds with high α2-$GABA_AR$ intrinsic activity and reduced α1-$GABA_AR$ specificity should avoid an unwanted sedation [50]. Therefore, the understanding of key elements of γ2 modulation of α2-$GABA_AR$ will constitute a promising therapeutic approach against chronic pain.

On the other hand, δ subunit determines the sensitivity of tonic GABAergic currents to general anesthetics, neurosteroids and Gaboxadol [51–53]. In addition, this subunit together with α4 or α6 is reported to be more sensitive to pharmacological modulation by ethanol [54–57].

The role of extra-synaptic δ-$GABA_AR$ has been less explored due to reduced pharmacological tools to specifically differentiate these types of receptors. Recent evidence suggests that δ-$GABA_AR$ play a critical role in central pain sensitization [58,59]. Behavioral characterization of *gabrd* knockout mice (*gabrd*−/−) which lack δ subunit, showed that injection of formalin in the hind paw induces a biphasic response, and have reduced formalin phase II response. This evidence suggests that tonic currents mediated by δ-$GABA_AR$ play a relevant role in spinal pain circuits control. Future studies to better understand this modulation will be necessary to improve the pharmacological tools against persistent pain development and establishment.

6. The glycine receptor auxiliary subunit

Glycine is the main inhibitory neurotransmitter in the spinal cord and brain stem, playing a significant role in motor respiratory control and pain sensitization [60]. In the mature spinal cord, glycine activates GlyRs, which are pentamers composed mainly of α1 (48 kDa) and β (58 kDa) subunits [61]. These subunits are encoded by *glra* and *glrb* genes [62]. The resulting receptors can be assembled as homopentamers (α1–α4) or heteropentamers (forming complexes with β subunits). Auxiliary β subunit is unable to form functional channels without association with α subunits. However, previous reports have demonstrated that β subunit determines the synaptic localization of GlyRs, as well as the pharmacological profile of glycinergic currents [63,64].

Different types of GlyRs can be differentially modulated by exogenous and endogenous moieties, including cations, cannabinoids, tropeines, neurosteroids, avermectins, butyrolactones, alcohols and general anesthetics [65]. Using site-directed mutagenesis, several domains that play a relevant role in allosteric modulation of homomeric GlyRs have been identified [66–68]. In contrast, little is known about allosteric modulation of GlyRs by auxiliary β subunit. This might result from reduced specific pharmacologic tools available to study this modulation. Bormann et al. described that residues within and close to domain TM2 of the β subunit determine the pharmacological profile differences between homo- and heteromeric GlyRs. For example, the EC_{50} and Hill coefficient values for α1 were 30 μM and 5.7 while for α1β were 54 μM and 2.5 [64]. On the other hand, N-terminal extracellular domains of β subunit determine ligand binding to α/β interface of GlyRs [69]. Furthermore, intracellular β subunit loop interacts with gephyrin protein and both regulate the receptor density during synapse formation, modification and elimination [70,71].

Some studies have described that α2β heteropentameric GlyRs are less sensitive to the inhibitory effects of zinc ion [72,73], while the homomeric receptors are more sensitive to the effects of dehydroxylcannabidiol (DH-CBD), a cannabidiol analogue [74–76]. Ahrens et al. showed that the general anesthetic propofol potentiated the glycine activated currents of α1β GlyRs [77]. In addition, our recent report indicates that homomeric α1 GlyRs are more sensitive to the effects of ethanol, therefore the β subunit may be allosterically modulating α subunits [78]. On the other hand, the role of auxiliary β subunit in the allosteric modulation of glycinergic currents in chronic pain stages has been less explored. Taking advantage of a rat model of chronic neuropathic pain, we have evaluated the expression and localization of β subunit in the spinal cord of such model. Western blot analysis showed that the levels of β subunit were increased in tissue lysates derived from animals subjected to pain for three days. Similar results were obtained by immunofluorescence analysis of β subunit in spinal sections. Taking together, these findings suggest an important, albeit underappreciated, role of the β subunit in the allosteric modulation of glycine receptors in chronic pain development (Mariqueo et al., unpublished data).

7. The ligand-gated ion channel glutamate receptor family

Ionotropic glutamate receptors (iGluRs) are a family of tetrameric ligand-gated ion channels that mediate most of the excitatory neurotransmission in the CNS. It is composed by three major receptor subtypes: *N*-methyl-D-aspartate receptor (NMDAR), Kainate receptor (KAR) and α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor (AMPAR) named according to their preferred synthetic agonist. All subunits share a common topology of extracellular N-terminal domain, three transmembrane domains (TM1, TM3, and TM4), a re-entrant channel lining TM2 with both ends facing the cytoplasm and an intracellular C-terminal domain (Fig. 1B).

8. The NMDA receptor auxiliary subunits

The NMDARs correspond to one of the three receptors able to recognize glutamate. The NMDA receptor is a tetramer and it has been described in association with several proteins, including scaffolding, peptide and signaling regulators proteins. Seven genes codify for the subunits of the NMDAR and are classified into three general categories, [GluN1/NR1 (*grin1*)], [GluN2/NR2 (*grin2A*, *grin2B*, *grin2C*, & *grin2D*)] and [GluN3/NR3 (*grin3A* & *grin3B*)]

[79–84]. The activated NMDAR is permeable to monovalent and divalent cations, as sodium, potassium and calcium, respectively.

There is controversy whether NMDARs posses or not auxiliary subunits, as there are several candidate proteins, but not obvious evidence. For example, the NETO1 protein has been structurally associated to NMDARs complex [85]. However, a study carried out in NETO1 Knockout mice demonstrated that NMDA mediated currents were not affected by this mutation [86]. On the other hand, Amyloid Precursor Protein (APP) infusion increases NMDAR evoked currents [87] and together with its related proteins, amyloid precursor-like proteins 1 and 2 (APLP1 & APLP2), enhance surface NMDA receptors expression *in vitro* [88]. Controversial results regarding the NETO1-NMDAR interaction led to overlook it as an auxiliary subunit (as discussed by Molnar [89]), while there is no evidence demonstrating amyloid-NMDAR direct interaction. Further studies are necessary to confirm or discard the putative existence of NMDAR auxiliary subunits.

9. The AMPA receptor auxiliary subunits

In the mammalian CNS, AMPAR regulates fast-synaptic transmission in response to glutamate, leading to depolarization of the neuronal membrane. This channel assembles as a homo- or heterotetramer formed by two dimers of GluA1-4 proteins, encoded in *gria1-4* genes, respectively [90]. Each gene can yield several splice variants, which may form channels with different kinetics. Additionally, GluA2 mRNAs editing allows further changes in channel properties [91].

In vertebrates, AMPARs have been found to interact with a variety of auxiliary subunits, as recently reviewed by Karataeva [92], including *transmembrane AMPAR regulatory proteins* (TARPs), Shisa9 and cornichon homologs. TARP family comprises six members divided into two groups: Type I TARPs (γ2-4, γ8) and Type II TARPs (γ5, γ7). They are grouped based on sequence homology and the inability of γ5 and γ7 subunits to fully restore functional AMPA receptors on rescue experiments in γ2 knockout mice [93]. These proteins are ubiquitously expressed within the CNS; furthermore, a recent analysis of postsynaptic density fractions from rat cerebral cortex, estimated an average stoichiometry of roughly two TARPs per AMPAR [94].

TARPs are capable of regulating the maturation, trafficking and the activity of AMPA receptors within the neuronal membrane. The consensus of studies affirms that TARPs lower receptor deactivation and desensitization rate, while they increase ion conductance, recovery from desensitization, glutamate affinity and open probability. In addition, γ4, γ7 and γ8 subunits confer receptor resensitization [93,95–97]. Different is the case of γ5 subunit, which increases conductance, deactivation and desensitization rate; while reducing the channels open probability [98].

Besides modulating AMPAR kinetics properties, some TARPs decrease the time constant value of desensitization induced by ethanol [99] . Moreover, TARPs improve the efficacy of the partial agonist kainate and change the nature of several quinoxalinedione, from antagonists to partial agonists [100].

It has been reported that TARPs are able to enhance the binding affinity of the radiolabeled [3H]-LY395153 modulator, but render it sensitive to displacement by 2,3-benzodiazepine and quinazolinone antagonists, which does not occur in recombinant receptors [101].

Other AMPAR auxiliary subunits recently identified in rat brain are cornichon homologous (CNIH-2, CNIH-3) [102]. These proteins increase conductance, however their reduction in the desensitization and the deactivation rate, is greater than the caused by TARPs, and likewise reduces the blockade by intracellular polyamines and increase Ca^{2+} permeability. Studies on the CNIH-2 subunit have shown that it promotes receptor trafficking to the membrane, although to a lesser extent than TARPs, and comparably to γ2 subunit [103,104]. In addition, Cornichon Homolog-2 increase total binding of [3H]-LY395153, and promotes its displacement by non competitive antagonists. Unlike CNIH-2, which does not regulate glutamate affinity, CNIH-3 reduces it [101,102].

The most recently described AMPAR auxiliary subunit is Shisa9 (originally CKAMP44), which was first identified in *Mus musculus* dentate gyrus, being able to interact with AMPAR and modulate its gating by increasing conductance, glutamate affinity and desensitization rate. Additionally, it reduces deactivation rate and delays the recovery from desensitization state [105]. Due to the interaction of Shisa9 with PSD-95 (scaffolding protein), it may be able to regulate trafficking and/or anchoring [92]. Recent work has shown upregulation of Shisa9 transcript in neurons of mice dorsal root ganglion in neuropathic pain [106].

In summary, AMPA receptors are tightly modulated by distinct regulatory subunits; nevertheless, there are still some interacting proteins that are good candidates that may qualify as auxiliary subunits, although more experimentation is required.

The knowledge of AMPAR regulation by these proteins is essential for the development of new drugs targeting this receptor. For instance, the scaffolding protein PSD-95 (which interacts with AMPARs through auxiliary subunits) has shown to be crucial in the mechanisms of central sensitization. This leads to think AMPAR as a possible target in the development of new pharmacological tools in treating persistent pain [107].

10. The kainate receptor auxiliary subunits

Kainate receptors (KARs) are tetramers of GluK1-5 proteins, in humans encoded by *grik1-5* genes. The Gluk1-3 subunits may assemble as homo- or heterotetrameric receptor, while GluK4-5 must form heteromers with the remainder subunits in order to give rise functional receptor channels. Some genes may yield different mRNAs due to alternative splicing and RNA editing of transcripts, generating splice variants that differ in their trafficking, protein interactions, channel conductance, ion selectivity, susceptibility to block by polyamines and *cis*-unsaturated fatty acids [108–117].

Kainate receptors have been found to interact with neurophillin and tolloid-like protein 1 and 2 (NETO1 and NETO2) in mammalian CNS [118,119]. These proteins have two extracellular CUB domains, an LDLa domain, a transmembrane domain and an intracellular C-terminal domain. These proteins are capable of modulating kainate receptors and their effect depend greatly upon the receptor composition [120,121]. Both proteins increase current amplitude, and glutamate affinity for all homomeric receptors, and significantly reduce inward rectification, but their modulation on desensitization kinetics is different [86,120,121].

The auxiliary protein NETO2 modulates homomeric kainate receptors by slowing down their desensitization and it may speed up or delay recovery from desensitization state. On the other hand, NETO1 subunit accelerates the onset of desensitization for GluK1 and GluK3 receptor, but slows GluK2 desensitization kinetics and reduces the recovery of desensitization time for all three homomeric KARs. Mutations of NETO proteins within the LDLa domain eliminated their effects on desensitization but not on rectification. And conversely, C-terminal domain deletion eliminated the reduction of inward rectification without modifying receptor kinetics. Both proteins also modulate synaptic targeting, but to a lesser extent than the GluK4 and GluK5 subunits, and also may interact with scaffolding proteins [120–126].

It has also been described that kainate receptor GluK2 forms a macromolecular complex with the predominant neuronal Cl^- transporter KCC2, which also interacts with NETO2. The receptor

and its auxiliary subunit are capable of critically regulating the function of the transporter *in vivo* and *in vitro* [127,128]. Interestingly, KCC2 and KARs function and expression have been implicated in neuropathic pain. The transporter KCC2 is downregulated after spinal cord injury, while KAR inhibition or ablation reduces pain behavior [129,130]. Both are tightly modulated by NETO2, therefore it may be as well a possible target in the development of new pharmacological tools for research and clinical treatment of pain. It is also worth to mention that NETO1 transcript is elevated more than 10-fold in dorsal root ganglion in mice model of neuropathic pain [106], which given its regulation of KARs, may be related with the central sensitization process.

11. The ligand-gated ion channel P2X receptor family

The P2X channels belong to the LGIC glutamate-like super family. P2X receptors have seven members; P2X1 to P2X7. P2X receptors are ion channels that form homo- or heterotrimeric receptors [131,132]. Each subunit has one extracellular domain, involved in the recognition of the endogenous agonist. Two transmembrane domains that form the channel pore and an intracellular segment [133] (Fig. 1C).

P2X channels permeate Na^+, K^+, Ca^{2+} and exceptionally Cl^- ions and they have been described in many species and a broad spectrum of tissues, ranging from unicellular to vertebrate organisms. Although ATP is the endogenous agonist of P2X receptors, they can be allosterically modulated by several other moieties and also by changes in temperature, ionic strength, glycosylation, phosphorylation of the receptor [134].

12. The P2X auxiliary subunits

P2X are encoded by seven genes, located in different chromosomes and several splicing variants of these receptors have been described [135]. P2X channels family are allosterically modulated by trace metals, protons, neurosteroids, reactive oxygen species and phosphoinositides [136,137]. However the literature is deficient in revisiting the modulation of P2X channels by auxiliary subunits, particularly by their interactions with other related proteins. This review is aimed to analyze the available literature regarding modulation of P2X receptors by Pannexin, as an auxiliary subunit.

Early studies demonstrate that the activation of P2X7 receptors in macrophages could lead to the intake of several high molecular weight markers [138]. Further, it has been established that the channel responsible for the incorporation of these markers is Pannexin-1. Another report shows that Pannexin-1 is also responsible for the release of interleukin 1β (IL-1β) [139,140].

Another type of interaction between P2X7 and Pannexin-1 has been proposed in T cells promoting an inflammatory status [141]. In a model of lung fibrosis has been demonstrated that ATP released from BLM-injured lung cells can activate the complex P2X7/Pannexin-1 by inducing the secretion of IL-1β and the progression of lung fibrosis [142].

On the other hand, P2X7R has been implicated in cellular death; in this context some authors have pointed out that the interaction between P2X7 and Pannexin-1 plays a critical role in the establishment of the large conductance complex shown in cellular death [143].

ATP liberated from human erythrocytes induces vasodilatation of microvascular circulation. In a similar manner, ATP is able to induce the release of phosphoinositides, which depends on activation of P2X7 and Pannexin-1 [144,145]. Recently published evidence demonstrates that complex P2X7/Pannexin-1 favor the pass through membrane of cardioprotective purines (ATP and related metabolites) in ischemic pre- and post-conditioning [146,147]. Thus, the pharmacological blockade of P2X7 and Pannexin-1 abolishes the cardioprotective effect [147].

12.1. Mechanism of interaction between P2Xand Pannexin-1

By using site directed mutagenesis it was described that C-terminal domain of P2X7 interacts with Pannexin-1 and with several other proteins, including laminin, actin, integrins, phosphatidylinositol 4-kinase and receptor protein phosphatase [148]. However, the functional relevance of these interactions has not been evaluated so far. Studies involving deletion of the P2X7C-terminal segment showed a reduction in membrane permeability [148–150]. Moreover, analysis of functional consequences of P2X7R polymorphism in the C-terminal segment, demonstrate a diminished capability of P2X7 activity in immune cells [151,152].

In the spinal cord it was demonstrated that the fibroblast growth factor 1 (FGF-1) is able to induce the activation of P2X7R and release of ATP by Pannexin-1, this mechanism could be extremely relevant for pathological chronic conditions that involve increase of specific neurotrophins as FGF-1 [153].

Future studies are required to gain insight about interaction of P2X and Pannexin-1 in other pathological conditions as chronic pain.

13. Conclusions and future perspectives

As we have reviewed in here, auxiliary subunits certainly modulate LGICs gating and pharmacological properties. In most cases this interaction has a huge significance *in vivo*, exhibiting its relevance as an essential element of the receptor complex for normal functioning. Because of that, it surprises us that studies aiming to develop novel pharmacological agents been able to modulate LGICs, do not consider auxiliary subunits in their experimental setup. We believe that these proteins should be taking in account as much as any other receptor subunit. It is necessary to point out that in some cases auxiliary subunits modulation of the channel properties is far more significant than subunit composition alone.

Because of their physiological relevance, ligand-gated ion channels are target for extensive pharmacological intervention in several diseases and medical conditions. Therefore, we believe necessary a wider knowledge of auxiliary subunits and a different criterion for research in order to maximize the targets of clinical relevance and a more efficient approach to treatment.

Conflict of interest

The authors declare there is no conflict of interest.

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