Baku State University Journal of Life Sciences & Biology ***2024****, v.1 (2), p. 19-30*

journal homepage: http://bsuj.bsu.edu.az/en

CONNEXINS PLAY A CRUCIAL ROLE AS PIVOTAL PROTEINS IN THE PROGRESSION OF INFLAMMATORY RESPONSES

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Received 11 january 2024; accepted 29 may 2024

Abstract

The connexin family comprises twenty-one distinct protein isoforms, eleven of which are localized within the central nervous system and are expressed by both neuronal and glial cells. These proteins assemble on the cellular membrane to generate a hydrophilic channel measuring approximately 1.5 nanometers in diameter, thereby facilitating the establishment of gap junctions between adjacent cells. The resulting structures, known as connexons and gap junctions or electric synapses, enable the coordinated exchange of functional and metabolic information between neurons and astrocytes. The modification of connexin (Cx) expression and functionality has been found to impact both inflammation and neurodegenerative diseases. Specifically, Cx-mediated hemichannels and channels have been implicated in the development of neurodegenerative disorders. The exacerbation of pathological processes within glial cells results in aberrant regulation of hemichannels, leading to the unregulated release of gliotransmitters and subsequent amplification of the inflammatory response. This highlights the pervasive pathophysiological mechanisms underlying neuroinflammation and suggests the therapeutic potential of targeting Cx-based hemichannels and channels for treatment purposes.

This comprehensive review examines the significance of Connexins (Cxs) in both neuroinflammatory and neurodegenerative disorders, where blocking connexin-mediated channels and hemichannels presents potential therapeutic opportunities for treating convulsive and degenerative neurological conditions. The review underscores the crucial role played by Cx-based channels, including gap junctions and electric synapses, as well as hemichannels, or connexons, in the development of neuroinflammation and neurodegeneration. Furthermore, this review delves into the mechanisms underlying Cx-mediated processing of neuro-inflammatory responses.

Keywords: Connexins, Neuroinflammation, Neurodegenerative diseases.

I. Cellular connections

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Intracellular connections coupled with highly communicated cells. These structures allow adjacent cells to be firmly connected. They prevent metabolites from being exchanged through or establishing rapid connections between nearby cells [1]. Connections have significant roles in tissue strength, transferring information between extracellular and intracellular spaces, controlling ion exchange and molecules across cell layers, directing ion transfer and molecules across cell layers, and exchanging ions and metabolites through the cell cytoplasm [2]. There are several types of animal cell connections, including anchoring gap junctions (GJ) and tight junctions.

II. Characteristics of Gap junction

Many invertebrate and vertebrate tissues are not independent of each other, and have intracellular connections that exchange ions and larger molecules. In this type of connection, the distance between the two membranes is 2 nm and is seen in the form of strips, plates, or small dots [3]. Each contains hexagonal objects with a diameter of 90-70 angstroms, each of which has a narrow central duct with a diameter of 15 Å that is interconnected in the intracellular space[4, 5]. his type of connection can be present in any part of the lateral membrane, mostly in the epithelial cells.

Connexin protein members exist in the cell membrane as hexameric proteins, which are recognized as connexons and act as connexin hemichannels that permit permeability to small molecules, ions, and hormones [6, 7].

III. Gap junction physiological structure

Unlike neurotransmitters, metabolite exchanging through GJ are both one-way and two-way GJs proteins have three widespread families: annexin 1, panxin 2, and C. Inxins form GPs in invertebrates. Mixins are sensitive to membrane potential closure and depolarization. A third family of inxin-like proteins called pannexins has recently been discovered in invertebrates and vertebrates [8]. Pantiescano forms intracellular channels. Thus semi-channels. Panxins may allow for exchange between intracellular and extracellular spaces. Panxins have no similarity to Cxs and are approximately 20% similar to connexins [9]. All of these proteins form four membrane-wide alpha-helices (M1-M4), two extracellular loops (E1 and E2), and a cytoplasmic loop subunit of connexin, pannexins, and Cx hemichannels [7, 10]. The presence of cysteine in the extracellular loop is crucial for GJ formation in two opposite hemichannels in interconnected cells. Cxs and connexins contain three and two cysteine amino acids per extracellular loop, respectively, and pannexins contain two cysteines per extracellular loop [11].

There are 21 different Cx genes in humans are expressed in various cell types. The Cx gene family in mice consists of 20 members [12]. Cxs belong to a unique vertebrate protein family [13].

Gap junctions are the channels that connect two adjacent cells. Each channel consists of two half channels [14]. Each half-channel contains six subunits, called Cxs. Cxs are integral membrane proteins known to produce their molecular weight.

Cx six-dimensional oligomerization begins within a hemichannel in the endoplasmic reticulum and ends in the Golgi apparatus transgene region. Vesicles containing connexons are then transferred to the cell membrane, along with microtubules and actin filaments. Dual-channel noncovalent bonds located in the plasma membrane of neighboring cells are formed through hydrogen bonds between the extracellular sides of Cx [15].

The Cx protomer consists of four loops, two extracellular loops, two cytoplasmic loops, and C and N terminals. The most common community of GJ is the gap junction plate, which is characterized by a reduction in space between membranes where cells are connected [16, 17].

Cytoplasmic regions, especially the C-terminal region, have revealed significant signal transduction, phosphorylation, kinase activation, and cytoskeletal evidence. Some cells express a single connexin that forms identical channels [18]. However, some cells express at least two Cxs; these different proteins are arranged in

heteroesophageal Cxs that form heterogeneous junctions. The presence of variability in the channel composition leads to differences in channel permeability [19].

IV. Gap Junctions in evolution

Transient and unstable pairing of neurons by GJs is a phenomenon in mammalian nervous system development. GPs are common in the early stages of neurogenesis [20]. Direct Intracellular metabolite exchange can be observed from the embryonic eight-cell stage forward, and protein precursor cleavage is detectable at the zygote stage (21). There is an inverse relationship between Cx expression and cell duplication [22]. It has been proposed that cell cycle and Cx expression may be related. In adult neurons, Cx protein expression is important for coordinating neural activity and synchronizing the oscillation network [23].

Most cell types in the brain express GJ, which are regulated during development and differentiation [24].

Examples of pathological changes in Cxs, including Cx 46, have been observed in Schwann cells during peripheral nerve regeneration. Studies have revealed several roles of the GJ during development. Observations show developmental changes in he ssassociationetween GJ and Cx 36 expression in neural differentiation, migration, and neural circuit formation and maturation [25].

It is assumed that GJ's role of GJs in these phenomena is through calcium, metabolites, and secondary messenger exchange between cells, which coordinates metabolic and transcriptional activity in developing neurons and often involves cooperation between GJ and neurochemical receptor receptors. Neurochemical transmitter receptors play a significant role in the development of gap junction communication [26]. Metabotropic group 2 receptors are regulated by glutamate receptors (mGLURs) via cAMP/PKA-dependent signals, which are regulated by GABA A receptors via repolarization and Ca2 + / PKC-dependent signals [27]. The rate of pairing and communication varies greatly between different anatomical sites [28].

V. Gap Junctions regulation

GJ control in the brain is regulated by dynamic processes. These channel conduction modifications depend on voltage changes, intracellular calcium, sodium, magnesium, and intracellular pH [29].

Voltage sensitivity is imperative for the configuration of intracellular connections between excitable cells. Cx 43 channels are relatively more sensitive to voltage changes than channels consisting of Cx 45 [24]. Studies have shown that GJs mediate the dependence of GJ valves on calcium levels. The binding of calcium to these domains causes structural changes and induces calmodulin interactions with the receptors [30]. Such interactions with Cxs 32, 37, 38, 43, 44, and 50 are approximately shown [31]. Recent experiments have shown that, after learning, the NMDA receptor and CamkII response require both hippocampal and independent memory for stabilization levels [26]. Furthermore, NMDA receptor responses are required for long-term storage of old memories in neural circuits and reactivation of NMDA in the amplification process when synaptic re-entry is associated with stabilization and storage of long-term memories representing a single cellular mechanism [32].

In all cells, acidification of the intracellular environment reduces intracellular communication through GJs [33, 34]. Protein kinase activity or phosphorylation may cause cell-to-cell communication and channel function modifications. Phosphorylation alters the metabolic and electrical connections between interconnected cells by altering the molecular structure of the channels. Modifications to the molecular structure affect the channel unit conduction, its average open time, or its opening probability. Furthermore, altering the etc-terminal may regulate the voltage or connector pH sensitivity [35].

Cytoplasmic side C-terminal tail f Cxs contain serine, threonine, and tyrosine subunits that may be phosphorylated by different protein kinases. Cxs are expressed in the nervous system and in other tissues [36]

Cxs are expressed in all tissues, besides the muscle system, erythrocytes, and mature sperm cells. Endothelial cells express Cxs40 and 43 [37]. Expression of Cxs45, 43, 40 along Cx 37 in blood vessels has been reported(38).

Cx 40 is abundant in endothelial cells and Cx 43 in smooth muscle cells. In the heart, Cxs 30, 40, 43, and 45 can form fissured connections between myocytes and atrial myocardium and ventricle [31]. In the nervous system, there are GJs between neurons, oligodendrocytes, astrocytes, microglia, ependymal cells, and also between neurons-astrocytes or astrocytes-oligodendrocyte s.[39].

In general, Cxs identified in the mammalian nervous system include: Cxs 26, 29, 30, 31, 32, 36, 37, 43, 47, and 45 [40].

GJs between ependymal cells are composed only of Cx 43 and leptomeningeal cells are composed of Cx 43 and 26 [41]. Cxs 26, 30, and 43 are expressed in astrocyte GJs, and connexin 43, which is associated with Cxs 30, 40, and 45, is abundant in astrocytes. Cxs 32 and 45 are predominant in oligodendrocytes [42]. GJs are formed in the hippocampus and neocortex between inhibitory intermediate neurons. GJs of these GABAergic inhibitory intermediate neurons include Cx 36, which forms between dendrites and is a prominent feature in the neural network of inhibitory neurons that produce and regulate neurons oscillating in the city of this region and other brain areas. is the predominant expression of Cx 36 occurs in neurons of the lower olive nucleus and hippocampus [43]. Cx 36 is a 321 amino acid protein and a member of the GJD2 gene family γ subunit (encoding Cx 36) located on chromosome 3 of rats in the human chromosome 15q14 kinetic region [44]. The sequence of the gene encoding the protein Cx 36 is 98% similar to that in mice, rats, and humans, and 8% is similar to the gene sequence of Cx 35 protein in fish. Cx 45 [45]. Cx 45 gene is expressed in almost all brain sections during embryonic development and up to two weeks after birth. Its expression is limited to the thalamus, CA3 area of the hippocampus, and cerebellum. Cx Gja7 consists of three exons and two introns. The amino acid sequence of the Cx 45 protein is located in exon 3 [46, 47].

Cx 45 is a 396 amino acid Cx and one of the four isoforms expressed in the heart. Recent studies using the Lacz reporter gene for Cx 45 in mice showed that: Cx 45 in the adult hippocampus showed poor expression in CA3 pyramidal cells and no signal in other areas of the hippocampus. Expression of Cx 45 has been shown in basal cells and cerebellar satellites Expression of Cx 45 has been shown in cortical pyramidal cells and thalamic neurons [48].

Expression of Cx 45 has been shown in neural populations other than oligodendrocytes and astrocytes. Cx45 expression has recently been observed in spinal cord alpha motor neurons. Research has shown that Cx 45 may be involved in homeostasis and cell survival beyond the coordination of oscillating activity. Cx 45 has a high sensitivity to moderate voltage and conduction compared to other Cxs such as Cx. Cx 36 can compensate for Cx 45 absence in mice brain but not in heart development, and this indicates the specific activity of fissured connective tissue, including Cx 36 [49].

VI. Gap junctions in synapses

GJs are synapses that can mediate neural communication in two ways: indirect exchange, chemical synapse activity through neurotransmitter release and direct exchange, and electrical synapse activity through ions and metabolites. Electrical synapses are GJ between neurons. In the mammalian central nervous system, neurons are paired by GJ [51]

Various studies have shown that intracellular communication via GJs is involved in learning and memory. Ravier et al. reported that in Cx36 knockout mice, 36 learning processes in the Y phase were disrupted, suggesting that GJs consisting of Cx 36 are involved in learning and memory [50]. S Beheshti et al. revealed that injecting carbenoxolone into hippocampus CA1 region impairs spatial learning process in rats in the Mauritius method and concluded that carbenoxolone prevents memory consolidation [51]. XZ Lieut al. mice without Cx 31 were impaired in learning to identify objects [51]. Pereda et al. reported that intracellular communication through electrical synapses between cerebellar olive nucleus neurons is essential for motor learning [52].

Pereda et al. reported that intracellular communication through electrical synapses between cerebellar olive nucleus neurons is essential for motor learning [53]. Allen et al. (2011) indicate that knocked out of Cx 36 in mice impairs short-term spatial memory. They concluded that interface neurons that communicate through gap connections are important for spatial information coding and cognition [54]. Limbäck-Stokin et al. reported that mice lacking Cx 36 have difficulty identifying objects in the hase in which calcium enzyme calmodulin kinase is depleted in their brain striatum region [55]. Anders, Stefanie et al. discovered that gap joints arm closure in rat hippocampus CA1 region is involved in memory consolidation so that their closure inhibits memory consolidation and opening them improves memory consolidation[56].

VII. Connexin signaling and inflammation

In the cerebral cortex, it has been observed that astrocytes primarily produce Connexin-43 (Cx43), while microglial cells predominantly exhibit Connexins-32 (Cx32) and -26 (Cx26). Additionally, other members of the Connexin family, such as Cx26, Cx30, Cx40, Cx45, and Cx46, are also present in astrocytes. Oligodendrocytes within the brain have been found to express Cx32, Cx47, and Cx29, whereas neurons synthesize Cx36 and Cx45 [57].

The blood-brain barrier exhibits expression of connexin proteins CX37, CX40, and CX43. Astrocytes, specifically, display expression of CX43, CX30, and CX26. Furthermore, during the developmental process of motor neurons within the spinal cord, mRNAs encoding CX36, CX37, CX40, CX43, and CX45 were detected. In contrast, spinal astrocytes exhibit expression of CX30 and CX43, whereas oligodendrocytes demonstrate positivity for CX29, CX32, and CX47 upon staining [58].

In instances of pathological disorders, pro-inflammatory cytokines are released into the circulatory system, triggering the migration of leukocytes towards specific chemical signals. This phenomenon leads to increased permeability of the blood-brain barrier, resulting in the infiltration of blood cells into the central nervous system and subsequent neuroinflammatory responses. The degradation of myelin sheaths surrounding nerve fibers, known as demyelination, serves as a hallmark characteristic of various neurodegenerative autoimmune diseases, including but not limited to multiple sclerosis, encephalomyelitis, and chronic inflammatory demyelinating polyneuropathy [59].

Under pathological conditions, pro-inflammatory cytokines are secreted into the bloodstream and chemotactic leukocytes. These cytokines cause the blood-brain barrier to become more permeable, consequential blood cell invasion to the central nervous system, and neuro-inflammation. Demyelination, a pathological condition in which myelin surrounding nerves has deteriorated, is a marker of many neurodegenerative autoimmune diseases, including multiple sclerosis, encephalomyelitis, and chronic inflammatory demyelinating polyneuropathy [60].

In addition, the expression of connexin 32 (Cx32) is inadequately controlled within myelinated axons, whereas connexin 47 (Cx47) is primarily found in precursor cells of oligodendroglia [61].

A recent study utilizing lipopolysaccharide (LPS) injections in rat models of autoimmune encephalomyelitis has elucidated the underlying mechanisms governing the expression patterns of connexin 43 (Cx43). The research findings indicate that the initiation of demyelination, characterized by the loss of Cx43 protein in the corpus callosum, is preceded by the activation of apoptotic pathways in oligodendrocytes [62]. Astrocytes play a pivotal role in initiating and regulating the production of pro-inflammatory cytokines, specifically interleukin-1 beta (IL-1β) and tumor necrosis factor-alpha (TNF-α), which are key mediators of inflammation [63]. In contrast, these cytokines have been shown to modulate gap junctions (GJs) and hemichannels (HCs) in astrocytes. The significance of astrocytes extends beyond their involvement in inflammatory processes, as they also contribute to various infectious and neurodegenerative disorders affecting the central nervous system (CNS). Notably, studies have demonstrated that intracerebral administration of lipopolysaccharide (LPS) in rat models leads to increased messenger RNA (mRNA) levels of connexin 32 (Cx32) in the hippocampus while concurrently reducing expression of connexin 43 (Cx43) [64].

When astrocytes are subjected to pro-inflammatory stimuli, including cytokines, lipopolysaccharides (LPS), and amyloid-beta peptides, their gap junctions (GJs) exhibit degradation. Additionally, interleukin-1 beta (IL-1β) has been found to impede GJ communication and conductivity by suppressing connexin 43 (Cx43) protein levels and messenger RNA (mRNA) expression in primary human fetal tissue samples. Similarly, tumor necrosis factor-alpha (TNF-α) treatment of primary rat astrocyte cultures leads to disruption of GJs accompanied by rapid phosphorylation of Cx43 proteins [65].

Nitric oxide (NO) is a potent metabolic product generated through the activity of phagocytes, leukocyte adhesion, and relaxation of smooth muscle cells. Intrinsic exposure of astrocytes to lipopolysaccharide (LPS) triggers a significant augmentation of nitric oxide synthase (NOS), thereby facilitating the production of NO and inducing a dose-dependent repression of gap junctions (GJs). Consequently, this process leads to a reduction in the expression of connexin 43 (Cx43) proteins and messenger RNA [66].

Upon exposure to Staphylococcus aureus, a gram-positive bacterial pathogen capable of inducing cerebral inflammation, pro-inflammatory mediators including tumor necrosis factor-alpha (TNF-α), interleukin-1 beta (IL-1β), and nitric oxide (NO) are activated in astrocytes and microglial cells. The identification of peptidoglycan, a key component of S. aureus's cellular wall structure, within these immune cells occurs through mediation by Toll-like receptor 2. Subsequent activation of this receptor triggers the p38 mitogen-activated protein kinase signaling cascade, ultimately regulating connexin 43 (Cx43) messenger RNA and protein expression, thereby facilitating gap junction formation. Notably, Cx43 mRNA and protein levels have been observed to be increased in primary microglial cultures [67].

GJA1, recognized as connexin43 (Cx43), is highly conserved among vertebrates. GJA1 is strongly related to amyloid and tau pathologies in AD. RNA sequencing analysis of Gja1astrocytes confirmed that Gja1 arranged the identified subnetwork in AD and numerous genes associated with Aβ metabolism. Astrocytes lacking Gja1 show decreased Apoe protein levels and weakened Aβ phagocytosis [68]. Cx43 also functions as a unitary channel that contributes to the pthe aracrine system, but this activity is often linked to pathological conditions [69]. GJA1 and GJB6 (connexin30) are mainly expressed in mature astrocytes, creating astrocytic networks that expedite calcium wave spread, potassium and glutamate buffering, and metabolic homeostasis. Astrocytic GJs are vital for neuronal function, as indicated by the neurological phenotypes in Gja1/Gjb6 double-knockout mice [70]. In a murine model of autoimmune thyroid disease, chronic inflammation downregulated (Cx26, Cx32, and Cx43. Interestingly, deficient thyrocytes persevered in primary cultures, signifying that the presence of inflammatory mediators is not essential to tolerate this difference [71].

Nutrition, connexin and neuroinflammation

In recent years, the role of nutrition and nutrients in relieving neuroinflammation in a time-limited manner has become more dramatic. Omega-3 fatty acids and simple sugars are among the dietary factors associated with chronic neuroinflammation. However, it should be noted that both of these nutrients are effective in altering Cx, and the expression and function of Cx are differentially regulated by nutrition.

Polyunsaturated fatty acids (PUFAs) are generally considered essential fatty acids, meaning they cannot be produced by mammals and need to be provided through the diet [72]. The brain contains high levels of n-3 long-chain polyunsaturated fatty acids such as docosahexaenoic acid (DHA) and arachidonic acid (AA) which have powerful anti-inflammatory properties [73]. Numerous studies have shown that PUFAs can help to reduce and resolve neuroinflammation [74]. Several studies have shown that PUFA dietary intake can induce changes in the expression, distribution, and post-translational modifications of Cx proteins. Cx43 content is positively associated with brain DHA levels [75]. In astrocytes of rats that had been supplemented with DHA for 10 days, an increase in gap junctional communication was observed [76]. DHA alone or eicosapentaenoic acid increases Cx43 phosphorylation in rat astrocytes [76]. In another study, an increase in the methylation of Cx43 induced by AA was described [77]. There is also evidence that fatty acids induce Cx responses via protein kinases that can be activated downstream of different G-protein coupled receptors (GPCRs) [78]. This evidence suggests

that the impact of fatty acids on neuroinflammation may be due, at least in part, to changes in Cx content. However, further research is required to confirm this hypothesis.

High glucose intake exacerbates astrocyte-mediated neuroinflammation in humans, and high glucose levels increase the mRNA expression of interleukin (IL)-6 and secretion of both IL-6 and IL-8 by astrocytes [79]. On the other hand, in several studies, downregulation of connexin expression by high blood glucose has been also observed [80] and high consumption of sucrose has disrupted signaling mediated by Cx [81] Therefore, the Lack of Cx expression following high glucose intake may exacerbate the inflammation caused by sugar intake. Connexin is also involved in obesity-induced inflammation. Obesity has been shown accompanied by oxidative stress and inflammation [20] as well as by Cx43 alterations, such as irregular Cx43 distribution [21, 22]. This evidence suggests that the effect of nutritional factors on neuroinflammation can be mediated by Connexin.

Conclusion

The involvement of GJs in the pathogenesis of neurodegenerative diseases such as AD has been revealed in numerous studies [82]. GJs expression has been revealed to be dysregulated in the postmortem AD brains [71] Recent studies have reported that GJs play critical roles in AD-related phenotypes in mouse models of AD. Alternatively, numerous studies have indicated that some GJs such as Gja1 have neuroprotective effects. Beyond studies, GJA1 expression is upregulated in AD, as well as its regulatory role in promoting a major astrocyte-specific molecular network underlying AD. Indeed, our functional analysis using Gja1 astrocytes showed that the Gja1 network contributed to neuronal differentiation and reduced their viability after Aβ1–42 treatment, which may explain synapse and neuron loss in the disease of brains [83]. Indeed, the Gja1 network is assumed to be involved in the phagocytosis of Aβ and the maintenance of neuronal activity under Aβ stress. It'sreported thatGja1 is taken together with the increase in Cx43 protein and its channel activity, specifically in astrocytes surrounding plaques. A study by, Li, Shuo et al. Unraveledadvantages of Cx32-knock outed mice revealed that the loss of this connexin gene affected LPS-induced inflammation. Nevertheless, no difference was detected between Cx32-deficient and wild-type liver acute-phase transcripts [84]. The contribution of GJ to the cell response to injury was also analyzed during experimentally induced acute inflammation. Interestingly, Cx32 deficiency causes a mild reversible form of inflammation into a strict disease with enhanced necrosis and edema. Cx32-deficient mice exhibit decreased sensitivity of nerve cells to apoptotic stimuli. Proinflammatory cytokines are considered to intensify cell lesions but also have main effects on tissue regeneration by stimulating apoptosis and cell proliferation [85]. Above all, Cxs and GJs in the central nervous system (CNS) have always been of research interest in homeostatic glia/neuron activities in various neurological disorders.

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