Chapter 15

Plasma Membrane Channels Formed by Connexins or Pannexins in Microglia: Possible Role in the Inflamed Brain

Juan A. Orellana

Additional information is available at the end of the chapter

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1. Introduction

In a healthy brain, microglia exhibit a resting surveillance state associated with active exploration of their environment for exogenous or endogenous signals representing a threat to the homeostasis [1-5]. When physiological balance is impaired in the central nervous system (CNS), resting phenotype of microglia shift to a reactive phenotype with different degrees of activation according to the nature of the stimuli and the context. During intense CNS inflammation, rather than show a repair-orientated activity profile, reactive microglia constitute a source of toxic factors and participate in the recruitment of non-resident brain cells involved in the innate immune response, which worsen brain damage. The brain performs exceptionally complex and dynamic tasks that depend on the coordinated interaction of glial cells, therefore it is conceivable that impairment of intercellular signaling and coordination among microglia could play an important role on several CNS disorders. In vertebrate cells, this synchronization is in part mediated by gap junctions [6-10]. They are aggregates of intercellular channels termed gap junction channels that allow direct, but selective, cytoplasmic continuity between contacting cells, promoting the exchange of ions (allowing eletrical coupling), metabolites (e.g., ADP, glucose, glutamate and glutathione) and second messengers (e.g., cAMP and IP_3)[11-16]. Whereas a gap junction channel is formed by the serial docking of two hemichannels each one contributed by one of two adjacent cells, each hemichannel is composed by six protein subunits termed connexins (Fig. 1). The latter belong to a highly conserved protein family encoded by 21 genes in human and 20 in mouse with orthologs in other vertebrate species [17-19]. Connexins are abundantly expressed in cells of the CNS, and they are named after their predicted molecular mass expressed in kDa, so that connexin43 (Cx43) has a molecular mass of ~43 kDa.

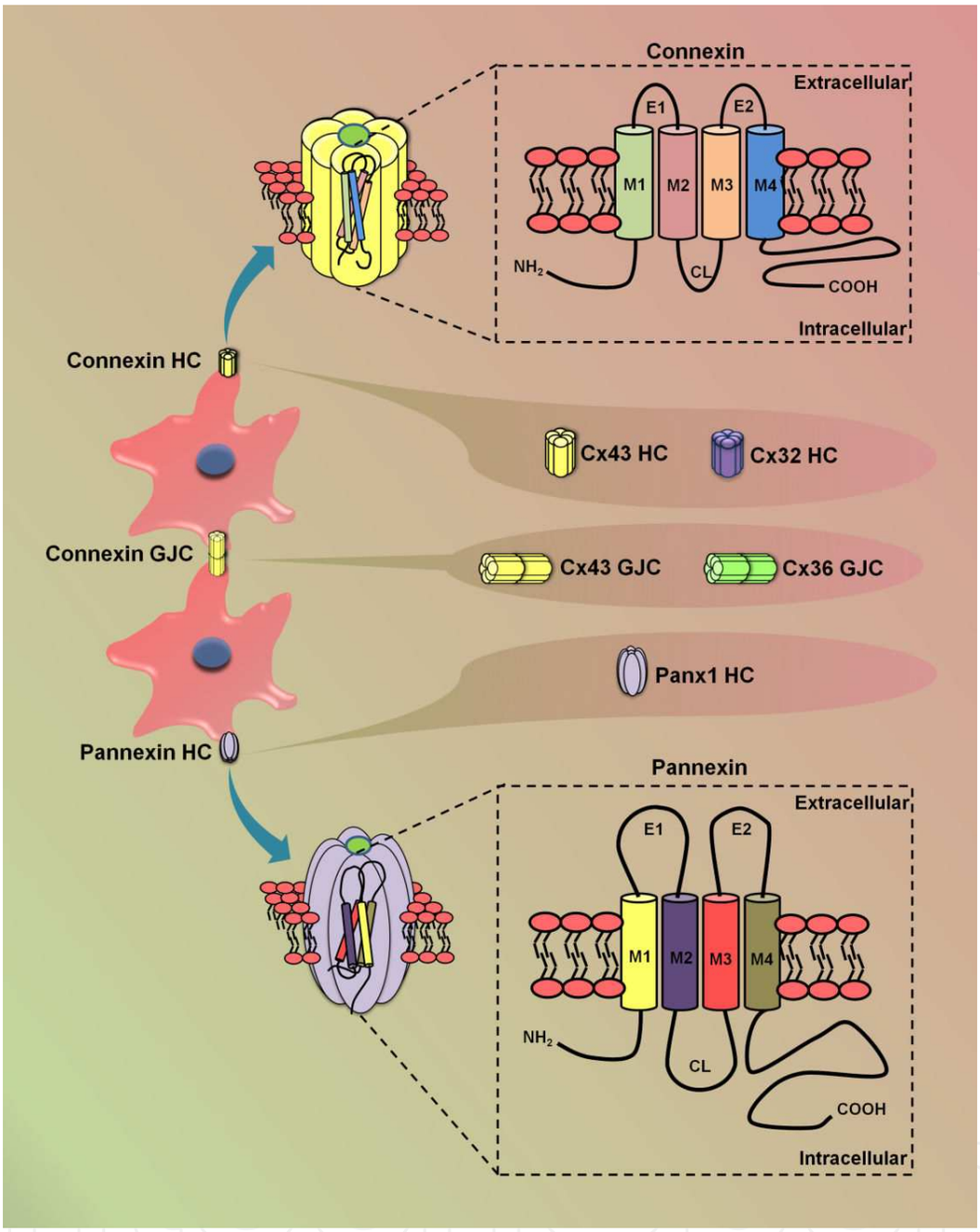

Figure 1. Diagram illustrating basic structures of gap junction channels and hemichannels formed by connexins or pannexins in microglia. Connexins and pannexins share similar membrane topology, with four α-helical transmembrane domains (M1-M4) connected by two extracellular loops (E1 and E2), one cytoplasmic loop (CL) where both amino (NH2)- and caboxy (COOH)-termini are intracellular. Top and bottom center show hemichannels formed by six connexin or pannexin subunits each, respectively. The middle center shows a connexin gap junction channel, at a close contact between two microglia. A hemichannel is formed by six connexins or pannexins that oligomerize laterally leaving a central pore in the activated state (open). Under resting conditions hemichannels remain preferentially closed, but they can be activated by diverse physiological and pathological conditions, offering a diffusional transmembrane route between the intra and extracellular milieu. In addition, it is depicted the types of hemichannels and gap junction channels expressed by microglia. This figure includes only the available information obtained under in vivo and/or in vitro studies using more than one experimental approach.

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For a long time the main function attributed to connexin hemichannels was the formation of gap junction channels. Nevertheless, in the last decade, the presence of functional connexin hemichannels in nonjunctional membranes has been demonstrated by several experimental approaches [20-24]. These channels serve like aqueous pores permeable to ions and small molecules that permit diffusional exchange between the intra and extracellular compartments, allowing cellular release of relevant quantities of autocrine/paracrine signaling molecules (e.g., ATP, glutamate, NAD^+ and PGE_2) to the extracellular milieu [25-30], as well as uptake of small molecules (e.g., glucose) [31]. One decade ago, a new gene family of gap junction proteins composed by three members was discovered in chordates [32, 33]. These proteins are the chordate homologs of innexins (the gap junction proteins of non chordates), and were denominated pannexins (panx1, 2 and 3) because apparently they are present in all eumetazoans except echinoderms [34] (Fig. 1). It has been suggested that gap junctional intercellular communication occur via Panx3 in osteoblasts [35], whereas other studies have shown that overexpression of exogenous Panx1 could form gap junctions *in vitro* [33, 36, 37]. Nevertheless, the absence of ultrastructural evidences for gap junction formation and demonstration of functional communication mediated by other endogenously expressed pannexins indicate that they apparently act mainly as hemichannels [38].

Current knowledge regarding brain hemichannels state that, under physiological conditions, they have a low activity, but enough to ensure the release of paracrine substances necessary for diverse functions of the CNS, including ischemic tolerance [39, 40], establishment of adhesive interactions [41]; fear memory consolidation [42], glucosensing [30], chemoreception [43], blood-brain barrier permeability [44], neuronal migration [45, 46] and metabolic autocrine regulation [47]. Nevertheless, under acute or chronic neurodegeneration dysregulation of hemichannel properties could be critical on the beginning and maintenance of homeostatic imbalances observed in diverse brain diseases [48-50]. Pioneering findings from Paul and colleagues showed that Xenopus oocytes transfected with Cx46 mRNA exhibited non-selective cation currents associated to depolarization and cell lysis within 24 h [51]. From then on, several studies supported the idea that dysregulated opening of hemichannels is incompatible with normal cell life. In the CNS, the first convincing evidence of hemichannel opening was provided by Contreras and colleagues, whose work showed that opening of Cx43 hemichannels accelerate astroglial cell death induced by ischemia-like conditions [52]. Such increased hemichannel activity induced by ischemia-like conditions has been observed in neurons [40, 53-55], oligodendrocytes [56], and also in brain cells subjected to other pro-inflammatory conditions [48]. Up to now, it is believed that sustained hemichannel opening contributes to increased intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$), which in turn may favor even more the hemichannel activity (De Vuyst et al., 2007, Schalper et al., 2008), inducing Ca^{2+} and Na^+ intracellular overload (Fig. 2).

Under these conditions, ionic (or electrolyte) imbalance leads to an osmotic imbalance that results in cell swelling and plasma membrane breakdown. Calcium overload induced in part by hemichannel opening may also activate phospholipase A_2, with the subsequent generation of arachidonic acid and activation of cyclooxygenase/lipoxygenase pathways leading to increased free radicals, lipid peroxidation and further plasma membrane damage. Possi-

bly, exacerbated or uncontrolled hemichannel opening could lead to cellular damage by several ways: 1) High increase of $[Ca^{2+}]_i$ by Ca^{2+} entry through hemichannels, 2) cellular swelling by increased entry of Na^{2+} and Cl^- through hemichannels, 3) release of metabolic products essential to cell viability as glucose, NAD^+ or glutathione via hemichannels and 4) alternatively, spread of toxic molecules released by hemichannels (e.g., glutamate) could affect the viability of healthy neighboring cells.

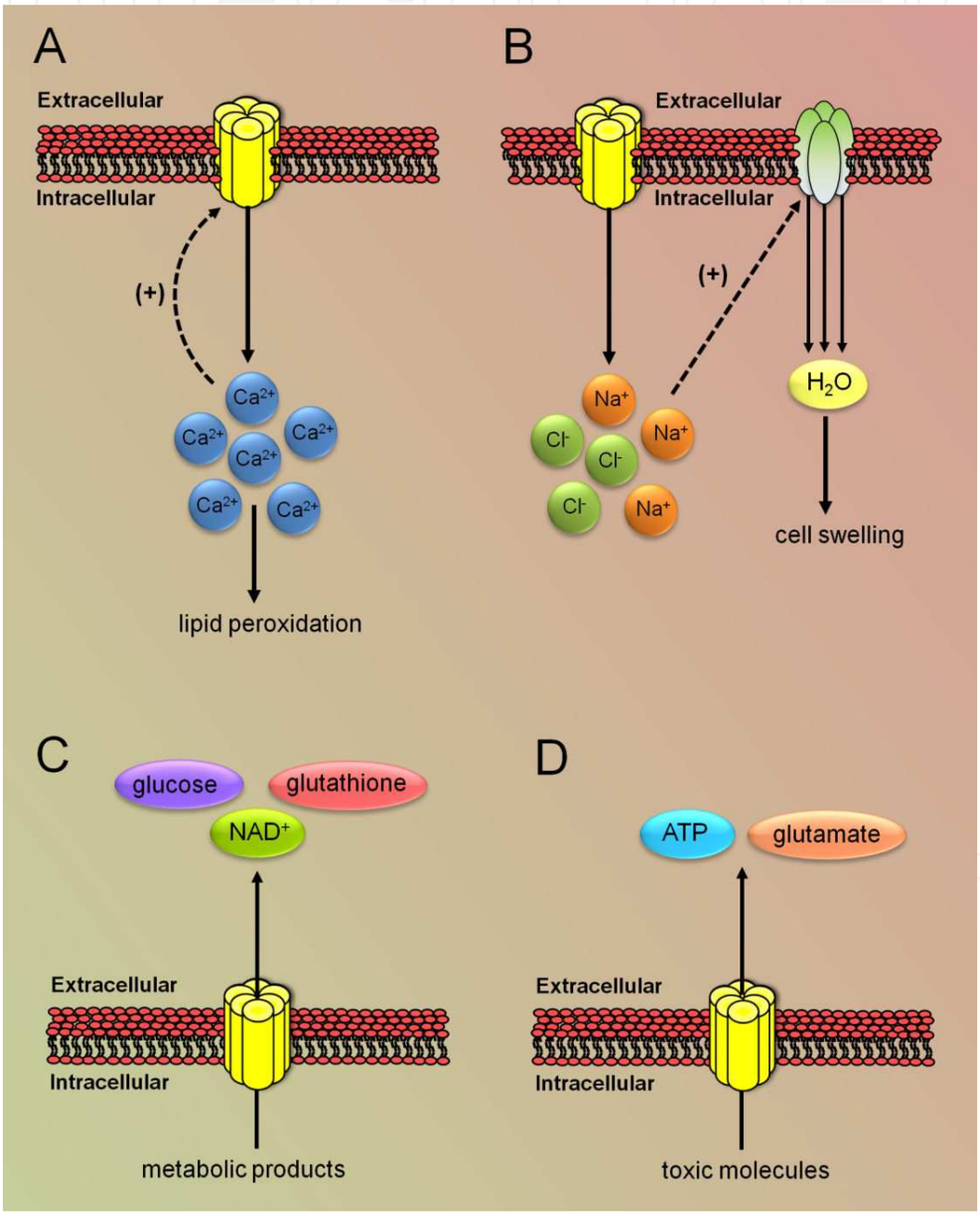

Figure 2. Dysregulated opening of hemichannels induces cell damage by different mechanisms. Under normal conditions, hemichannels (yellow channels) exhibit a low activity. However, upon exposure to inflammatory conditions, hemichannels undergo a dysregulation proccess leading to an uncontrolled opening which further results in cellular

damage by various mechanisms. (A) Ca^{2+} entry through hemichannels activate phospholipase A_2, with the subsequent generation of arachidonic acid and activation of cyclooxygenase/lipoxygenase pathways leading to increased free radicals, lipid peroxidation and further plasma membrane damage. Note that increased levels of $[Ca^{2+}]_i$ may activate even more hemichannel opening as demonstrated previously [57, 58]. (B) Na^{2+} and Cl^- entry through hemichannels could produce cellular swelling by increased influx of H_2O via aquoporins (green channels). (C) Release of essencial metabolic products via hemichannels (eg., glucose, NAD^+ or glutathione) could increase cell vulnerability. (D) Release via hemichannels of molecules that in high amounts are toxic (e.g., ATP and glutamate) could affect the viability of healthy neighboring cells and spread damage.

Taking into account that hemichannels participate in the paracrine signaling among brain cells, the current chapter attempts to review and discuss the role of gap junction channels and hemichannels in microglia on normal and inflamed brain.

2. Gap junction channels in microglia

In a resting surveillance state, microglia express almost undetectable levels of Cx43 and Cx36 [59-65]. Nevertheless, when microglia are subjected to pro-inflammatory conditions, they exhibit expression of Cx43 and are able to form gap junction channels among them, as evaluated by dye-coupling experiments. In fact, Cx43 expression and gap junctional communication is induced in microglia by LPS, TNF-α plus IFN-γ [61], calcium ionophore plus PMA [66], or Staphylococcus aureus-derived peptidoglycan [64]. Despite the above, cultured human or mouse microglia treated with LPS, granulocyte-macrophage colony-stimulating factor, INF-γ or TNF-α do not exhibit modifications in connexin expression [60, 63]. Recently has been showed that resting microglia exhibit detectable levels of surface and total Cx43, whereas upon treatment with amyloid-β peptide (Aβ) a high increase in Cx43 expression is observed (Orellana 2011a). The discrepancy in the above mentioned studies may be related to different types of animal used to obtain brain tissue, dissimilar methods to take out cells and different culture conditions.

The ability to establish gap junctional communication among microglia, requires a rise in $[Ca^{2+}]_i$ [66], while cAMP, cGMP or activation of PKC have been ruled out as possible inductors gap junction-mediated coupling [66]. In this regard, different degrees of microglial activation may trigger intracellular pathways that further result in a specific pattern of expression of gap juntion proteins. Communication via gap junctions may allow to activated microglia to recruit resting microglia at the site of injury, resulting in more damage or repair depending on the circumstances. Interestingly, microglia stimulated with cytokines or LPS exhibit reduced levels of Cx43 expression and gap junctional communication in astrocytes when both cell types are in co-culture or when conditioned media from activated microglia is used [31, 55, 59, 60, 67, 68]. Interestingly, gap junctions among dendritic cells ensure sharing of antigenic peptides [69-74], suggesting the possibility that these channels in microglia also could coordinate the CNS immune response. Importantly, recently it has been shown that the release of TNF-α and IL-1β by microglia depend on the activity of gap junction channels, because secretion of those cytokines was partially blocked by a gap junction blocker, α-glicirretinic acid [75]. Thus, it was proposed that gap junction channels play a key role into coordinate the microglial mediated inflammation.

3. Hemichannels in microglia

Up to now only few studies have documented the expression of functional hemichannels in microglia. Contrary to the expectations regarding as Cx43 the most possible protein to form hemichannels in microglia, TNF-α treatment was shown to induce release of glutamate through a pathway inhibited by a Cx32 (^{32}Gap27), but not Cx43 (^{43}Gap27) mimetic peptide [76]. Moreover, surface levels of Cx32 were increased in microglia treated with TNF-α. Noteworthy, the increased neuronal death associated with the release of glutamate was inhibited completely with the ^{32}Gap27 mimetic peptide [76]. Later, the same group of authors proposed that glutamate released via Cx32 hemichannels play a key role in neuronal damage originated by brain ischemia [77] and experimental autoimmune encephalomyelitis [78]. Accordingly, microglial cells from Mecp2 null mice, a model of a neurodevelopmental disorder known as Rett syndrome, promote neuronal death through glutamate release via a cell membrane pathway inhibited by ^{32}Gap27 and ^{32}Gap24, two Cx32 hemichannel mimetic peptides [79]. It is relevant to kept in mind that these and other mimetic peptides are homologous to extracellular domains of the respective connexin sequences, but their effects on hemichannel activity have not been documented, thereby some studies have questioned their specificity [80-82]. The use of cell cultures derived from connexin null mice and/or performing knockdown of the respective connexin, along the appropriate use of mimetic peptides could ensure the involvement of Cx32 hemichannels in these studies.

Almost two years ago, the opening of Cx43 and Panx1 hemichannels, evaluated by dye uptake and macroscopic cell membrane currents, were shown to be increased in microglia by $A\beta_{25-35}$ exposure (Orellana et al. 2012a). These observations were confirmed by using microglial cultures from Cx43 KO mice and Panx1 mimetic peptides. These currents were recorded at negative holding potential (-60 mV) in the presence of external divalent cations, suggesting that opening of microglial hemichannels may occur in Alzheimer's disease (AD). Importantly, ATP and glutamate released from microglia treated with $A\beta_{25-35}$ trigger hemichannel opening in neurons causing deleterious effects on them [83]. Supporting the idea of hemichannels as possible regulators in damage observed in AD, a novel putative hemichannel blocker (INI-0602) that crosses the blood brain barrier was recently shown to inhibit *in vivo* the LPS-induced glutamate release from microglia and to improve memory deficits in APP/PS1 mice [84]. Due the pharmacological pattern of this response," it was proposed the involvement of Cx32 hemichannels. However, the possible implication of other hemichannel forming proteins or even other channels was not ruled out and studies on the specificity of INI-0602 require further demonstration using, for example, *in vivo* experiments with $Cx32^{-/-}$ microglia or knockdown of Cx32. To demonstrate the participation of hemichannels in this disease it is necessary to analyze the functional state of microglial hemichannels in brain slices from AD model mice (APP/PS1) by using patch-clamp and membrane permeability assays.

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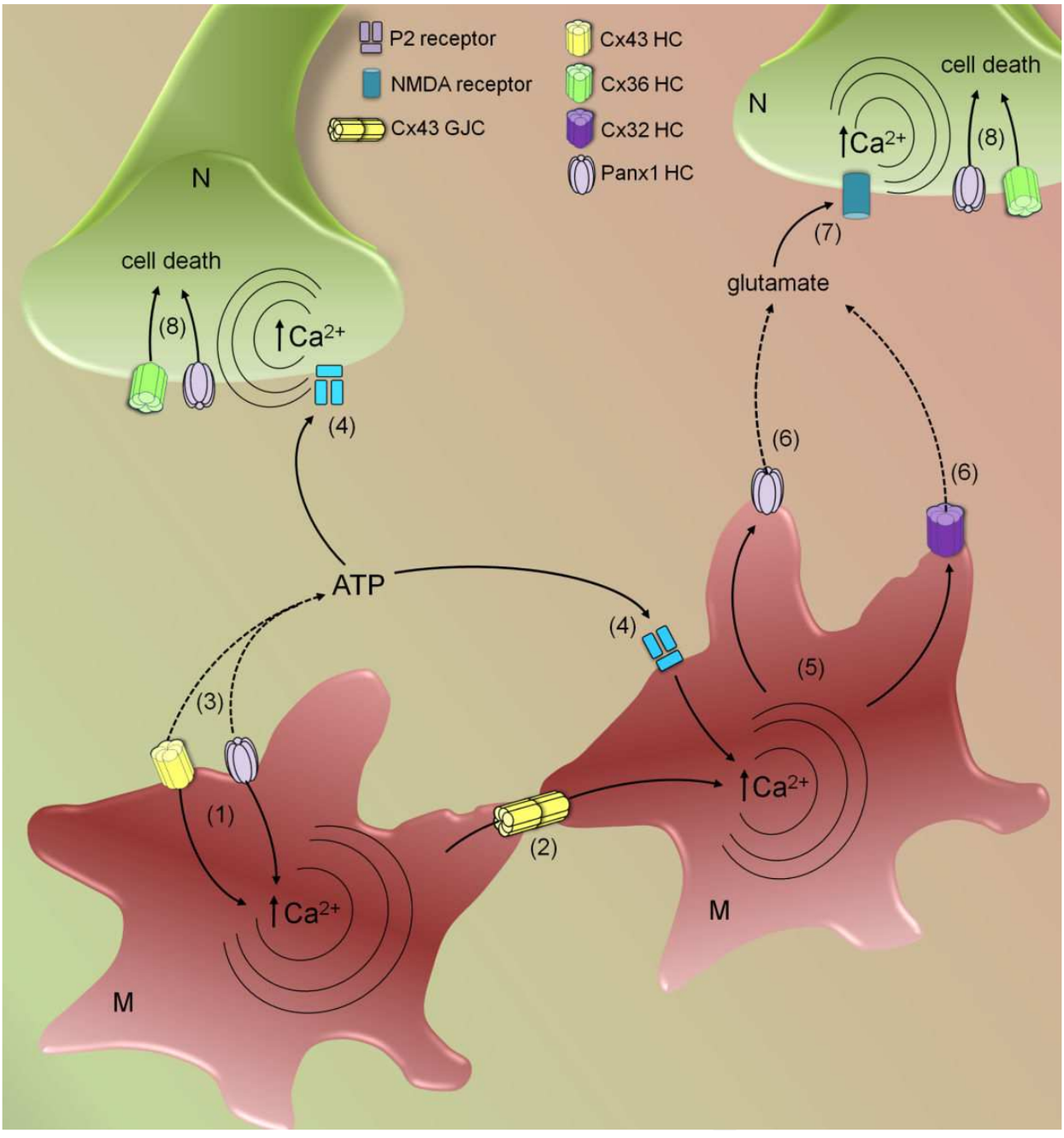

Figure 3. Role of microglial cell hemichannels and gap junction channels during neuroinflammation. Chronic or acute inflammation increases hemichannel (HC) activity in microglia allowing the influx of Ca^{2+} (1) and its spread to neighbor cells through gap junctions (GJCs) (2) raising the intracellular free Ca^{2+} concentration ($[Ca^{2+}]_i$). HC opening induced by inflammation in microglia leads to ATP release (3), which diffuses through the extracellular space and activates membrane purinergic (P2) receptors (4). High levels of $[Ca^{2+}]_i$ (5) allow the release of glutamate through microglial cell HCs (6) and further activation of neuronal NMDA receptors (7). P2 and NMDA receptor activation in neurons increase the activity of neuronal Panx1 and Cx36 HCs, affecting electrochemical and Ca^{2+} imbalance in neurons, which leads to cell death (8).

4. Conclusions

Microglial cells are known to play a relevant role in neuronal survival [3]. In pathological situations, dysregulation of connexin- and pannexin-based channels expressed by microglia, contribute importantly to determine the neuronal fate [48, 50]. Microgliosis and brain inflammation are associated with most, if not all, brain injuries and pathologies. Hemichannel activation in microglia could play a crucial role in the reinforcement of the neuronal death, due to their capacity to release glutamate and ATP (Fig. 3) [55, 76, 83, 85]. Opening of Cx43, Cx32 and Panx1 hemichannels could increase $[Ca^{2+}]_i$ in microglia, which further propagate Ca^{2+} waves via gap junction channels to neighbor cells (Fig. 3). Moreover, in distant microglia, Ca^{2+} waves can activate hemichannels, as demonstrated previously [57, 58, 86]. Then, opening of neuronal Panx1 hemichannels could be triggered by the rise in $[Ca^{2+}]_i$ via activation of NMDA and P2X receptors by glutamate and ATP, respectively. Panx1 hemichannels are likely to contribute to the intracellular Ca^{2+} overload that activates neurotoxic intracellular cascades during excitotoxicity [87] (Fig. 3). Thus, the prevention of hemichannel activation under pro-inflammatory conditions may represent an unexplored strategy to prevent neuronal damage and death. Altogether these observations strengthen the emerging concept that unregulated membrane permeability through enhanced hemichannel permeability and dysfunctional gap junction channels may contribute to the development of CNS pathologies and connexins as well as pannexins might represent potential and alternative targets for therapeutic intervention in neuroinflammatory diseases.

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Author details

Juan A. Orellana

Departamento de Neurología; Escuela de Medicina, Pontificia Universidad Católica de Chile, Santiago, Chile

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