Neuroscience 286 (2015) 45–59

NEUROSCIENCE FOREFRONT REVIEW

HEMICHANNELS: NEW PATHWAYS FOR GLIOTRANSMITTER RELEASE

T. D. MONTERO AND J. A. ORELLANA *

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

Abstract—Growing evidence suggests that glial cells express virtually all known types of neurotransmitter receptors, enabling them to sense neuronal activity and microenvironment changes by responding locally via the Ca^{2+}-dependent release of bioactive molecules, known as ''gliotransmitters''. Several mechanisms of gliotransmitter release have been documented. One of these mechanisms involves the opening of plasma membrane channels, known as hemichannels. These channels are composed of six protein subunits consisting of connexins or pannexins, two highly conserved protein families encoded by 21 or 3 genes, respectively, in humans. Most data indicate that under physiological conditions, glial cell hemichannels have low activity, but this activity is sufficient to ensure the release of relevant quantities of gliotransmitters to the extracellular milieu, including ATP, glutamate, adenosine and glutathione. Nevertheless, it has been suggested that dysregulations of hemichannel properties could be critical in the beginning and during the maintenance of homeostatic imbalances observed in several brain diseases. In this study, we review the current knowledge on the hemichannel-dependent release of gliotransmitters in the physiology and pathophysiology of the CNS.

Key words: connexin, pannexin, astrocyte, microglia, neuron, hemichannel.

Contents

INTRODUCTION

Connexins are the protein subunits forming the hexameric ring of a connexon, one half of a gap junction channel (GJC) (Hervé and Derangeon, 2013). These ''half channels'' are usually referred to as ''hemichannels'' and form a full GJC when they dock with another hemichannel in the apposed membrane of an adjacent cell (Fig. 1). GJCs allow for the intercellular exchange of metabolites (e.g., ADP, glucose and glutathione), second messengers (e.g., cAMP and IP_3) and ions (e.g., Ca^{2+}, K^+ and Na^+). Because of their ubiquitous expression throughout different organs and their ability to allow intercellular communication, connexins are crucial for several physiological functions, including the transmission of intercellular Ca^{2+} waves, spread of electrotonic potentials, local blood flow regulation and spatial buffering of ions and metabolites (Saez et al., 2003).

For a long time, the main function attributed to hemichannels was the construction of GJCs. However, in the early 90s, pioneering studies by Paul and colleagues identified the first nonjunctional currents mediated by hemichannels in *Xenopus* oocytes expressing connexin 46 (Cx46) (Paul et al., 1991). They observed that overexpression of Cx46 resulted in oocyte lysis, thus the opening of hemichannels was believed to be incompatible with cellular life. Therefore, the rationale was as follows: if hemichannels are open to the extracellular space, ionic imbalance would make maintaining an appropriate membrane potential impossible, resulting in further cell death. In the late 90s, this idea was supported by several groups showing that hemichannel opening occurred under very specific circumstances: during ischemia-like conditions (John et al., 1999; Kondo et al., 2000; Li et al., 2001; Contreras et al., 2002; Vergara et al., 2003); low extracellular Ca^{2+} concentrations ($[Ca^{2+}]_e$) (Ebihara and Steiner, 1993; Li et al., 1996; John et al., 1999; Quist et al., 2000; Valiunas and Weingart, 2000) and upon depolarization

*Corresponding author. Address: Departamento de Neurología, Escuela de Medicina, Pontificia Universidad Católica de Chile, Marcoleta 391, Santiago, Chile.
E-mail address: jaorella@uc.cl (J. A. Orellana).

Abbreviations: AD, Alzheimer's disease; APP, amyloid-β precursor protein; Aβ, amyloid-β peptide; BBB, blood–brain barrier; BLA, basolateral amygdala; CBX, carbenoxolone; CM, conditioned media; $[Ca^{2+}]_e$, extracellular Ca^{2+} concentrations; $[Ca^{2+}]_i$, intracellular Ca^{2+} concentrations; GJC, gap junction channel; KO, knockouts; LTD, long-term depression; LTP, long-term potentiation; MBH, mediobasal hypothalamus; PCO_2, pressure of CO_2; PSD95, postsynaptic density 95; SFKs, Src family kinases; VMS, ventral medullary surface.

http://dx.doi.org/10.1016/j.neuroscience.2014.11.048

(Ebihara and Steiner, 1993; Trexler et al., 1996; Valiunas and Weingart, 2000; Contreras et al., 2003). During those years, different approaches relying on channel reconstitution (e.g., unilamellar liposomes) and electrophysiological recordings revealed that hemichannels exhibit distinct unitary conductance, molecular permeability/selectivity and electric and chemical gating (similar to GJCs), depending on their protein subunit composition (Saez et al., 2005; Harris, 2007; Moreno and Lau, 2007; Wang et al., 2013). Based on their subunit composition, hemichannels can be homomeric if they are composed of one connexin isoform or heteromeric if they are composed of more than one connexin isoform.

Later, a new gene family of gap junction proteins composed of three members was described (Panchin et al., 2000; Bruzzone et al., 2003; Baranova et al., 2004). These proteins are the chordate homologs of innexins (the gap junction proteins of non-chordates). Because they are present in all eumetazoans (except echinoderms), they were called pannexins (Panx1, 2 and 3) (Fig. 1A) (Shestopalov and Panchin, 2008; Dahl and Keane, 2012; Bond and Naus, 2014). Although pannexins have no sequence homology with connexins, they share a similar topology, secondary and tertiary structures and somewhat overlap on pharmacological properties (Lohman and Isakson, 2014). Most of evidence indicates that pannexins act mainly as plasma membrane channels similar to hemichannels formed by connexins (MacVicar and Thompson, 2010; Wang et al., 2013). Pioneering studies indicated that Panx1 hemichannels are non-selective channels with large unitary conductances, including 475 pS in oocytes (Bao et al., 2004), 450 pS in insulinoma cells (Iglesias et al., 2009b), and 300 pS in cardiac myocytes (Kienitz et al., 2011). Nevertheless, under determined experimental conditions, Panx1 hemichannels seem to act as anion channels with conductances in the range of ~60–68 pS (Ma et al., 2012; Romanov et al., 2012).

Gap junctional communication through pannexins has only been reported in a few overexpressed systems (Bruzzone et al., 2003; Vanden Abeele et al., 2006; Lai et al., 2007; Ishikawa et al., 2011), and several research groups have advocated this idea (Sosinsky et al., 2011). Nevertheless, a recent study has reintroduced this issue. Using molecular approaches, Sahu and colleagues showed that stable expression of Panx1 resulted in the formation of functional GJCs in HeLa cells but not in Neuro-2a or PC-12 cells because Panx1 showed a high degree of glycosylation in these cells (Sahu et al., 2014). Moreover, both Panx1 and Panx3 GJCs showed different pharmacological and channel properties when compared to Cx43 GJCs, as measured by dye coupling and electrophysiological experiments. Indeed, in contrast to Cx43 GJCs, pannexin GJCs were insensitive to heptanol, carbenoxolone (CBX), probenecid and changes to the trans-junctional voltage (Sahu et al., 2014). This study suggested that pannexins form gap junctions with properties that are distinct from junctions formed by Cx43; a finding that is necessary to claim a GJC function is formed by pannexins. Nevertheless, the existence of pannexin GJCs must be demonstrated not only in exogenous expression systems but in native cells and *in vivo*.

Currently, most data indicate that under physiological conditions hemichannels have a low activity, but this activity is sufficient to ensure the release of relevant quantities of autocrine/paracrine signaling molecules (e.g., ATP, glutamate, NAD^+, adenosine and PGE_2) to the extracellular milieu (Bruzzone et al., 2001; Stout et al., 2002; Cherian et al., 2005; Locovei et al., 2006; Lin et al., 2008; Iglesias et al., 2009a), as well as the uptake of small molecules (e.g., glucose and dyes) (Fig. 1B) (Retamal et al., 2007; Orellana et al., 2012b). They have been involved in numerous biological functions, including colonic transit (McClain et al., 2014), flow regulation of collecting ducts (Svenningsen et al., 2013), glucosensing (Orellana et al., 2012b), ischemic tolerance (Lin et al., 2008; Schock et al., 2008); fear memory consolidation (Stehberg et al., 2012), monocyte–endothelial adhesion (Yuan et al., 2012), bone development (Batra et al., 2012), potentiation of skeletal muscle contraction (Riquelme et al., 2013), wound healing (Makarenkova and Shestopalov, 2014), synaptic transmission (Klaassen et al., 2011; Prochnow et al., 2012), chemoreception (Wenker et al., 2012), blood–brain barrier (BBB) permeability (De Bock et al., 2011), cell proliferation (Pinheiro et al., 2013), chemotaxis of neutrophils (Bao et al., 2013), neuronal migration (Liu et al., 2010, 2012) and cellular adhesion (Cotrina et al., 2008). Nevertheless, as previously mentioned, it has been proposed that dysregulations of hemichannel properties could be critical in the beginning and during the maintenance of homeostatic imbalances observed in several diseases (Orellana et al., 2009, 2011b, 2012c; De Vuyst et al., 2011; Davidson et al., 2013; Fasciani et al., 2013; Salameh et al., 2013). Based on this idea, exacerbated or dysregulated hemichannel opening could lead to cellular damage via several mechanisms: (1) an increase in $[Ca^{2+}]_i$ mediated by hemichannel permeation to extracellular Ca^{2+}, (2) cellular swelling induced by uncontrolled influx of Na^{2+} and Cl^- through hemichannels and (3) the spread of toxic molecules released by hemichannels (e.g., glutamate) from injured cells (Fig. 2). In this study, we review current knowledge on the role of hemichannels in the release of gliotransmitters in health and disease.

HEMICHANNEL-MEDIATED GLIOTRANSMITTER RELEASE IN THE NERVOUS SYSTEM

Based on the increasing body of evidence suggesting that astrocytes are an active partner in synaptic transmission, the *tripartite synapse* concept was coined in the late 90s. This idea suggested that, in addition to the presynaptic and postsynaptic neurons, astrocytes are crucial components that determine synaptic function (Araque et al., 1999). More recently, *in vivo* studies have shown that microglia constantly extend toward and retract from synapses, participating in a new range of undiscovered functions, including neuronal surveillance, synapse elimination and regulation of cell death, among others (Tremblay et al., 2010; Schafer et al., 2013; Wake et al., 2013). Indeed, it has recently been proposed that the tripartite synapse concept of the chemical synapse should