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Battle of the hemichannels – Connexins and Pannexins in ischemic brain injury

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ABSTRACT

Perinatal ischemic brain injury can occur as a result of a global ischemic insult or focal ischemic stroke in the preterm or full-term neonate. One of the most striking features of HI injury is that, after initial recovery of cellular oxidative metabolism, there is a delayed, 'secondary' mitochondrial failure that spreads over time from the most severely damaged areas outwards, into previously undamaged regions. This secondary failure is accompanied by transient seizure activity and cytotoxic edema.

The specific mechanisms of this spread are poorly understood, but it is at least partly associated with spreading waves of depression that can trigger cell death in neighboring uninjured tissues. Both Connexin and Pannexin hemichannels may mediate release of paracrine molecules that in turn propagate cell death messages by releasing intracellular mediators, such as ATP, NAD^+, or glutamate or by abnormally prolonged opening to allow cell edema. This review will discuss the controversy around the relative contribution of both Connexin and Pannexin hemichannels and mechanisms by which they may contribute to the spread of ischemic brain injury.

1. Introduction

Ischemia is a reduction in tissue blood flow that leads to a reduction in delivery of both oxygen and energy substrates (e.g., glucose) below metabolic requirements. During the perinatal period, this can occur as a result of focal ischemic stroke, where a blood vessel in the brain becomes blocked, leading to localized tissue damage, or global hypoxia-ischemia resulting from birth asphyxia or cardiovascular collapse (Fernandez-Lopez et al., 2014; Wassink et al., 2014). Perinatal arterial ischemic stroke occurs in approximately 1/2300–1/5000 live infant births, and is more common in the term or near-term infant, but can also occur in the preterm infant (Fernandez-Lopez et al., 2014; Benders et al., 2008; Golomb et al., 2008). Moderate to severe hypoxic-ischemic encephalopathy resulting from a global insult occurs in approximately 1–3/1000 live full-term births (Vannucci, 2000). Currently, the only treatment for full term infants suffering from hypoxic-ischemic encephalopathy is therapeutic hypothermia. Although hypothermia significantly reduces death and disability, it is only partially effective (Edwards et al., 2010). There is no clinical treatment available for infants suffering perinatal arterial ischemic stroke, likely due to difficulties in and timing of diagnosis. HI also contributes to the high burden of neurodevelopmental disability in infants born preterm, who now constitute 7–12% of all live births (Committee on Understanding Premature Birth and Assuring Healthy Outcomes, 2007). There are no clinical treatment options available for preterm infants suffering ischemic brain injury. Therefore, novel treatment strategies for preterm and full-term infants exposed to focal or global ischemic brain injury are needed.

Propagation of injury from damaged areas of the brain into previously healthy regions is common to all forms of ischemic injury. In ischemic stroke, a characteristic pattern is a focal ischemic core comprised of dead tissue and a surrounding penumbral region that has the potential to either progress to death or be salvaged. The central focal ischemic stroke core extends out into the penumbral regions over the following hours and days. In the case of perinatal HI, brain injury is not uniform and some areas are more prone to early damage. This lesion also propagates over a number of days to encompass initially uninjured regions (Thornton et al., 1998). This results in a biphasic pattern of evolving neural injury, with the acute period of HI per se representing the primary phase of injury, followed by a latent phase after tissue reperfusion in which most parameters transiently normalize. From 6 to 15 h after moderate to severe insults there can be a phase of secondary deterioration that

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extends the volume of affected tissue, in association with metabolic failure, seizure activity, edema and cell death (Williams et al., 1991, 1992; Gunn et al., 1997).

Gap junctions have been implicated in the spread of injury in a wide array of central nervous system disease models including ischemic brain injury, spinal cord injury, epilepsy, and even trauma (Rawanduzy et al., 1997; de Pina-Benabou et al., 2005; Fonseca et al., 2002; Nakase et al., 2006; Cronin et al., 2008; Danesh-Meyer et al., 2008; Xie et al., 2011; Tamura et al., 2011; Belousov et al., 2012). However, many early studies mainly relied on the use of non-specific compounds, such as octanol and carbenoxolone, which can block multiple membrane channels, including both gap junctions and hemichannels. Long-term blockade of gap junctions can be deleterious after ischemic brain injury, likely due to disruption of the astrocytic syncytium (Nakase et al., 2003, 2004). In our own studies, infusion of a gap junction channel blocking peptidomimetic, peptide 5, in the near-term fetal sheep at dose levels likely to cause uncoupling of gap junctions as well as blocking hemichannels (O'Carroll et al., 2008), was associated with impaired EEG recovery, increased secondary cell swelling and an apparent trend to higher lactate concentrations and mortality after 30 min of global cerebral ischemia (Davidson et al., 2012a).

There is, however, increasing evidence that it is specifically Connexin hemichannels that are associated with the spread of brain injury after ischemic stroke and global hypoxic-ischemic brain injury in the fetus/neonate (Davidson et al., 2014, 2013, 2012b; Orellana et al., 2010), as well as other central nervous system disorders, such as spinal cord injury and retinal stroke (O'Carroll et al., 2008; Danesh-Meyer et al., 2012; O'Carroll et al., 2013). Recent evidence suggests that Pannexin hemichannels, membrane channels that are similar in structure to Connexin hemichannels, may also contribute to the spread of ischemic brain injury, although this has not been studied directly in the developing brain (Madry et al., 2010; Orellana et al., 2011).

The specific roles and the relationship between Connexin and Pannexin hemichannels in the spread of ischemic brain injury are highly controversial. This review discusses the evidence for roles of the Connexin and Pannexin hemichannels in ischemic brain injury, with a particular emphasis on how they might contribute to injury in the fetus and neonate.

2. Introduction to Connexin and Pannexin hemichannels

Gap junction plaques are clusters of intercellular channels that link adjacent cells directly and are permeable to small molecules, ions, and second messengers with a molecular weight less than one kilodalton (kDa) (Kumar and Gilula, 1996; Sohl et al., 2005). Gap junctions are formed by docking of two connexons together, one contributed from each adjacent cell (Fig. 1). A connexon or hemichannel is a hexamer that consists of six sub-units called Connexins. There are 21 known Connexins in the human genome, 11 of which are expressed in the central nervous system and are named according to their theoretical molecular mass in kDa [as reviewed in (Willecke et al., 2002)]. The type of Connexin expressed is highly dependent on the brain region, cell type, and stage of development. Connexin 43 is the predominant Connexin found in astrocytes, along with Connexin 30 (Rash et al., 2001). Astrocytes are linked by gap junctions to other astrocytes and oligodendrocytes, but not to mature neurons (Rash et al., 2001). Connexin 43 is also abundant at the astrocytic end–foot processes that surround blood vessels, and contribute to the blood–brain barrier, and on the astrocytic processes in close proximity to chemical synapses (Chew et al., 2010). Connexin 43 is also expressed in normal capillary endothelium and at low levels in microglia (Pepper et al., 1992; Eugenin et al., 2001).

It is now becoming clear that Connexin hemichannels are active under normal physiological conditions, including contributing to purinergic signaling by regulated release of ATP (Stout et al., 2002; Kang et al., 2008). Connexin 43 hemichannels also mediate nicotinamide adenine dinucleotide (NAD^+) transport, and functionally interact with the plasma membrane ectoenzyme cyclic adenosine diphosphate (ADP)-ribose hydrolase CD38 that converts NAD^+ to the calcium ion mobilizer, cyclic ADP-ribose (cADPR) (Bruzzone et al., 2001a, 2001b). Although the mechanism is not yet clear, there is recent evidence that Connexin hemichannels may contribute to spontaneous depolarizations within the human fetal cortex during the second trimester of gestation (Moore et al., 2014).

Pannexins share 20% sequence homology with the invertebrate innexin proteins that form invertebrate gap junctions, but have no homology with Connexins (Panchin et al., 2000; Yen and Saier, 2007). However, Connexin and Pannexin membrane topology is very similar and both channels are blocked by a number of commonly used compounds, such as carbenoxolone and flufenamic acid (Bruzzone et al., 2005). It has been suggested that in vertebrates Pannexins cannot form gap junctions, as interaction between Pannexin hemichannels is prevented by their extensive glycosylation and therefore, they exist only in the hemichannel form (Boassa et al., 2007). Only a single study has shown Pannexin hemichannels being involved in cell–cell channel formation, following Pannexin expression in mouse C2C12 cells (Ishikawa et al., 2011).

Three Pannexin genes have been identified, Pannexins 1–3. The Pannexins 1 and 2 are expressed in neurons extensively throughout the brain including in the cortex, striatum, olfactory bulb, hippocampus, thalamus, and cerebellum (Bruzzone et al., 2003). In contrast, Pannexin 1 mRNA has not been detected in glial cells in vivo (Ray et al., 2005; Vogt et al., 2005). Pannexin 1 has been shown to form hemichannels when expressed in Xenopus oocytes (Bruzzone et al., 2003).

Similar to Connexin hemichannels, Pannexin hemichannels are also thought to be involved in purinergic signaling under physiological conditions. Knockdown of Pannexin 1 hemichannels has been shown to significantly reduce ATP release from astrocytes in response to 3-*O*-(4-benzoyl)benzoyl adenosine triphosphate (BzATP), a $P2X_7$ agonist (Iglesias et al., 2009). However, this concept has recently been challenged by evidence that ATP release was no different in Pannexin 1/Pannexin 2 deficient astrocytes compared to wild type in response to BzATP stimulation (Bargiotas et al., 2011). This release of ATP was blocked by carbenoxolone, an inhibitor of both Pannexin and Connexin hemichannels, suggesting that Connexin hemichannels were mediating the release of ATP from these astrocytes.

2.1. Connexin hemichannels in ischemic brain injury

In addition to their roles in normal physiological functioning of the brain, Connexin hemichannels may contribute to the spread of injury under pathological conditions, such as ischemia. Unregulated opening of such a large and relatively non-specific channel linking the intra- and extra-cellular space could compromise ionic gradients required for resting membrane potentials and membrane transport as well as permitting unregulated movement of metabolites and second messenger molecules.

The first convincing evidence of Connexin hemichannel opening came from Paul et al. (1991), who showed that Xenopus oocytes transfected with Connexin 46 mRNA depolarized and lysed within 24 h unless osmolyte was included in the bathing medium. A voltage dependent current was subsequently shown to result from reduced extracellular Ca^{2+} concentrations in the bathing medium of the Connexin 46 cDNA injected oocytes (Ebihara and Steiner, 1993). Opening of Connexin 43 hemichannels has also been shown in Connexin 43 transfected cells that display sensitivity to low