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Invited review

Connexin targeting peptides as inhibitors of voltage- and intracellular Ca^{2+}-triggered Cx43 hemichannel opening

Nan Wang [a], Marijke De Bock [a], Elke Decrock [a], Mélissa Bol [a], Ashish Gadicherla [a], Geert Bultynck [b], Luc Leybaert [a,*]

[a] Department of Basic Medical Sciences, Physiology Group, Faculty of Medicine and Health Sciences, Ghent University, Ghent, Belgium
[b] Laboratory of Molecular and Cellular Signaling, Department of Cellular and Molecular Medicine, Campus Gasthuisberg O/N-1 Bus 802, K.U. Leuven, Leuven, Belgium

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ABSTRACT

Connexins form gap junctions that function as intercellular channels and hemichannels that form a conduit between the cytoplasm and extracellular fluid when open. Peptide inhibitors of connexin channels, especially those identical to defined connexin sequences, are interesting experimental, and possibly also therapeutic tools because they may have better selectivity than general inhibitors like carbenoxolone. Over the past ten years, several peptides have been demonstrated to block hemichannels, including Gap26, Gap27, peptide5, L2 and Gap19; some of these specifically block hemichannels but not gap junctions. Most of these peptides have only recently been investigated towards their actions at the single hemichannel level, bringing up interesting information on how they interact with the connexin protein and how they affect hemichannel gating. Hemichannels can be opened by electrical, mechanical and chemical stimuli. We here review the effect of the prototypic peptides Gap26/27 and L2/Gap19 with specific focus on their inhibition of Cx43 hemichannel opening triggered by positive membrane potentials and changes in intracellular Ca^{2+} concentration. Both Gap26/27 and L2/Gap19 peptide families block Cx43 hemichannel opening triggered by voltage as well as intracellular Ca^{2+} stimulation. Interestingly, these peptides as well as intracellular Ca^{2+} elevation modulate the voltage activation threshold for hemichannel opening, pointing to a common target. Moreover, L2 and Gap19 peptides are part of a sequence on the cytoplasmic loop that acts as a Ca^{2+}/calmodulin interaction site. We here review the interesting network of interactions between Cx43 targeting peptides, voltage gating and intracellular Ca^{2+} as major modulators of hemichannel function.

This article is part of the Special Issue Section entitled 'Current Pharmacology of Gap Junction Channels and Hemichannels'.

1. Introduction

Connexins are tetraspan transmembrane proteins with two extracellular loops (ELs), a cytoplasmic loop (CL) and both amino- and carboxyl-termini (NT and CT) facing the cytoplasm. They engage in forming gap junctions that facilitate electrical coupling and direct cell–cell transfer of chemical/metabolic signals. Beyond gap junctions, a plethora of data has substantiated the functional existence of unapposed hemichannels at non-junctional sites of the plasma membrane (Goodenough et al., 1996; Wang et al., 2013a). Typically closed under resting conditions, these half gap junction channels open in response to a variety of physiological and pathological stimuli including membrane depolarization, mechanical stimulation (Gomes et al., 2005), decreased external Ca^{2+} concentration (Allen et al., 2011; Muller et al., 2002; Quist et al., 2000), elevated intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) (Braet et al., 2003b; De Vuyst et al., 2009), lowering of intracellular redox potential and post-translational modifications including S-nitrosylation and altered phosphorylation status (Johnstone et al., 2012; Retamal et al., 2007a, 2007b). Open hemichannels provide a direct conduit between the cytoplasm and the extracellular compartment, allowing passage of ions and signaling molecules with molecular weight below 1–2 kDa. Distinguishing unapposed hemichannels from other molecular uptake/release pathways relies on knock-down and knock-out approaches of specific connexins. However, a critical limitation of these genetic tools is that such approaches deplete both pools of gap junction channels and

* Corresponding author. De Pintelaan 185 (Block B – Rm 031), B-9000 Ghent, Belgium. Tel.: +32 9 332 33 66; fax: +32 9 332 30 59.
E-mail address: Luc.Leybaert@UGent.be (L. Leybaert).

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hemichannels. More recently, mutants with a 'gain-of-function' of hemichannels and a 'loss-of-function' of gap junctions have been characterized, allowing better distinction between hemichannel and gap junctional functions in knock-in studies. For example, the point mutations G138R and G143S identified in oculodentodigital dysplasia give rise to leaky Cx43 hemichannels and deficient junctional communication (Dobrowolski et al., 2007, 2008). Recent work making use of transgenic mice with an astrocyte-targeted Cx43G138R knock-in established the involvement of Cx43 hemichannels in a neuron-astrocyte-neuron inhibitory feedback loop (Torres et al., 2012). Pharmacological tools (reviewed in (Evans et al., 2012)) are still pretty disappointing in terms of selectivity towards inhibiting hemichannels or gap junctions although some interesting tools have recently emerged. Peptides derived from highly conserved regions located on the first (Gap26, VCYDKSFPISHVR) or second (Gap27, SRPTEKTIFII) extracellular loop of Cx43 rapidly inhibit hemichannels while block of gap junctions occurs with some delay (Decrock et al., 2009; Desplantez et al., 2012) (Fig. 1). Peptide5 (VDCFLSRPTEKT) is a peptide shifted 5 amino acids in NT direction compared to Gap27 and inhibits hemichannels at low (5 μM) concentration while block of gap junctions occurs at higher (100 μM range) concentrations (O'Carroll et al., 2008). *In vitro* work with these peptides has demonstrated involvement of connexin hemichannels in a variety of cellular processes including dynamic Ca^{2+} signals (Ca^{2+} waves and Ca^{2+} oscillations) (Braet et al., 2003b; De Bock et al., 2011), release of neurotransmitters (Romanov et al., 2007) as well as propagation of cell death (Decrock et al., 2009). *In vivo*, Gap26/27 peptide confers cardioprotection against ischemia/reperfusion while peptide5 reduces tissue damage secondary to spinal cord injury and attenuates a vascular permeability increase following retinal ischemia (Danesh-Meyer et al., 2012; Hawat et al., 2012; O'Carroll et al., 2013). The growing interest and frequent application of these peptides contrasts with the rather limited characterization of their hemichannel effects. Initial studies of our group reporting Cx43 hemichannel block by Gap26 and Gap27 peptides were based on indirect measures such as ATP release and hemichannel-permeable dye uptake assays (Braet et al., 2003a, 2003b; De Bock et al., 2011; De Vuyst et al., 2007; De Vuyst et al., 2009). This work was complemented by macroscopic hemichannel current recordings in Cx43 expressing taste bud cells (Romanov et al., 2008) and HeLa cells (Desplantez et al., 2012), but contrasts to other findings claiming little effect of Gap26 on macroscopic hemichannel currents in *Xenopus* oocytes (Wang et al., 2007). To address these opposing findings, we investigated the effect of Gap26/27 at the single-channel level, which allows unequivocal identification of Cx43 hemichannels by their typical unitary conductance of ~220 pS and their highly positive activation threshold (+50 mV) for opening (Wang et al., 2012). More recently, we characterized Gap19 which is composed of a nonapeptide sequence present in the L2 domain of the cytoplasmic loop of Cx43. This peptide inhibits hemichannels without inhibiting gap junctions and is specific for Cx43 and does not affect Cx40 hemichannels or Panx1 channels (Wang et al., 2013b). The primary purpose of this review is to highlight the progress made towards a mechanistic understanding of how these various peptides inhibit Cx43 hemichannels. Furthermore, biophysical studies of Cx43 hemichannel function necessitate stimulation with strongly positive voltage steps to open the channels and therefore a secondary aim is to discuss our understanding of how a moderate $[Ca^{2+}]_i$ increase lowers the voltage threshold for Cx43 hemichannel activation. Importantly, Gap26/27 and Gap19/L2 peptides inhibit voltage-activated hemichannel opening as well as Ca^{2+}-promoted hemichannel opening, indicating that they block electrically as well as chemically triggered hemichannel activities

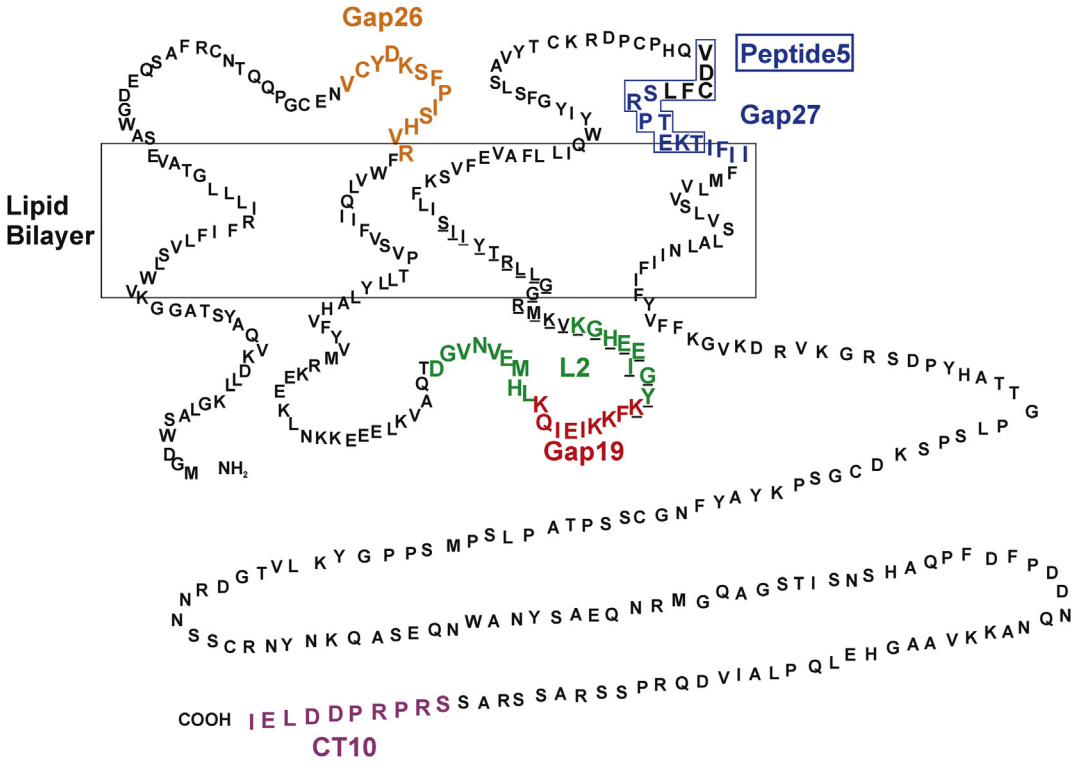

Fig. 1. Overview of Cx43 targeting peptide sequences on the Cx43 protein as well as the Ca^{2+}/CaM interaction site. Orange, Gap26 sequence; Blue, Gap27 sequence; Purple, CT10 sequence; Red, Gap19 sequence; Green, L2 sequence that spans through the Gap19 region; Box: Peptide5. Amino acids involved in the Ca^{2+}/CaM binding domain contiguous to the Gap19 region are underlined.